Rapid Diagnosis and Treatment of Hospital-acquired and Ventilator-associated Pneumonia in Intensive Care Unit Patients

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DECLARATION

I, Zaneeta Dhesi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Signature

…18th August 2021……..

Date

ABSTRACT

Background. This study aimed to define pathogens causing hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) using the polymerase chain reaction (PCR) based FilmArray™ Pneumonia Panel Plus (BioFire Torch system); and determine its potential in antimicrobial stewardship alongside a bespoke prescribing algorithm. The FilmArray™ panel, used on lower respiratory tract samples, includes 34 bacteria/viruses/resistance gene targets.

Methods. FilmArray™ results and conventional diagnostic sputum results were analysed for participating intensive care unit (ICU) patients with clinically-suspected HAP/VAP from 12 UK hospitals. Antibiotic prescribing and adherence to the prescribing algorithm were evaluated. Comparisons were made between COVID-19 and non-COVID-19 patients.

Results. The study included 326 patients; 126 were COVID-19 positive. 112 FilmArray™ tests were available for 104 non-COVID-19 adult patients; 44 FilmArray™ tests for 44 paediatric patients; 157 FilmArray™ tests for 126 COVID-19 adult patients. Amongst the non-COVID-19 group the most common organism was *Haemophilus influenzae* (n=23), followed by *Staphylococcus aureus* (n=20). Significantly more viruses were identified amongst children (P<0.0001). The proportion of negative result samples amongst conventional diagnostic microbiology results (50.7%) was significantly higher than for FilmArray™ (36.6%); P=0.024. The most common organism in COVID-19 patients was *Klebsiella pneumoniae* (n=30); its prevalence significantly higher than in non-COVID-19 patients (P=0.005). In both

patient groups approximately one third of patients' antibiotics adhered to the prescribing algorithm. A negative FilmArray™ result, in COVID-19 patients, was associated with stopping more antibiotics, compared to non-COVID-19 patients: 95% CI: 1.0%, 28.2%; and with significantly more patients not starting antibiotics: 95% CI: 8.8%, 33.6%. Significantly more COVID-19 patients had antibiotics stopped after a positive FilmArray™ result: 95% CI: 2.3%, 19.4%.

Conclusions. *Klebsiella* spp. were more prevalent in COVID-19 patients than in a similar pre-pandemic population. FilmArray™ identified more bacteria than conventional diagnostic microbiology, and there was evidence for its use as an antimicrobial stewardship tool – especially amongst COVID-19 patients.

IMPACT STATEMENT

Currently, clinicians treating intensive care unit (ICU) patients with a pneumonia wait approximately 2-3 days for a sputum culture result, but with the use of the FilmArray™ molecular diagnostic tool this turn-around-time can be reduced to just 75 minutes.

This test panel includes 34 bacteria and viruses associated with pneumonia as well as resistance gene targets. The output of such a test, reporting which organisms and resistance genes are identified in the sputum, would assist clinicians with both cessation of antibiotics and switching to a narrower spectrum agent if deemed clinically appropriate.

The results presented in this thesis show that implementation of the FilmArray™ rapid diagnostic tool at the bedside has the potential to benefit critically ill patients. Such a test would enable both a swifter diagnosis for hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), as well as swifter appropriate treatment. The findings presented also provide novel insight into the differences between pathogens responsible for HAP and VAP in COVID-19 and non-COVID-19 patients.

This thesis describes a prescribing algorithm developed for a rapid diagnostic test that seeks multiple pathogens in a critical care setting. The algorithm aims to advocate the narrowest-spectrum agents predicted to give good coverage, promoting antimicrobial stewardship; and importantly to translate complex

microbiological results into a clinical prescribing decision. Approximately one third of antibiotic prescriptions adhered to the algorithm; this highlights the multi-factorial decision making process which happens when making treatment decisions. The clinical picture and clinicians' own past experiences have an impact on these decisions; as does the use of a new diagnostic test.

There is much debate surrounding the results from such molecular tests, with belief that it can be difficult to determine whether organisms identified are colonising organisms or pathogens. Current ongoing work, using the same patient group as described in this thesis, aims to examine the role of blood biomarkers in conjunction with the FilmArray™ Pneumonia Panel Plus helping to answer the complex question: Is this a pathogen or a non-pathogen?

The evidence in this thesis supports the hypothesis that a rapid molecular test, the FilmArray™ Pneumonia Panel Plus, could provide us with a welcome antimicrobial stewardship tool to help improve antibiotic prescribing, especially amongst COVID-19 patients.

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DISCLAIMER

INHALE trial[1] data is presented in this thesis. I worked as a clinical fellow on the trial whilst working on my MD (Res).

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PUBLICATIONS AND PRESENTATIONS

Publications

Z. Dhesi, V.I. Enne, J. O'Grady, V. Gant, D.M. Livermore. *Rapid and Point-of-Care Testing in Respiratory Tract Infections: An Antibiotic Guardian?* ACS Pharmacol Transl Sci (2020) May 12;3:401-417.

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Conference Presentations

2020 – **e-ISICEM.** Poster Presentation – The INHALE trial: Designing a Prescribing to Aid Antibiotic Choices for the FilmArray™ Pneumonia Panel Plus.

2020 – **ECCVID.** Oral Presentation – Organisms causing secondary pneumonias in COVID-19 patients at 5 UK ICUs as detected with the FilmArray™ Test.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
BAL	Broncho-alveolar lavage
BSAC	British Society for Antimicrobial Chemotherapy
CAP	Community-acquired pneumonia
CDC	Centres for Disease Control and Prevention
CFU	Colony Forming Unit
CI	Confidence Interval
COVID-19	Coronavirus Disease 2019
CRP	C-reactive protein
ESBL	Extended-spectrum β-lactamase
ETA	Endotracheal aspirate
HAP	Hospital-acquired pneumonia
ICNARC	Intensive Care National Audit and Research Centre
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IL-6	Interleukin 6
IQR	Inter-quartile Range
KPC	*Klebsiella pneumoniae* carbapenamase
LRTI	Lower Respiratory Tract Infection
MDR	Multi-drug resistant

MERS	Middle East respiratory syndrome-related coronavirus
MHRA	Medicines and Healthcare Products Regulatory Agency
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MRSA	Methicillin-resistant *Staphylococcus aureus*
NDM	New Delhi metallo-β-lactamase
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NP	Nasopharyngeal
OP	Oropharyngeal
PCR	Polymerase Chain Reaction
PELOD-2	Paediatric Logistic Organ Dysfunction scores
POC	Point of care
POCT	Point of care test
RCT	Randomised control trial
RT- PCR	Real-time Polymerase chain reaction
SARS	Severe acute respiratory syndrome-related coronavirus
SD	Standard Deviation
SOFA	Sequential Organ Failure Assessment scores
VAP	Ventilator-associated pneumonia

WCC	White cell count
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1: MICROBIOLOGY AND DIAGNOSTICS OF HAP/VAP

1.1.1 Pneumonia

Pneumonia is a common cause of admission to intensive care units (ICU) in the UK, and is associated with significant mortality.[2] The National Institute for Health and Care Excellence (NICE) states (in pre COVID-19 times) that hospital-acquired pneumonia (HAP) is estimated to increase hospital stay by 7-9 days; and of those patients admitted to hospital with a community-acquired pneumonia (CAP), 1.2-10% are managed in ICU.[2] At present, if a patient is suspected to have a pneumonia – be it a CAP, HAP, or ventilator-associated pneumonia (VAP), the routine practise is to take a sputum/ endotracheal aspirate (ETA), or rarely a broncho-alveolar lavage (BAL) sample and send it to the microbiology laboratory for processing in line with the Standard UK Laboratories Operating Procedures.[3] Investigation of clinically-suspected bacterial pneumonia is complicated by the poor sensitivity of sputum culture and by the considerable interval (typically *circa* 72h) from sample to susceptibility test results.[3] Patients are started on empiric antibiotics, and these are often broad-spectrum, a concern for antimicrobial resistance (AMR). The utility of rapid diagnostics in this setting may prove a game-changer, providing results far more swiftly than routine methods, aiding appropriate use of antimicrobials.

AMR is a worldwide problem, and the World Health Organization (WHO) developed a Global Action Plan on AMR in 2015. As a result of this, countries were

asked to develop their own National Action Plan by 2017. In 2019 the UK Government devised a 5 year plan to help tackle this ever-rising issue of AMR.[4] The Review on Antimicrobial Resistance by Jim O'Neill (2016) stated that the use of rapid diagnostic tools could reduce unnecessary antibiotic use, and recommend that governments in high-income countries 'should consider incentives to facilitate the uptake and use of rapid point-of-care diagnostics in primary and secondary care'.[5] Prizes such as the Longitudinal Prize are available as a fund which will 'reward a team of innovators who develop a point–of–care diagnostic test that will conserve antibiotics for future generations; the test must be accurate, rapid, affordable and easy to use anywhere in the world.'[6] Therefore, the deployment of a rapid molecular tool at the bedside, as described in this thesis, is highly topical and relevant.

1.1.2 Pathogens associated with HAP/VAP

Pathogens responsible for HAP/VAP are generally more diverse than those causing CAP, and the infections can often be polymicrobial.[7] The common causative HAP/VAP organisms are Enterobacterales (including *Escherichia coli, Enterobacter spp.* and *Klebsiella pneumoniae*), *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Acinetobacter* spp. as demonstrated in a paper by Jones et al.[8] INHALE, a five year National Institute for Health Research (NIHR) Programme Grant for the antimicrobial themed call, reported the aetiology of HAP/VAP in ICU patients.[1,9] The study team (Appendix 1 and 2) analysed sputa, ETAs and BALs from patients on 15 UK ICUs who were about to receive either new antibiotics or a change in antibiotics for a HAP/VAP. Conventional diagnostic microbiology for 652

patient samples found the most common organism to be *S. aureus,* followed by *P. aeruginosa.*[10]

The aetiology of HAP/VAP varies across the world; with Gram-negative multi-drug resistant (MDR) organisms being far more prevalent in some countries than others.[11,12] The emergence of such MDR organisms gives rise to the use of broad-spectrum antibiotics as reflected in the Infectious Diseases Society of America (IDSA) guidelines.[13] These guidelines recommend that if a unit has >10% resistance to anti-Gram-negative agents, and a methicillin-resistant *Staphylococcus aureus* (MRSA) rate of 10-20%, then empirical treatment for HAP/VAP should be two anti-pseudomonal agents as well as an anti-staphylococcal agent which has activity against MRSA. In contrast, a review by Leone et al. has advocated the use of monotherapy in patients with HAP wherever possible.[14]

According to The British Society for Antimicrobial Chemotherapy (BSAC), 12.7% of *Klebsiella pneumoniae* from respiratory samples have extended-spectrum β-lactamases (ESBLs), and 0.9% have carbapenemases[15], whilst in India the picture is very different; 86.9% of *K. pneumoniae* have ESBLs, and 56.6% have carbapenemases.[16] This demonstrates how stark the difference is globally. A study by Dey et al. from India, looked at ICU patients with VAP and found that 80% of *E. coli* isolates and 100% of *K. pneumoniae* isolates produced ESBLs.[17]

Carbapenemases vary in their distribution worldwide: OXA-48 being common in most of Europe (important to note that within Europe itself there is a lot of variation) and the Middle East; *Klebsiella pneumoniae* carbapenamase (KPC) enzyme in China, Israel, Americas, Greece and Italy; and New Delhi metallo-β-lactamase (NDM) in India.

In countries where MDR organisms are commonplace, e.g., India, it is no surprise that the use of broad-spectrum agents is high, however if diagnostics provided a swifter result, then there would be scope to rationalise antibiotics sooner. The current approach is rational, given the practicalities of conventional testing, but is sub-optimal in respect of antibiotic stewardship. Many patients are given unnecessarily broad-spectrum antibiotics when they have susceptible pathogens, but some receive agents to which their pathogens prove resistant.[18,19] These limitations are exacerbated by the slowness of conventional diagnostic microbiology, by delays in sample transport to laboratories, by failures to act on laboratory results once received, and by frequent failures to grow pathogens from clinically-diagnosed patients, particularly those with pneumonia, where as few as 29% of clinically-diagnosed patients have an organism cultured.[20]

Certainly, risk-factors associated with HAP/VAP must be considered, as ultimately prevention is better than cure. Such risk factors include: aspiration, use of antacids, enteral nutrition, nasogastric tube, reintubation, tracheostomy, age, head trauma, previous antibiotic use, need for intracranial pressure monitoring.[21] A systematic review on reducing non-ventilator associated HAP concluded that interventions should include improving oral care, increasing mobility and dysphagia management.[22]

1.1.3 The FilmArray™ Pneumonia Panel Plus

The BioFire FilmArray™ Pneumonia Panel Plus (bioMérieux, Utah), performed using the BioFire Torch system, was selected for the INHALE trial. The panel has targets for 18 bacterial, nine viral, and seven antibiotic resistance genes. The turn-around-

time for the test is 75 mins, considerably faster than conventional diagnostic microbiology which can take 2-3 days.[3]

Work carried out by the INHALE trial team (Appendix 1 and 2) prior to commencement of the randomised control trial (RCT), data from which is presented in this thesis, evaluated two PCR platforms for HAP/VAP diagnosis against conventional diagnostic microbiology.[10] The platforms compared were the BioFire FilmArray™ Pneumonia Panel and the Curetis Unyvero™ Hospitalised Pneumonia Panel.

From this earlier work, the FilmArray™ was decided upon as the best test for INHALE. In order to determine the best test, a complex scoring system was devised. This consisted of overall concordance of results with conventional diagnostic microbiology; sensitivity; failure rate; breadth of panel; time to result; cost per test; footprint; consumable logistics; quality of customer service; and ease of use. The main reasons for choosing the FilmArray™ were firstly, from the 652 respiratory tract samples analysed, FilmArray™ had higher sensitivity compared with Unyvero™. FilmArray™ had sensitivity of 91.7-100.0% and specificity of 87.5-99.5%, whereas Unyvero™ had sensitivity of 83.3-100.0% except for *Klebsiella aerogenes* (50.0%) and *Serratia marcescens* (77.8%), and specificity of 89.4-99.0%.[10] Secondly the turn-around-time was faster for FilmArray™; and thirdly the footprint was smaller for the FilmArray™ - important as the platform was to be placed as a point of care test (POCT) on the ICU.

A large multinational evaluation of the Pneumonia Panel compared with standard of care testing was performed by Ginocchio et al at bioMérieux.[23] They reported results from 2,476 samples from 14 countries. The FilmArray™ detected

95.8% of bacteria compared to 57.1% for standard of care; and had a sensitivity ranging from 85.54-100%.

1.1.4 PCR tests used as rapid diagnostic tools

Rapid diagnostics, including polymerase chain reaction (PCR), have considerable potential for the improved investigation of pneumonia, increasing diagnostic yield and providing early treatment guidance.[24] Rogers et al. suggest that the use of a rapid respiratory panel test in hospital could reduce length of stay, and decrease duration of antibiotic use.[25] Rogers' study was performed using the FilmArray™ Respiratory Panel, on children, who typically present with more respiratory viruses compared with adults. A Japanese study by Kitano et al. also used this panel on paediatric patients with respiratory infections.[26] They compared use of this panel against rapid antigen tests and found that the length of hospital stay reduced from 8.18 days to 6.83 days (P=0.032). Furthermore, a Chinese study again using the same panel, but this time in adults, and comparing it with their in-house PCR assays also reported a reduction in duration of hospital stay: 8 days versus 9 days (P<0.001).[27]

Using such rapid diagnostics as a POCT adds another level of complexity. This would mean the test is available for clinicians to use at bedside, and a laboratory or medical microbiologist may well not be involved. The advantages of this would be eliminating the transport time to the laboratory and the time taken to book samples in at specimen reception, enabling faster patient management decisions. Transport time is an increasing issue in the UK with hospital laboratories being centralised. This was highlighted by Andrews et al. who used the FilmArray™

Respiratory Panel on 606 patients.[28] The FilmArray™ was used as a POCT on two adult medical wards and the medical assessment unit. This platform was compared with routine laboratory respiratory PCR and serology. They found no reduction in length of stay between the two groups and commented that the FilmArray™ was only performed when study investigators were on the ward. This translates to any POCT being maximally useful only if it is close to the patient and performed without delay without needing specialist input to interpret results.

It is important to remember that PCR-based diagnostics carry their own limitations. First, they do not seek all possible pathogens; they can only detect targets for which they have PCR primers. The organisms represented on the BioFire FilmArray™ Pneumonia Panel cause around 90-95% of pneumonia infections.[13] *Stenotrophomonas maltophilia* is a notable omission from the FilmArray™ Pneumonia Panel, accounting for around 1-6% of VAP cases.[29,30] *Citrobacter koseri* and *Raoultella* spp. are also absent from the panel – both reported to be a cause of pneumonia.[31,32] Issues of omission are far greater in respect of resistance genes. FilmArray™ seeks only the determinants of carbapenemases (*bla*$_{KPC}$, *bla*$_{OXA-48-like}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$), along with *mecA* (conferring methicillin resistance in staphylococci) and *bla*$_{CTX-M}$ (encoding the principal family of extended-spectrum β-lactamases, ESBLs). The carbapenemases sought are significant both in respect of infection control and treatment choice, but remain extremely rare, being present in <1% of Enterobacterales from HAP and VAP in the UK.[15] CTX-M ESBLs (encoded by *bla*$_{CTX-M}$) and methicillin resistance in *S. aureus* (encoded by *mecA*) are more prevalent worldwide, and have implications for treatment choice; however only 50-70% of ESBLs in *Klebsiella* spp. are CTX-M types (most of the others are TEM and

SHV variants), meaning that a negative result does not exclude the possibility that an isolate has an ESBL.

Secondly, PCR based diagnostic tools commonly find more organisms than are reported by conventional microbiology, often reporting combinations of organisms and perhaps promoting greater antibiotic use.[33] Determining the pathogen from the colonising organism will be more difficult if several organisms are reported, especially if platforms are used as a POCT and the role of the clinical microbiologist is removed. The role of the clinical microbiologists and healthcare scientists is to advise which organisms identified are likely to be clinically relevant and to suggest the best treatment options. These steps would be removed if laboratory tests were used as POCT. Thirdly, PCR based diagnostics seek only a narrow range of acquired antibiotic resistance genes and do not link these genes with particular organisms. This will make it difficult to know which is the resistant organism, and will have implications for infection control/ contact tracing. The relevant isolates would need to be cultured via conventional microbiological methods and resistance mechanisms determined; if it were an outbreak situation typing would at the reference laboratory would be necessary. In situations like these, the FilmArray™ does not give you results in as much detail as conventional methods do. Last, they may be operated, on a 24 hour a day basis by non-laboratory staff at the point of care, meaning that they need to be supported by a new type of stewardship, converting the test output – in terms of pathogens and resistance genes detected – into prescribing advice.

Crucially, we have to remember the behavioural psychology and subsequently the decisions making of clinicians when a new test is deployed. Systematic reviews have highlighted the antibiotic prescribing decisions on ICU are complex.[34,35] It will

take time for novel technologies such as the FilmArray™ to embed into day-to-day routine tests. Removing the laboratory from the equation will also have implications such as appropriate use of the platform – clinicians may be uncertain when to use it or be apprehensive about performing tests which the microbiology service usually performs. Results may not be presented in the same way as they are by the laboratory, often with a helpful comment on how to interpret the result in a clinical context. A clinician's own experience also has marked impact on the decision-making process. The use of these rapid molecular tests as a POCT will raise many questions including how much does the clinician trust or believe the test? Trusting a new platform and basing treatment decisions on such a device will develop as experience of using it develops. These issues have been discussed in detail by Pandolfo et al.[36]

1.1.5 Clinical Governance and introduction of a POCT service

Clinical governance is an essential part of any POCT service. Setting up a POCT in the NHS is a complex procedure and involves the steps described in Table 1.1. The relevant stakeholders, i.e., laboratory manager, medical microbiologist, chief biomedical scientist, infection control lead, ward consultant, ward matron, information technology lead, and pharmacy, should all be involved in the discussion.

Table 1.1: Introduction of a POCT

Steps Involved	Details of Steps
Needs assessment	Describe the clinical need for the POCT
POCT lead	Identify someone responsible for implementation
Funding/ business case	Business case will need to be written and cost effectiveness described
Service level agreement with the laboratory	This may be useful to help with arranging ordering of reagents/ servicing/ training.
Risk analysis	Identify risks associated with the use and interpretation of results.
Health and safety policy	Hazards of handling and disposing of body fluids and sharps, outside of a laboratory setting. Infection control risks.
Reporting adverse incidents	This could be a result of limitations of the test.
Standard operating procedure	Must be written and include details on safe working practice, maintenance procedures, interpretation of error messages, the recording of data and quality control procedures.

Training of staff	Staff must be trained. Only trained staff can use the machine.
Documentation of results	Results must be recorded correctly, treated as confidential and kept in a secure place.
Quality assurance	Measures taken to ensure that investigations are reliable and fit for purpose.
Audit	To ensure standards are maintained

A period of evaluation would be necessary prior to implementation of the POCT – this would involve a validation and verification process. Laboratories have standard operating procedures in place to follow; they help maintain both the correct running of platforms and the up-to-date training of staff. Similar procedures would be necessary if a POCT were to be implemented on the ward. Further to this an internal quality control would be necessary to ensure the quality of the results from the POCT. The platform would also require regular servicing – as equipment in a laboratory does.

Results from platforms used as a POCT would need to have an interface with the hospital IT system/ have a way to make sure they were entered into notes so that any prescribing advice can be linked to them. Record keeping must be robust. Quality assurance is key in laboratories. It involves participation in external quality assurance procedures; analysis of the specimen and documenting the results promptly; correct interpretation of the result; appropriate and timely action to be taken based on the result produced; and documentation of all procedures for

reference. A similar procedure would be necessary in the clinical area where the POCT was placed. The POCT department should be accredited to the Clinical Pathology Accreditation (UK) Ltd Standards for the Medical Laboratory, and the POCT should be enrolled in a recognised external quality assurance scheme with assessment of the service by an external accreditation body.

Central to introduction of a POCT is responsibility – who is responsible for aspects such as the quality control of the tests, reagents, training of staff, interface with IT, reporting of adverse incidents associated with the test, and platform maintenance. Pearson et al., describes a 'whole-system approach in POCT.'[37] This involves establishing a POCT Group, with representatives of all stakeholders - the group should develop a POCT Policy for the hospital.[37] They go on to say that should be a POCT lead/ manager – ideally a member of laboratory staff who will have a major responsibility in leadership/ training. In a document written by The Medicines and Healthcare Products Regulatory Agency (MHRA), on management of POCT devices, it suggests that the POCT lead 'should also be aware of their responsibility for clinical governance and of the medico-legal implications of an erroneous result.'[38]

The Carter Report states that, 'in pathology we also see the need for accreditation of POCT (irrespective of site of provision) with this preferably being integrated with that of the laboratory service because of the close synergies – from the patient's perspective – between the two modes of testing.'[39] The MHRA similarly recommends, 'there should therefore be close liaison between users and the local hospital pathology laboratory on all issues relating to POCT'.[38] If the POCT lead/ manager is not associated with pathology services, then advice must be sought from the laboratory manager as to how best to implement such a tool. With the

centralisation of laboratories in the UK, these POCT scenarios are becoming more commonplace – therefore familiarity with setting up these services is key.

1.1.6: Disruptive Technologies

Introduction of a new technology requires considerable thought. Feldman et al. comment on how new technologies can disrupt existing patterns of team interaction in ways that can prove more complex than initially anticipated.[40] Such technologies can be described as disruptive technologies, examples include e-commerce and online news sites - they disrupt the market by replacing long-standing established competitors. Implementation of the FilmArray™ as a POCT could also be considered a disruptive technology. It would by-pass the conventional microbiology investigations and also, in some sites, the medical/ clinical microbiologist. This could introduce friction between clinicians and microbiologists, especially if results are acted on in a manner which microbiology would not have advised. Taking diagnostic tests out of the laboratory and onto the ward is no straight-forward task; clinical governance must taken into account (Table 1.1) and so must interpretation of test results.

Greenhalgh et al. describe a new framework for theorizing and evaluating non-adoption, abandonment, and challenges to the scale-up, spread, and sustainability of health and care technologies (NASSS framework).[41] They suggest that use of the NASSS framework can help with implementation of new technologies. The NASSS implementation framework consists of six domains: the condition i.e., nature of illness, the proposed technology, value proposition, adopters of the technology (staff/ patients), health care organisation involved, wider system (social/

political/ professional) and embedding of the technology over time. Frameworks like this highlight the complexities involved as well as ways forward.

1.2: MICROBIOLOGY AND LABORATORY DIAGNOSIS OF HAP/VAP in COVID-19

COVID-19, the respiratory disease caused by SARS-CoV-2, first identified in December 2019 in Wuhan, China, has become a global pandemic. This novel coronavirus has caused a total of 130,086 deaths in the UK, and 4,244,541 deaths worldwide, to date.[42,43] The treatment of respiratory co-infections in COVID-19, remains challenging; both in terms of both diagnosis and treatment. The emergence of SARS-CoV-2 as a pandemic virus of global importance has driven the need for clinical and pathological evidence upon which to base optimal therapeutic decisions. Whilst purely viral infections should not be treated with antibiotics, several respiratory viruses, notably influenza, and exacerbation of chronic lung diseases, are associated with secondary bacterial infection and additional pathology.[44]

1.2.1 Co-infections associated with respiratory viral infections

A systematic review looking at bacterial co-infection in influenza by Klein et al., including 27 studies, reported that the most common coinfecting species were *Streptococcus pneumoniae* accounting for 35% (95% CI, 14%-56%) of infections, and *Staphylococcus aureus* 28% (95% CI, 16%-40%) of infections.[44] A retrospective cohort study, by Shah et al., of patients with severe influenza infection, reports

findings over a 8 month period from 33 ICUs.[45] They compared 507 adult and paediatric patients with and without co-infection. Of these, 22.5% developed bacterial co-infections, and were found to have a longer ICU stay and higher mortality.

Arabi et al. performed a retrospective multicentre cohort study of critically ill MERS-CoV patients in 14 Saudi Arabian hospitals, and reported that 18% had bacterial co-infections.[46] Of course, it must be taken into account that the pathogens likely to be found in the setting of a Saudi Arabian hospital will not be the same as those in a UK hospital, in particular MDR organisms. Balkhy et al., described drug resistance in ICU patients with VAP in centre in Saudi Arabia.[47] They reported that *Acinetobacter* spp. was 60-89% resistant to tested antimicrobials including carbapenems. From the findings reported by Arabi et al. it would follow that a proportion of critically ill COVID-19 patients would also likely have a bacterial co-infection. Secondary bacterial infections are facilitated by a combination of viral damage to the protective mucosa as well as by virally-induced immunosuppression.[48,49] Viral and bacterial respiratory co-infections exacerbate disease severity, and can prompt ICU admission.[50]

1.2.2 Co-infections in COVID-19

The extent to which COVID-19 is associated with secondary bacterial infection of the respiratory tract is unknown, with studies reporting differing results.[51] A rapid review by Rawson et al.[52] reports that 72% of COVID-19 patients received antibiotics, whilst fewer than 8% had evidence of bacterial infection, highlighting the difficulty associated with decisions around prescribing antibiotics in this patient group.

Nonetheless some patients do develop secondary infection. A retrospective multicentre cohort study from Wuhan by Zhou et al., including 191 inpatients, found that secondary infections developed at a median of 17 days after COVID-19 illness onset, commenting that they were found in 50% of patients who did not survive, and in 15% of overall patients.[53] Povoa et al., in a commentary piece, highlight the importance of the diagnosis and treatment of VAP in COVID-19 patients, arguing that it prolongs hospital stays, and increases mortality.[54]

Another study by He at al., a retrospective data analysis for 918 COVID-19 cases from Wuhan, reported that the healthcare associated infection rate in this patient group was 7%.[55] Zhang et al. reported similar from Wuhan, amongst 221 patients the bacterial coinfection rate was 7.7%.[56] A retrospective cohort study across two London hospitals, by Hughes et al., compared bacterial and fungal co-infection in 836 COVID-19 patients with a control group 216 of influenza patients, infections were divided into community and healthcare-associated.[57] They reported respiratory samples for 112/836 (13%) COVID-19 patients, of which 39/112 (35%) identified bacterial pathogens; compared with 38/217 (18%) respiratory samples obtained for influenza patients of which 8/38 (21%) identified bacterial pathogens. In terms of respiratory pathogens in COVID-19 patients, they reported *S. aureus* to be the most common in community acquired infections, and *Pseudomonas* spp. the most common in healthcare-associated infections. The significance of these sputum results is uncertain, and the authors report similar overall significant bacterial pathogens across the two groups of patients. They reported three invasive fungal infections in the COVID-19 group, likely secondary to line infections; and no co-infection with influenza viruses, possibly a reflection of the timing of the study which looked at COVID-19 patients between February- April 2020.

A retrospective study, by Lehmann et al., looked at co-infection in 321 COVID-19 patients during the first 5 days of admission – specifically to focus on community-acquired infections.[58] Overall, co-infection was reported in just 3.7% of patients, with 1.2% being bacterial. Of the 17 ICU patients in the study, 41% had a co-infection ($P<0.005$) – this is a small sample size of ICU patients. Respiratory cultures were performed on 21% of patients, and of these 3% were suggestive of a co-infection. One patient grew *S. aureus* and *P. mirabilis* from a respiratory culture, and another has a positive *S. pneumoniae* urinary antigen test. Despite these findings, 69% of patients received antibiotics. Sharifipour et al. performed a small study in Iran examining ETA samples from 19 ICU patients with COVID-19.[59] Samples were taken at an interval of 3 days for each patient on the ICU. They reported all patients to be positive for bacteria, the most common organism being *Acinetobacter baumannii* (17/19 patients). Interestingly, there was no difference in the bacteria detected at the various sampling times.

A UK based retrospective study by Baskaran et al. looked at co-infections in 254 COVID-19 patients, across seven ICUs.[60] They reported early (<48 hrs after hospital admission) and late-onset (>48 hrs after hospital admission) infections, finding that infections increased with duration of stay. The organisms responsible for hospital acquired infections were mainly Gram-negative including *K. pneumoniae* and *E. coli.* These findings are similar to those of other studies. Clancy et al. found that, although there is little in the way of focus on AMR in COVID-19 patients, Gram-negative organisms are involved in many such cases – commonly *Acinetobacter baumannii*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.*[61] The rapid review by Rawson et al. on co-infection in COVID-19, reviewing 1007 abstracts,

concluded that there are insufficient data to inform empiric or reactive antibiotic decisions in a reasonable timeframe for critically-ill patients.[52]

1.2.3 Use of the FilmArray™ in COVID-19 patients

To date (June 2021), there is little literature on the use of the FilmArray™ in COVID-19 patients. This thesis reports the results of the COVID-19 sub-study which took place while INHALE was suspended during the first peak of the pandemic.[62] A monocentre retrospective study by Contou et al. reported results of microbiological investigations (blood cultures, respiratory samples, urinary antigen tests) for 92 patients with COVID-19.[63] They looked at investigations performed within 48 hours of admission, and found 28% of patients were co-infected with a bacteria, this represents 32 bacteria the most common of which was *S. aureus.* The FilmArray™ Pneumonia Panel was used on 30 patients, and the most common organism detected was *S. aureus,* 80 blood cultures were sent, only one was positive and this was again *S. aureus,* this was also the most common organism isolated form respiratory tract cultures. These findings are similar to those of the UK study by Baskaran et al., with *S. aureus* commonplace in early infections.[60]

Two further studies by Verroken et al. and Kreitmann et al. used FilmArray™ to sample COVID-19 patients, again on admission to ICU, rather than late in their stay. They predominantly found *S. aureus, Moraxella catarrhalis*, streptococci and *H. influenzae*.[64,65] The smaller of the two studies was conducted by Verroken et al.; this was a single-centre study and used nine sputum samples and 23 ETA samples.[64] Kreitmann et al. looked at 47 patients from a prospective cohort study across three ICUs, using ETAs (n=45) and BALs (n=2), to analyse with the

FilmArray™.[65] They reported the FilmArray™ method to have detected early bacterial infection in 12/47 samples, compared with 1/47 samples by conventional methods. Kreitmann et al. concluded that despite PCR methods detecting more organisms, it does not necessarily translate to infection; therefore, it is important to remember that results need to be interpreted in the clinical context.[65]

A prospective study by Camelena et al. looked at microbiological tests performed immediately after intubation in 43 critically ill patients, and included use of the FilmArray™ Pneumonia Panel, the panel used in the INHALE trial.[66] They reported the most common organism to be *P. aeruginosa* followed by *S. aureus;* and concluded that the use of this rapid diagnostic tool could speed up the diagnosis of bacterial infection in this patient group. A larger prospective study by Kolenda et al. comprising of 99 patients on ICU used the same panel.[67] They reported both routine culture results, and the results of the FilmArray™ Pneumonia Panel. Culture reported a bacterial co-infection in 15.1% of patients; and the FilmArray™ had a sensitivity of 100% and overall specificity of 98.7%. The most common organisms identified were *S. aureus* and *H. influenzae.* However, 60.5% of the FilmArray™results reported as positive were positive by culture, demonstrating the importance of interpreting these results in the clinical context. A review by Lai et al., commented that 13 studies reported the prevalence of COVID-19 co-infections, and stressed that clinicians must have a high index of suspicion for coinfection among COVID-19 patients, however further studies are necessary to investigate co-infections in COVID-19 patients.[68]

1.3: ANTIBIOTIC PRESCRIBING IN PATIENTS WITH HAP/VAP

Unfortunately, the diagnosis of pneumonia is often uncertain, and can be difficult to make.[69] Antimicrobial stewardship plays a central part in AMR. It follows that given the current diagnostic tool of sputum culture for HAP/VAP is suboptimal, the patients are often on broad-spectrum empirical antibiotics.

1.3.1 NICE Recommendations

NICE (2019) recommends that patients with suspected HAP who have severe symptoms/ higher risk of resistant organisms, are started on intra-venous antibiotics, listing the following antibiotics as options: piperacillin/ tazobactam, ceftazidime, ceftriaxone, cefuroxime, meropenem, ceftazidime-avibactam, or levofloxacin.[70] NICE define 'higher risk of resistance' to include 'symptoms or signs starting more than 5 days after hospital admission, relevant comorbidity such as severe lung disease or immunosuppression, recent use of broad-spectrum antibiotics, colonisation with multidrug-resistant bacteria, and recent contact with a health or social care setting before current admission'.[70] They go on to suggest that antibiotics are reviewed at 48 hours once microbiological results are available.

An opinion piece by Wootton et al. highlights the shortcomings of the NICE recommendations, stating that NICE acknowledges that *P. aeruginosa* is a common cause of HAP, yet they suggest using antibiotics which do not cover it.[71] The article goes on to say that rapid diagnostics may well have a part to play here.

1.3.2 BSAC Recommendations

Similarly the BSAC recommendations for treatment of HAP are as follows: co-amoxiclav or cefuroxime for patients with infection fewer than 5 days following admission to hospital, provided they have not previously received antibiotics and in the absence of other risk factors; if these patients had recently received antibiotics and/or who have other risk factors, they recommend: third-generation cephalosporin (cefotaxime or ceftriaxone), a fluoroquinolone or piperacillin/tazobactam.[72] They state that antibiotics should last no longer than eight days. Recommendations are made for broad-spectrum agents, for a duration of at least five days. These antibiotics may be switched to a narrow-spectrum agent once sputum cultures are back from the laboratory.

Hospitals have their own antibiotic prescribing guidelines which vary from site to site. Their choices will depend on local resistance rates, and patient population. Further to this, some medical microbiologists will have preference for certain agents which will add to the variation.

1.3.3 Role of rapid diagnostics in antimicrobial prescribing

Rapid diagnostics have a role to play in antimicrobial stewardship, the focus here is specifically on PCR tests. A review written in parallel with this thesis, explores in detail the role of such tests as antibiotic guardians.[33]

Lee et al. in Taiwan, used the FilmArray™ Pneumonia Panel to identify respiratory pathogens in ICU patients, concluding that its use *could* have changed antibiotic prescribing in 41% of 51 patients reviewed.[73] A larger study from the USA

by Buchan et al. used the same test on BAL samples from 259 patients, suggesting that antibiotics *could* have been adjusted in 70.7% of cases, and *could* have been stopped or de-escalated in 48.2%.[74] However, neither study deployed the test, nor examined whether the gains were realisable in practice.

Two further studies using the same FilmArray™ panel as INHALE warrant mention. Firstly, Alviset et al. performed a prospective cohort study on four wards, using the FilmArray™ Pneumonia Panel on ETAs or induced sputa from 63 pneumonia episodes among 61 patients, concluding that the test results c*ould* have led to an early switch of antibiotics in 79% of these episodes.[75] Secondly, Lejeune et al. analysed 60 samples (30 BAL, 21 mini-BAL, 5 sputa and 4 tracheal aspirates), again using the FilmArray™ Pneumonia Panel, and estimating that the approach *could* have led to an earlier change in antibiotics in 53% of patients.[76] Whilst these authors point to scope for better stewardship, neither demonstrated that the gain was realised in actuality.

A retrospective observational analysis by Li et al. used the FilmArray™ Respiratory Panel and reviewed patients at three A&E departments in California between October 2016 and March 2017, presenting with viral respiratory tract infection.[77] Three hundred and twenty-three of 424 (76.2%) patients had a positive viral PCR result from a nasopharyngeal (NP) swab tested using the FilmArray™ Respiratory Panel, this was available to the clinician before they were discharged from A&E. The remainder of patients had results available post discharge. Among the former 323 patients, only one-fifth were prescribed antibiotics – far fewer than would ordinarily be expected – underscoring the potential of this multiplex PCR as a stewardship tool. Patients diagnosed with influenza by PCR were unlikely to receive antibiotics. Antiviral prescribing was not reviewed. Multivariate analysis identified

factors influencing the antibiotic prescribing decision, many related to concerns over secondary bacterial infection. This highlights again that decision-making is multi-factorial with several aspects to consider.

1.3.4 The INHALE Prescribing Algorithm

The INHALE trial aims to answer questions including can rapid diagnostics improve antibiotic use, and can they be used as an antimicrobial stewardship tool. These rapid tests have a panel of organisms which they detect, as well as some (such as the FilmArray™ Pneumonia Panel) reporting antimicrobial resistance genes. For the INHALE trial the trial team has written a prescribing algorithm (Appendix 3), in order to help translate what can be a complex output from the FilmArray™.

In developing the present algorithm, we sought to identify antibiotics that: (a) were reliably active against the pathogens sought but otherwise had the narrowest spectra possible, (b) evaded critical resistance mechanisms where detected, (c) were licensed or in accepted use for HAP/VAP, and (d) had acceptable safety and tolerability. All agents advocated are in routine hospital use, no new/trial drugs are advocated. Details of the development of the prescribing algorithm are specified in Chapter 5.3.2.

With this algorithm in place, the translation of platform output into treatment occurs in real-time, avoiding the need to wait for microbiological advice. This is pertinent, especially because some of the ICUs participating in INHALE do not have daily microbiology input.

1.4: ANTIBIOTIC PRESCRIBING IN COVID-19 HAP/VAP

1.4.1 Antibiotic use and NICE/WHO guidelines in COVID-19 patients

There are concerns surrounding antibiotic use in COVID-19 patients, with both Langford et al.[78] and Rawson et al.[52,79] highlighting the frequent use of antibiotics in this patient group, where evidence of bacterial infection is often not present. The meta-analysis by Langford et al.[78] suggest that over 71.9% of COVID-19 admissions are prescribed empirical antimicrobials.

Both NICE[80,81] and WHO[82] suggest prescription of antibiotics for suspected bacterial infections in COVID-19 patients. The NICE guideline focusing primarily on community patients, discourage use of antibiotics for prevention of pneumonia or if symptoms are mild. The NICE guideline relating to antibiotic use in hospitalised patients with pneumonia, suggest prescribing antibiotics for moderate or severe CAP pneumonia. They advocate the use of doxycycline or co-amoxiclav and clarithromycin; with a review at 24/48 hours and a stop date of 5 days (if continued) where possible. However, for HAP, the guideline recommends using doxycycline or co-amoxiclav if non-severe; and piperacillin-tazobactam or ceftazidime if severe. The use of vancomycin is advocated should MRSA be suspected/confirmed. The WHO document is far broader, and they also advise against the use of antibiotics in terms of prophylaxis, instead using them only when there is clinical suspicion of bacterial infection.

1.4.2 Co-infections in COVID-19 patients and use of antibiotics

A systematic review and meta-analysis by Lansbury et al. looking at co-infections in COVID-19 patients, searched for relevant studies published between 1 January 2020 to 17 April 2020.[83] The authors included 30 studies, with 3834 patients in total. They reported that a low proportion of COVID-19 patients had a bacterial co-infection: overall 7% of patients in hospital, increasing to 14% when looking at ICU patients only. In terms of antibiotic use, they report that in 10 studies, over 90% of patients received empirical antibiotics. Unfortunately, as the team only looked at literature until mid-April, a lot more of the recent data will not have been included. Nonetheless, these findings are in line with those of Langford et al.[78] and Rawson et al.[52,79], both of whom report the frequent use of antibiotics in COVID-19 patients. In a reply to the findings reported by Lansbury et al., Youngs et al. comment that early (<48 hours of ICU admission) co-infection is rarer in COVID-19 compared with influenza.[84] They go on to emphasise that in terms of antimicrobial stewardship, COVID-19 is not like influenza – an important point to remember; stating that most patients with COVID-19 do not present to ICU with a bacterial co-infection but instead have a viral pneumonitis. They agree with Lansbury and advocate against the use of empirical antibiotics in COVID-19 patients, and where possible narrow-spectrum agents should be used.

Phua et al. remark that most patients in China with COVID-19 were given empirical antibiotics and the need to rapidly de-escalate this as guided by the microbiology and clinical condition of the patient.[85] A paper by Mirzaei et al. states that the use of antibiotics will result in an increase in drug-resistant infections, and the need for antimicrobial guidelines to be developed.[86] Furthermore, they suggest

that the use of antibiotics can result in a reduction of antibody production due to a depletion in the gut microbiome. Indeed, this would be of concern when it comes to vaccinations.

A study by Vaughn et al. using a randomly sampled cohort of 1705 patients with COVID-19 from 38 hospitals, analysed antibiotics prescribed within the first two days of admission, and community onset bacterial co-infection.[87] They reported that 56.6% of patients were prescribed early empirical antibiotics, yet only 3.5% had confirmed bacterial infection. Over a quarter of patients received antibiotics targeting MRSA and *Pseudomonas* spp. Only 7.7% of patients had a sample sent for respiratory culture in the first 3 days of admission. Importantly, this study only looks at patients when they arrive through the hospital doors, and not at antibiotic therapy through what could be a long hospital stay. Similar high antimicrobial usage is reported in a smaller single-centre retrospective study of 242 patients by Goncalves et al.: they found bacterial co-infection in 19% of patients, but 67% of patients received antibiotics.[88]

Townsend et al. reported the duration of antibiotic use in 117 hospitalised COVID-19 patients.[89] A total of 72% were prescribed antibiotics (median duration of 7 days) for a lower respiratory tract infection, with pathogens identified in only 6% of patients. Importantly, they found that positive cultures were associated with a longer antibiotic duration (P=0.0041); as was increased oxygen requirements (P=0.026) and elevated CRP (P=0.0009). A way forward would be to flag patients with high oxygen demands and raised inflammatory markers on antibiotics to the antimicrobial stewardship/ microbiology team, helping monitor/ tailor their antibiotic treatment.

Conversely, Chang et al. write of an underestimation of co-infections in COVID-19 patients secondary to antibiotic use.[90] They state that in past pandemics

(SARS and MERS), most patients were given broad-spectrum antibiotics as a prophylactic measure, so it would follow that the same may happen in COVID-19. Furthermore, they hypothesise that the low co-infection rate may be due to the volume of empirical antibiotics prescribed, or the limited clinical examination performed in a busy pandemic setting; concluding that evidence-based guidelines, when possible, would be the best way forward.

It is pertinent to remember that the use of interleukin 6 (IL-6) inhibitors such as tocilizumab for COVID-19 is now seen more often than at the start of the pandemic. As a result of this clinicians must be alert to the fact that such agents can suppress common signs of sepsis.[91] We already know that patients who receive IL-6 inhibitors for other conditions, e.g., rheumatological disease, are a far higher risk of bacterial infections.[92] A prospective observational study by Falcone et al. looked at predictors of co-infection in hospitalised patients with COVID-19.[93] The study included 315 patients, of these 69 (21.9%) patients had documentation of co-infection. One of the factors studied were the immunomodulatory agents, tocilizumab and baricitinib. This was a statistically significant predictor of infection ($P<0.001$).

1.4.3 Dutch Working Party Antibiotic Policy

To this effect, The Dutch Working Party on Antibiotic Policy have written evidence-based recommendations for antibiotic use in COVID-19 patients with a respiratory infection.[94] They recommend antibiotics are initiated in patients with radiological findings plus or minus inflammatory markers suggestive of bacterial co-infection. Other recommendations include: sputum and blood culture samples should be taken

at the earliest opportunity; a treatment course of 5 days is recommended for patients with a secondary bacterial infection; if patients are started on antibiotics at admission, they should be stopped after 48 hours if cultures are negative. In line with these recommendations, the Scottish Antimicrobial Prescribing Group also suggested five days of antibiotics if there was clinical suspicion of bacterial pneumonia.[95]

1.4.4 Survey of antimicrobial prescribing in COVID-19

A survey of antibiotic and antifungal prescribing by Seaton et al. anaylsed antimicrobial data from 15 hospitals in Scotland on a single day between 20th and 30th April 2020. The study included 820 patients (666 suspected of having COVID-19 on admission, and 531 tested positive up to and including the day of the survey), and revealed that 38.3% of those with confirmed COVID-19 were prescribed antibiotics.[96] This is less than what is reported in the studies discussed in Chapter 1.4.2. On the day patients were admitted, 62.4% of patients were prescribed antibiotics, with the most frequent indication being a suspected respiratory tract infection.

When looking at ICU patients only with confirmed COVID-19, 45.9% were receiving antibiotics – this is a greater proportion as would be expected on a critical care unit. ICU was where most of the broad-spectrum agents were used. The authors comment that a relatively low prevalence of antibiotics were used in this patient group – lower than what other studies report. They also suggest that their results show that clinicians were reviewing and rationalising antibiotic use in the

context of the virology result, with fewer of the confirmed COVID-19 patients receiving antibiotics compared with those who had just been admitted to hospital.

1.4.5 Use of the FilmArray™ in COVID-19 as a stewardship tool

The study performed by Verroken et al. using the FilmArray™ was a prospective cohort study in 32 COVID-19 patients on the ICU, and reported on antibiotic usage.[64] In terms of samples sent, either a sputum or ETA was obtained for FilmArray™ analysis. They reported that one third of the patients remained off antibiotics, and in 5 patients antibiotic therapy was stopped due to a negative FilmArray™ result. The authors stress that molecular diagnostic tools are key to antimicrobial stewardship in COVID-19, however the small sample size must be kept in mind.

Maataoui et al. evaluated the performance and the impact of the FilmArray™ Pneumonia Panel on 112 respiratory samples from 67 COVID-19 patients on ICU suspected of having a co-infection.[97] Samples analysed included: 77 mini-BAL, 28 BAL, 4 sputa, and 3 ETA. Overall, the FilmArray™ led to antibiotic changes in 38/112 (34%) episodes: 16 withdrawal of antibiotics, 13 initiations, 3 adaptations, 5 de-escalations, and one change resulting in inadequacy. The authors concluded that in patients with a suspected HAP/VAP who had a negative FilmArray™ result, 19% had antibiotics discontinued and 24% remained antibiotic-free.

On balance it appears that antibiotics are used frequently and often in COVID-19 patients: this is not surprising given that it is a new infection which clinicians have little experience of managing. With time, as confidence, and experience grows we hope to see a reduction in antibiotic use, with the aid of stewardship.

1.5: THE INHALE RANDOMISED CONTROL TRIAL

The INHALE trial[1] (ISRCTN16483855) is a National Institute for Health Research (NIHR) funded RCT, in place across 12 UK ICUs (10 adult and 2 paediatric).[98] The first patient was recruited on 05/07/2019 and the last patient is expected to be recruited by 30/09/2021. INHALE has deployed a rapid molecular test (FilmArray™ Pneumonia Panel Plus[99]) as a POCT. This specific platform was chosen after earlier INHALE work deemed it the best choice for the trial (Chapter 1.1.3). The FilmArray™ test is available for ICU staff to use 24 hours a day, and results become available to clinicians in *c*. 75 min, without immediate review and interpretation by a medical microbiologist. In practise most sites chose not to use the test overnight, unless a trained staff member was on shift.

Eligible ICU patients must have a suspected HAP or VAP, be about to receive a new antibiotic or a change in antibiotics, and produce sufficient lower respiratory tract sample for testing. INHALE compares clinical outcomes and antimicrobial usage/stewardship in these patients, who are randomised to either: (a) treatment guided using the FilmArray™ Pneumonia Plus Panel, employed at point of care (POC) in the ICU or (b) conventional management, with empirical broad-spectrum agents, as specified in the participating hospitals' HAP/VAP guidelines (should sites choose to follow these). The FilmArray™ Pneumonia Panel Plus seeks 34 organism and resistance gene targets (Table 2.1). Patients in both arms have conventional diagnostic microbiological culture performed to allow later (or further) refinement of treatment. Patients randomised to the FilmArray™ arm have use of a prescribing algorithm (Appendix 3) to help translate the output into treatment. This algorithm aims to encourage use of narrow-spectrum antibiotics to cover the pathogens and

resistances found by the FilmArray™ panel. A specially designed REDCap database was used for data collection and storage.[100] One of the trial's main aims is to see whether this rapid molecular test can aid antimicrobial stewardship, and in-turn help prevent AMR.

AIMS

The overall theme of my thesis is to answer the question: What is the role of rapid pneumonia diagnostic tests in pathogen detection and antimicrobial prescribing, and how does this differ in patients with and without COVID-19?

The thesis aims to answer the following broad questions:

1. Which organisms cause HAP/VAP in COVID-19 and non-COVID-19 patients on ICU?
2. Which clinical factors, if any, influence positive FilmArray™ and conventional diagnostic microbiology culture results?
3. How are antibiotic prescribing decisions affected by the FilmArray™ in patients with and without COVID-19?
4. Did clinicians adhere to the prescribing algorithm in both the COVID-19 and non-COVID-19 patient groups?

CHAPTER 2

GENERAL METHODS

2.1: TRIAL DESIGN

2.1.2: INHALE RANDOMISED CONTROL TRIAL

The INHALE RCT was conceived and designed by the NIHR funded INHALE programme grant investigators. The trial protocol for the INHALE RCT was developed by the Norwich Clinical Trials Unit (Appendix 1) in collaboration with study investigators and with input from the broader study team (Appendix 2).[98] The Norwich Clinical Trials Unit also developed the bespoke REDCap database and ran the trial day to day in conjunction with the research assistant based at University of East Anglia, the programme manager based at UCL and myself, in my role as clinical fellow at University College London Hospital.[100] The first patient was recruited to the trial on 05/07/2019.

Twelve ICUs participated in the INHALE RCT – ten adult and two paediatric. The hospitals were: Aintree University Hospital (part of Liverpool University Hospitals NHS Foundation Trust), Birmingham Women's and Children's Hospital NHS Foundation Trust, Chelsea and Westminster Hospital NHS Foundation Trust, BUPA Cromwell Hospital, Dudley Hospital NHS Foundation Trust, Great Ormond Street Hospital NHS Foundation Trust, James Paget University Hospitals NHS Foundation Trust, Royal Free London NHS Foundation Trust, Royal Liverpool University Hospital, Royal Stoke University Hospital, University College London NHS Foundation Trust, and Watford General Hospital (part of West Hertfordshire NHS Trust). Both Great Ormond Street Hospital NHS Foundation Trust, and James Paget

University Hospitals NHS Foundation Trust have closed to recruitment with their last patients recruited on 08/03/20 and 14/02/20 respectively. To replace these two sites, The Royal Brompton Hospital (part of Royal Brompton and Harefield NHS Foundation Trust, including both adult and paediatric patients) and Newham Hospital (part of Barts Health NHS Trust) joined the trial. The work presented in this thesis does not include patients from these last two sites as they joined after my data analysis was performed.

Patients were randomised, using the online REDCap database, into the control or intervention arm. The control arm patients had two respiratory samples collected (i.e., sputum/ ETT exudate/ BAL): one was sent to the participating hospital's microbiology laboratory for routine processing performed according to the Standard UK Laboratories Operating Procedures[3], and the other was sent to the central INHALE laboratory at The Centre for Clinical Microbiology Royal Free Campus UCL, for FilmArray™ testing; the results of which would not be made available to the treating clinicians. The intervention arm patients also had two respiratory samples taken: one processed on the FilmArray™ platform on ICU using the FilmArray™ Pneumonia Panel Plus, and the other was sent to the participating hospital's microbiology laboratory for processing.[98] The BioFire FilmArray™ Pneumonia Panel Plus seeks 34 organism and resistance gene targets as shown in Table 2.1.

Table 2.1: BioFire FilmArray™ Pneumonia Panel Plus targets

Bacteria	**Viruses**	**Antibiotic resistance genes**
Acinetobacter baumannii complex	Adenovirus	*mecA or mecC*
Chlamydia pneumoniae	Coronavirus (NL-63, OC43, HKU1 and 229E)	*bla*$_{IMP}$
Enterobacter cloacae	Influenza A	*bla*$_{NDM}$
Escherichia coli	Influenza B	*bla*$_{VIM}$
Klebsiella aerogenes	MERS-CoV	*bla*$_{KPC}$
Haemophilus influenzae	Metapneumovirus	*bla*$_{OXA-48}$
Klebsiella oxytoca	Parainfluenza	*bla*$_{CTX-M}$
Klebsiella pneumoniae	Respiratory Syncytial Virus	
Legionella pneumophilia	Rhinovirus/ Enterovirus	
Moraxella catarrhalis		
Mycoplasma pneumoniae		
Proteus spp.		
Pseudomonas aeruginosa		
Staphylococcus aureus		
Serratia marcescens		
Streptococcus agalactiae		
Streptococcus pneumoniae		
Streptococcus pyogenes		

The FilmArray™ Torch platform (bioMérieux, Utah, USA) was placed on the ICU as a POCT, operated by the ICU research teams. The ICU research teams (Appendix 4 and 5) at each site were trained in how to use the platform. Training was carried out by a member of the INHALE team; it involved face to face training at the local participating hospital with their FilmArray™ platform.[101] Eligible patients had to be ICU in-patients; in hospital for at least 48 hours; have sufficient volume of airway specimen for routine testing plus 200μL for the FilmArray™ test; and be about to receive an antimicrobial to treat a suspected HAP/VAP for the first time, or a change in existing antimicrobial for a lower respiratory tract infection (LRTI) because of deteriorating clinical condition. The FilmArray™ test result was immediately given to the ICU clinical team along with the prescribing algorithm (Appendix 3). This algorithm was site specific and developed with the site medical microbiologist. The specific details of this development process will be covered in the final results chapter. Repeat tests were permitted in the study provided the patient was randomised to the intervention arm, they were no less than 72 hours apart from the prior specimen and a specimen would have been taken regardless of trial participation. Prior to site opening a site initiation visit was performed by the trial team. The visit included FilmArray™ platform training, a presentation on the trial/ data collection, and a presentation on use of the prescribing algorithm.

The two primary outcomes were i) to determine whether there was non inferiority in clinical cure of pneumonia at 14 days post -randomisation between patients treated according to the FilmArray™ test's molecular results plus trial-based prescribing algorithm versus those treated with standard care; ii) to determine whether there was improvement in antimicrobial stewardship at 24 hours post randomisation for participants treated according to the FilmArray™ test versus those

treated with standard care. There were several secondary outcomes including all-cause mortality within 28 days of randomisation, change in Sequential Organ Failure Assessment Score (SOFA) from randomisation to seven days post-randomisation, as well as outcomes relating to antibiotic use.

To summarise, INHALE is a multicentre, parallel group, randomised controlled trial to investigate clinical, safety and cost effectiveness of the FilmArray™ test plus trial based prescribing algorithm versus standard care, with the aim of showing non-inferiority in participant outcomes and superiority in antimicrobial stewardship.

2.1.3: Design of COVID-19 sub-study

Five of the ten adult ICUs participated in the COVID-19 sub-study: Aintree University Hospital (part of Liverpool University Hospitals NHS Foundation Trust), Chelsea and Westminster Hospital NHS Foundation Trust, Royal Free London NHS Foundation Trust, University College London NHS Foundation Trust and Watford General Hospital (part of West Hertfordshire NHS Trust). The two paediatric hospitals did not take part. This sub-study used the infrastructure in place for INHALE as described above. Patients were recruited to study this from 03/04/2020 – 23/06/2020. Due to COVID-19, recruitment to the INHALE trial, and instead the COVID-19 sub-study was performed.

The COVID-19 sub-study was not randomised. In order to be eligible for the COVID-19 sub-study, patients had to be in-patients in a participating ICU and to have clinically-diagnosed or PCR-proven COVID-19, with clinical features compatible with a suspected secondary bacterial pneumonia over and above those expected for COVID-19 viral pneumonia. As was the case for INHALE: patients also needed to

have sufficient surplus lower respiratory tract sample (200 μl sputum/ BAL or ETA) for the FilmArray™ test. In order to minimise COVID-19 infection risk, all FilmArray™ tests were performed in designated COVID-19 clinical areas by staff wearing full personal protective equipment suitable for invasive procedures, according to local guidelines. Test results were immediately delivered to the clinical ICU team along with the INHALE RCT prescribing algorithm. A second FilmArray™ test ≥ 5 days from the first test was permitted if a new or continuing bacterial pneumonia was suspected. In parallel, a respiratory sample was sent to the hospital laboratory for routine microbiological investigation, performed according to the Standard UK Laboratories Operating Procedures.[3]

The BioFire FilmArray™ Pneumonia Panel Plus was used on all patients – again as a PCOT on ICU. The test has a run time of 1h 15 min, with a loading time of approx. 2 min; utilisation followed the manufacturer's instructions[102] with samples loaded by clinical ICU staff. The panel does not seek SARS-CoV-2, and diagnosis of COVID-19 was based on separate testing by the hospitals.

2.2: DATA COLLECTION

Data was collected into the REDCap database[100]; this provided multiple features to maintain data quality, including an audit trail, ability to query spurious data, search facilities, and validation of predefined parameters/missing data. A separate REDCap database was designed for the COVID-19 sub-study by the Norwich Clinical Trials Unit (Appendix 1).

The research teams at the sites (Appendix 4) collected the datapoints described in Table 2.2.

Table 2.2: Datapoints collected for INHALE and COVID-19 sub-study

Datapoint	INHALE	COVID-19 sub-study
Demographics (age, gender)	✓	✓
Comorbidities	✓	✓
Reasons for ICU admission	✓	×
Dates of ICU and hospital admission	✓	✓
Dates of ICU and hospital discharge	✓	×
Hospital and ICU stays in the 3 months prior to the current admission	✓	×
Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Paediatric Index of Mortality 3 (PIM3), recorded on admission	✓	×
Temperature, White Cell count including neutrophils, and C-Reactive Protein at enrolment	✓	×
Sequential Organ Failure Assessment (SOFA) score, paediatric SOFA score and Paediatric Logistic Organ Dysfunction (PELOD-2) score recorded daily while in ICU. Data collected for up to 14 days after randomisation or until discharge from ICU.	✓	×
Additional vasopressor use	✓	×
Ventilation status (at enrolment)	✓	✓

Ventilation status (daily)	✓	✗
Type of ventilation	✓	✓
Mortality - all cause, up to 28 days after randomisation	✓	✗
Clinical outcome of patient and date recorded	✓	✓
Details of any other infections (identified by routine microbiology), from 7 days prior to randomisation to 21 days after	✓	✗
Antimicrobial prescriptions (including antibiotics administration in the 7 days prior to randomisation and 21 days after)	✓	✗
Antimicrobial prescriptions (including antibiotics administration in the 7 days prior to and 7 days after FilmArray™ test)	✗	✓
Indication for antimicrobial prescription	✓	✗
Time of sputum sample collection	✓	✓
Results from conventional diagnostic microbiology for sputum specimens	✓	✓
Results from FilmArray™ test	✓	✓
Routine chest x-ray and/or CT scan, dates closest to screening, day 14 and	✓	✗

day 21 and whether it showed evidence of pneumonia		
Clostridium difficile infections and any other adverse events potentially related to antibiotic use	✓	✗
Health service resource use data relating to cost of the ICU/ hospital stay	✓	✗
Details of follow-up FilmArray™ tests	✓	✓
Date sample taken for COVID-19 testing	✗	✓
Date of positive COVID-19 test	✓	✓

2.3: STATISTICAL ANALYSIS PLANS

I developed the statistical analysis plan to answer my specific aims, and analysed the data presented; these are detailed in the individual results chapters. My statistical analysis plan was reviewed by the trial statistician, and any analyses thought to overlap with the INHALE trial outcomes were removed. Both my analysis plan and the data I intended to use were approved by the trial steering committee and data monitoring committee to ensure that the analyses did not interfere with the primary and secondary outcomes of the INHALE RCT, given that this thesis will be submitted before the main trial reports.

In summary, data were described using mean (standard deviation) or median (interquartile range) as appropriate; P-values and 95% CI from Chi-squared tests were used to compare proportions; medians were compared using a Mann-Whitney

test, and associated 95% CIs; and the t-test was used to compare means. Logistic regression models were preformed to examine the effect of multiple covariates on an outcome.

Due to the pause in the trial the full dataset was not available to analyse, so instead data from the first 200 patients recruited from 05/07/2019 until 19/08/2020 were analysed.

2.4: ETHICS STATEMENT

Before initiation of the trial at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective participant were submitted to the relevant Research Ethics Committee and to the Health Research Authority for approval. The research protocol was approved by the London, Brighton and Sussex Research Ethics Committee (19/LO/0400) and the Health Research Authority. The COVID-19 sub-study had ethics approval as an amendment under the main INHALE trial approvals.

CHAPTER 3

MICROBIOLOGY OF HAP/VAP PATIENTS IN ICU

3.1: INTRODUCTION

Patients with HAP/VAP receive antibiotics for varying durations and often these are broad-spectrum agents. This is associated with the range of pathogens involved varying between locations and also the difficulty of definitive diagnosis.[18] Current practice does not lend itself to swift diagnosis and treatment, with laboratory culture results taking 48-72 hours. The rapid tool deployed in the INHALE trial (FilmArray™ Pneumonia Panel Plus) dramatically reduces this time to c.75 mins.[99] The panel detects 34 organism and resistance gene targets (Table 2.1). How detection of these organisms translates in antibiotic selection/ prescription will be analysed in Chapter 5.

This chapter described the patient population and organisms causing HAP/VAP in the ICU setting in patients recruited to the INHALE RCT. Detection of organisms from 12 ICUs across the UK, as reported by the FilmArray™ and conventional diagnostic microbiology, were analysed. Factors thought to influence whether microbiological tests were positive or negative were examined.

3.2: AIMS

1. To describe organisms, as detected by the FilmArray™ and conventional diagnostic microbiology causing HAP/VAP.
2. To assess whether positive or negative FilmArray™ results/ positive or negative conventional diagnostic microbiology results have any correlations with the clinical variable often associated with pneumonia.

3.3: METHODS

3.3.1: Contribution

Data was collected by the research teams at the sites (Appendix 4). I worked with the Norwich Clinical Trial Unit to check and clean the data prior to analysis. Queries were raised with sites on any spurious datapoints. Once data input was complete, the data pages were locked so no further changes could be made. I developed the statistical analysis plan, and performed all analyses in this chapter.

3.3.2: Data Collection

For the purpose of this analysis the patients in INHALE RCT, were used. These patients did not have COVID-19. Baseline data, inflammatory markers (neutrophil count and CRP), antibiotic use in the 72 hrs prior to sample collection, imaging results, and the microbiology/FilmArray™ results were collected for this analysis. Permission was granted from the trials team for me to download relevant data (which

was collected/input by research teams at the hospital sites) from the REDCap database and this was exported into Excel spreadsheets. Both adult and paediatric patients were included in this analysis.

This data had all been checked and cleaned prior to analysis. Both the trials unit team and I reviewed the data in the database.

3.3.3: Statistical analysis plan

Stata v.16 was used for statistical analyses. Patient demographics, sample type, length of time in hospital, reason for admission, number of patients with two FilmArray™ tests, number and type of bacteria, viruses and resistance genes identified using FilmArray™ and by conventional diagnostic microbiology were described using frequency (%), mean (standard deviation) or median (interquartile range), as appropriate. Bacterial species were counted once per patient for any method, even if they were detected repeatedly.

When comparing the number of days of hospital admission prior to a positive or negative FilmArray™ test result, medians were compared using a Mann-Whitney test, and associated 95% confidence intervals (CI). P-values and 95% CI from Chi-squared or Fishers exact tests were obtained to work out whether there was a difference between positive and negative FilmArray™ results according to reason for hospital admission.

For those patients with two FilmArray™ tests, agreement by species groupings and resistance were examined for both tests. Frequency of organisms detected were compared as differences in paired proportions of positive tests with 95% CI, if numbers allowed.

The effect of independent factors on whether a patient had a double negative result (i.e., a negative FilmArray™ result, and negative conventional diagnostic microbiology) or at least one positive result (i.e., either FilmArray™ or conventional diagnostic microbiology positive, or both), was analysed. The independent factors of interest were: neutrophil count, CRP, whether imaging showed evidence of pneumonia, and antibiotic use in 72hr prior to sample collection. The effect of these separate variables on the outcome was analysed using Chi squared tests or Fishers exact tests, associated 95% CIs to compare proportions; and Mann-Whitney test with associated 95% CIs to compare medians. In addition, all covariates were fitted into a logistic regression model, and adjusted results reported in addition to the unadjusted results.

Characteristics of patients with negative FilmArray™ results were compared with patients who had a positive FilmArray™ result. Characteristics of interest were age, gender, date of hospital admission, date of FilmArray™ test, reason for hospital admission, temperature when FilmArray™ test was done, inflammatory markers, imaging showing pneumonia (yes/ no), and whether they on antibiotics in the 72hr prior to FilmArray™ test being done (yes/no). These were compared using Chi squared tests or Fishers exact tests, associated 95% CIs to compare proportions; t-test to compare means; and Mann-Whitney test with associated 95% CIs to compare medians. All covariates were fitted into a logistic regression model, and adjusted results reported in addition to the unadjusted results.

Any missing data has been accounted for in the analyses presented.

3.4: RESULTS

3.4.1 Demographics and sample types used in HAP/VAP patients

The first 200 patients recruited to the INHALE trial were included in this analysis; their data was cleaned and checked, and their records locked on the REDCap database. The recruitment timeframe for these 200 patients was 05/07/2019 – 19/08/2020. (Due to COVID-19 recruitment to the INHALE trial was paused from 16/03/2020 – 01/07/2020). Of these patients, 146 (73%) were adults, and 54 (27%) children. Demographic data are summarised in Table 3.1. Across the 10 adult sites, recruitment per site varied from 2-52 patients per site; and across the 2 paediatric sites from 17-37 patients per site.

Table 3.1: Demographics of HAP/VAP patients

	Adult Patients (N=146)	**Paediatric Patients (N= 54)**
Male (%)	107 (73.3)	29 (53.7)
Female (%)	39 (26.7)	25 (46.3)
Median Age (years); IQR^	66; 52-73	1; 3m-3y
Mean Age (years); SD^{+}	61.5; 17.1	2.9; 4.5
Max Age (years)	93	16
Min Age (years)	20	0*

* REDCap has recorded month of birth only, not the date, so it is not possible to determine how many weeks old a '0 month' baby is. ^IQR = Inter-quartile Range; $^{+}$SD = Standard Deviation

The sample types used for analysis are summarised in Table 3.2 (all samples were included here including those for repeat tests and those with missing FilmArray™ reports). Of note, a third of paediatric patients had BALs samples, compared with 5.8% of adults.

Table 3.2: Sample type used for analysis in HAP/VAP patients

	Adult samples (N=154)	**Paediatric samples (N=54)**
BAL (%)	9 (5.8)	18 (33.3)
ND BAL (%)	4 (2.6)	-
ETT exudate (%)	94 (61.0)	32 (59.3)
Sputum (%)	41 (26.6)	4 (7.4)
Tracheal aspirate (%)	3 (2.0)	-
Tracheostomy (%)	3 (2.0)	-

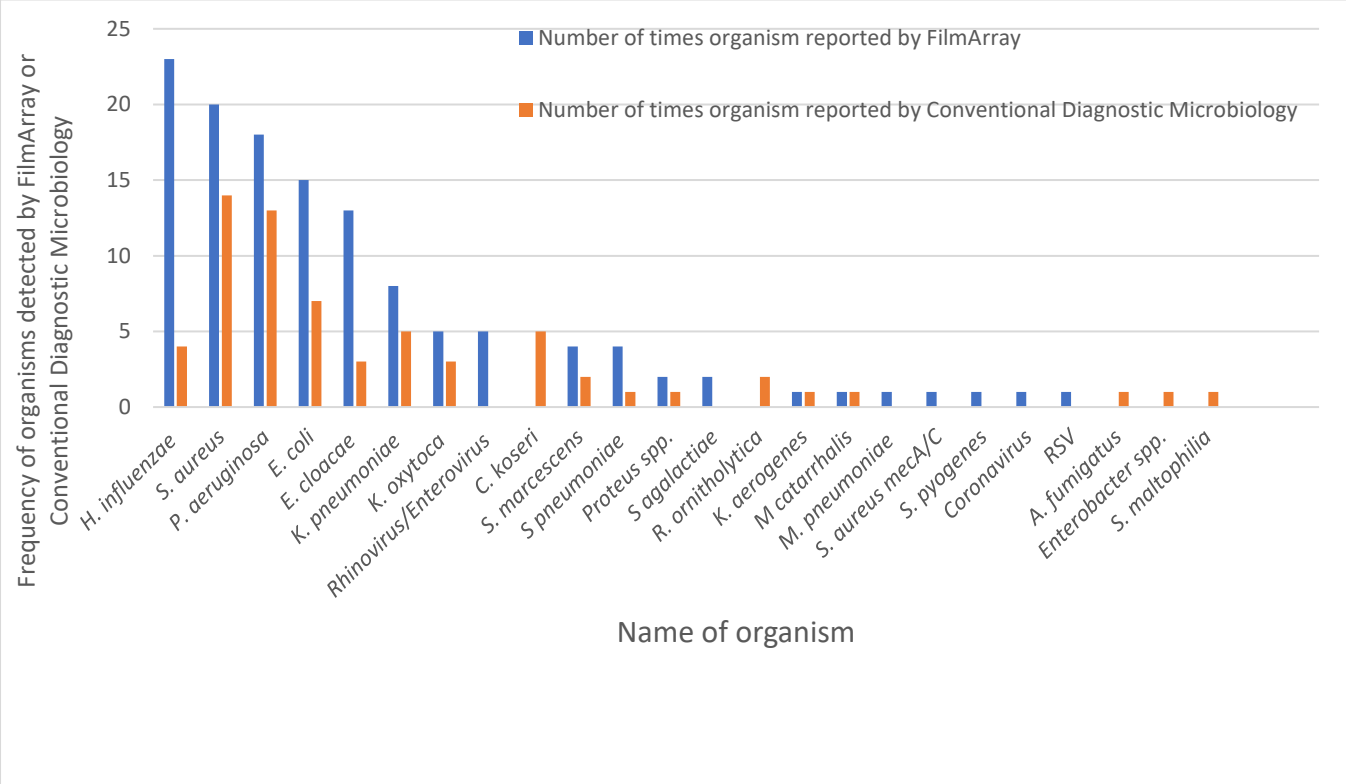
Of the 146 adult patients, 106 (72.6%) were invasively ventilated; 4 (2.7%) were on non-invasive ventilation; and 36 (24.7%) were not ventilated. Of the 54 paediatric patients, 49 (90.7%) were ventilated, and 5 (9.3%) were not ventilated. None of the paediatric patients described here required non-invasive ventilation.

3.4.2 FilmArray™ Results for Adult Patients

One hundred and four adult patients had FilmArray™ test results available for analysis. Of the 146 adults, 3 patients in the intervention arm had no FilmArray™

result uploaded to the REDCap database (i.e., results were unavailable to view), and 38 patients in the control arm did not yet have a result recorded; and in one case the test failed due to a machine error. Therefore, data was not available for these FilmArray™ instances. Eight repeat FilmArray™ tests were performed, giving a total of 112 FilmArray™ result for analysis from 104 adults. Of the 112 adult FilmArray™ test results, 71 (63.4%) were positive, and 41 (36.6%) were negative. If patients had repeat tests performed, multiple instances of the same species from a single patient were excluded, this avoided counting the same organism more than once in the same patient (i.e., the organisms counted are non-duplicate). A total of 126 non-duplicate organisms were identified by the positive FilmArray™ tests, as show in Figure 3.1. Some tests reported more than one organism.

Figure 3.1: Organisms reported by the FilmArray™ Pneumonia Panel Plus and Conventional diagnostic Microbiology across all samples (excluding multiple instances of the same species from a single patient and negative results).



The most prevalent organism as detected by the FilmArray™ was *Haemophilus influenzae,* followed by *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* There were 5 detections of Rhinovirus/Enterovirus, and one detection each of Coronavirus (FilmArray™ report does not specify which Coronavirus) and RSV. No other viruses were detected by FilmArray™. It is important to note that COVID-19 is not part of the Pneumonia Panel Plus, therefore when a Coronavirus was reported by the test it was not COVID-19.

3.4.3 Conventional Diagnostic Microbiology Results for Adult Patients

In total there were 148 conventional diagnostic microbiology test results available for the 146 adult patients, (six results not entered into the database by sites). Of these 148 results, 75 (50.7%) were reported as no growth, no significant growth or normal respiratory flora; 13 (8.8%) were reported as *Candida* spp. or yeasts; and 3 were positive in-house virology results. This left 57 specimens with a total of 65 relevant bacteria reported (some specimens reported more than one bacterium). Figure 3.1 demonstrates these 65 non-duplicate organisms. Among the bacteria identified, the most prevalent was *S. aureus,* followed by *P. aeruginosa.* The three viruses identified were Rhinovirus (n=1) and Coronavirus (n=2); note that these were reported from two samples – one with Rhinovirus and Coronavirus (and no bacteria), and the other reported Coronavirus (*E. coli* was also reported from this specimen).

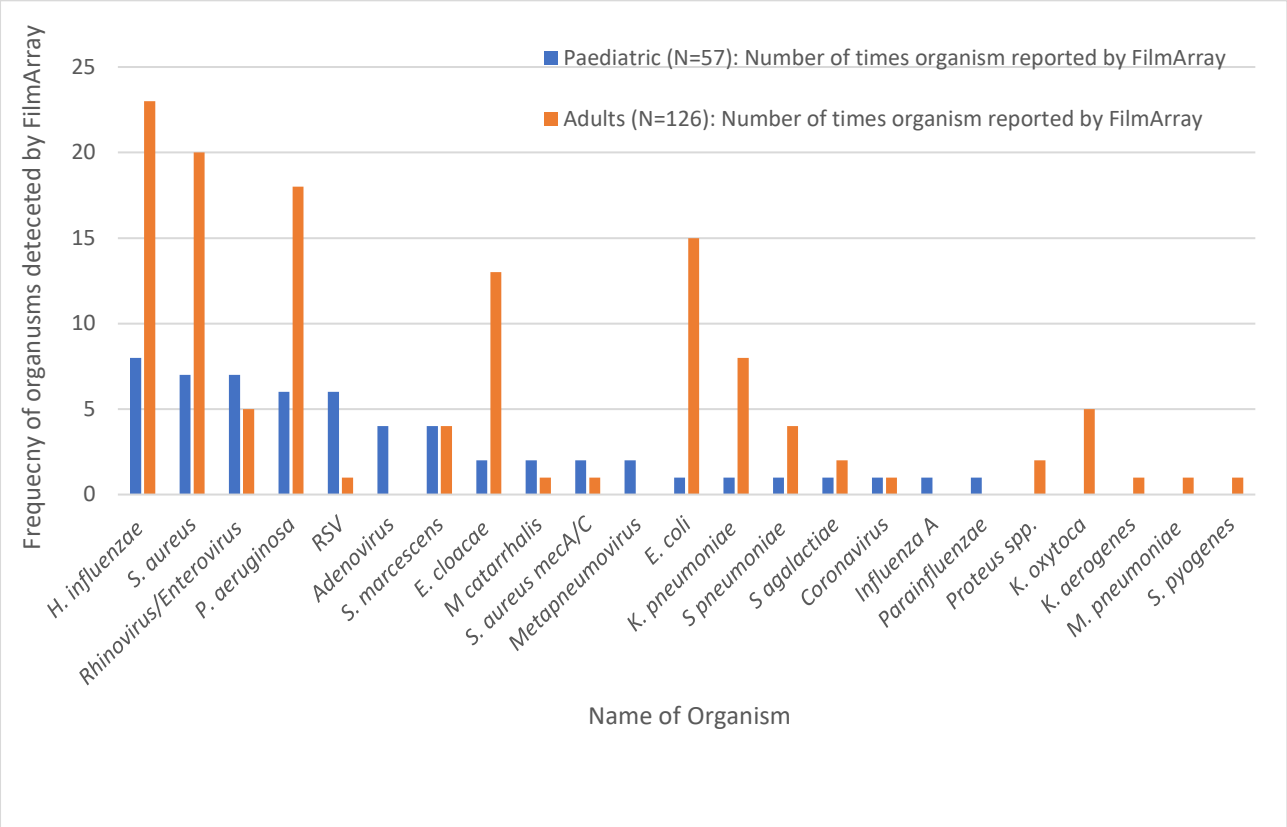
The proportion of negative result samples amongst the conventional diagnostic microbiology results (i.e., the 50.7% reported as no growth, no significant growth or normal respiratory flora versus 36.6%) was 14.1% higher than for the FilmArray results; P=0.024, 95% CI for difference in proportions: 2.1%, 26.1%. This suggests evidence of a significantly higher proportion of negative conventional diagnostic microbiology results compared with negative FilmArray results.

3.4.4 FilmArray results for Paediatric Patients; and comparison with Adults

Of the 54 paediatric patients no repeat tests had been performed. Data was not available for 10 paediatric control arm samples. This gave a total of 44 FilmArray™ results for analysis. Of the 44 FilmArray™ test results, 33 (75%) were positive, and

11 (25%) were negative. A total of 57 non-duplicate organisms were identified by the positive FilmArray™ tests, as show in Figure 3.2.

Figure 3.2: Organisms reported by the FilmArray™ Pneumonia Panel Plus in Adult and Paediatric Patients across all samples (excluding multiple instances of the same species from a single patient and negative results).



The most prevalent organism amongst paediatric patients as detected by the FilmArray™ was *H. influenzae;* followed by *S. aureus,* Rhinovirus/Enterovirus, and *P. aeruginosa.* When compared with adult FilmArray™ results, in both groups the two most common organisms were *H. influenzae* and *S. aureus.* The proportion of *E. coli* amongst adult patients was 10% higher than for paediatric patients (95% CI

for difference in proportions (adult-paediatric): 3.55%, 16.75%), suggesting there was evidence of a significant difference between the two groups. There was also some evidence of a difference in the proportion of *K. oxytoca* amongst adult patients being higher (by 4%) than for paediatric patients; 95% CI for difference in proportions (adult-paediatric): 0.56%, 7.38%. Significantly more viruses were reported by the FilmArray™ in children compared with adults: 33% higher in children; P<0.0001, 95% CI for difference in proportions: 19.7%, 46.3%. The remainder of the organism detections as reported by FilmArray™ were not significantly different between the adult and paediatric groups (CIs for difference in proportions included zero).

FilmArray™ positivity according to sample type was examined in the paediatric cohort (there were too few BAL samples to perform this analysis for adults). The deeper BAL specimens were compared with the more proximal ETT and sputum samples. Amongst the 44 FilmArray™ results available for analysis in children, 10 were BAL and 34 were ETT and sputum samples (ETT: n=30, sputum: n=4). There was no evidence of a difference in positivity of FilmArray™ results between BAL samples and ETT/sputum samples – CIs for difference in proportions included zero.

3.4.5 Reason for hospital admission to hospital, and how this influences the number of positive FilmArray™

Reason for hospital admission was recorded by sites as ‘medical’, ‘surgical’ or ‘trauma’. The category into which a patient was placed was decided upon by the site research nurses. Surgical patients were those requiring surgery; an example of trauma would be a patient involved in a road traffic accident; and medical was

anything related to a medical problem from chest pain to cancer treatment. In both the adult and paediatric patients, the reason for admission was analysed (Table 3.3). Medical admissions were the most common, followed by surgical and then trauma.

Table 3.3: Reason for hospital admission in HAP/VAP patients

	Adult (N=146)	**Paediatric (N=54)**	**Adults and Paediatric (N=200)**
Medical (%)	86 (58.9)	33 (61.1)	119 (59.5)
Surgical (%)	45 (30.8)	18 (33.3)	63 (31.5)
Trauma (%)	15 (10.3)	3 (5.6)	18 (9)

Of the 148 patients (i.e., adult and paediatric) with available FilmArray™ results (duplicate tests not included as reason for admission would be the same), 56/85 (65.9%) of medical patients had a positive FilmArray™ result, and 29/85 (34.1%) had a negative FilmArray™ result. Of the surgical patients: 36/52 (69.2%) had a positive FilmArray™ result, and 16/52 (30.8%) had a negative FilmArray™ result; of the trauma patients: 8/11 (72.7%) had a positive FilmArray™ result, and 3/11 (27.3%) had a negative FilmArray™ result. There was no evidence of an association between reason for hospital admission and whether or not the FilmArray™ tests delivered a positive result; P=0.856 (Fishers exact). There was no difference in the proportion of patients who had a positive versus negative FilmArray™ tests in the trauma, surgical or medical groups (CIs for proportions included zero).

3.4.6 Patients with more than one FilmArray™ test performed

All patients with a FilmArray™ result were included. No paediatric patients had more than one FilmArray™ test performed; of the adults, 7/146 (4.8%) had more than one test performed. Of these seven patients, six had one repeat test and one patient had two repeat tests, giving a total of eight repeat tests. Repeat tests were permitted provided the patient was randomised to the intervention arm, they were no less than 72 hours apart from the prior specimen and a specimen would have been taken regardless of trial participation. These were performed at the discretion of the ICU teams provided the aforementioned criteria were met. Six of these seven patients with repeat tests were male; the median age was 67 years (range 20-83 years). This group of patients was in hospital for a median number of nine days (IQR 6-25.5 days) before the first FilmArray™ test, and a median of 17 days (IQR 12-34.5 days) before their second FilmArray™ test. Only one patient had a third test, and this was done on day 18 of admission. The median number of days difference between the first and second test was five (IQR 3-8 days).

No new organisms were identified on the second FilmArray™ tests. Five of the seven second tests were identical to first tests. Of these five results: three of were negative results on both first and second tests; one detected a coronavirus on first and second tests; and one detected *P. aeruginosa* on both tests. The remaining two second tests identified one of two organisms identified on the first test. In the first patient: the first FilmArray™ test reported *P. aeruginosa and E. coli,* whereas the second reported only *P. aeruginosa;* in the second patient: the first FilmArray™ test reported *H. influenzae and S. aureus,* whereas the second reported only *S. aureus.*

For the patient who had three FilmArray™ tests performed: the third FilmArray™ test was negative and this was the same as both the first and second test results from that patient.

3.4.7 Comparing patients with both negative FilmArray™ and negative conventional diagnostic microbiology results, with those who have at least one positive result

All adult and paediatric patients with FilmArray™ and conventional diagnostic microbiology results were included. Each patient contributed one pair of tests i.e., repeated tests were excluded. Patients were divided up into a double negative group i.e., those with both a negative FilmArray™ result and a negative conventional diagnostic microbiology result; or into a group where patients had at least one positive result i.e., either a positive FilmArray™ result or a positive conventional diagnostic microbiology result. Table 3.4 summarises the patient demographics for both these groups.

Table 3.4: Demographic Data for patients with negative FilmArray™ and negative Conventional Diagnostic Microbiology and those with at least one positive result

	Double negative group*			**At least one positive result group^**		
	Adult (N=31)	**Paediatric (N=9)**	**Adult and Paediatric (N=40)**	**Adult (N=89)**	**Paediatric (N=42)**	**Adult and Paediatric (N=131)**
Male (%)	18 (58.1)	4 (44.4)	22 (55.0)	68 (76.4)	24 (57.1)	92 (70.2)
Female (%)	13 (41.9)	5 (55.6)	18 (45.0)	21 (23.6)	18 (42.9)	39 (29.8)
Median Age - years (range)	64 (22-83)	1 (0-16)	54.5 (0-83)	66(20-93)	1 (0-16)	50 (0-93)

*This does not include patients who have no FilmArray™ result.

^This does not include patients who have both no FilmArray™ and no conventional diagnostic microbiology result, but does include patients with no FilmArray™ result who have positive conventional diagnostic microbiology (and vice versa) – as only need either positive FilmArray™ or positive conventional diagnostic microbiology.

The proportion of all patients who had a 'double negative' result was 23.4%, and those who had at least one positive result was 76.6%. The following factors were examined to see whether they were associated with a patient having a double negative result or at least one positive result: imaging reporting pneumonia, neutrophil count, CRP, and antibiotic use in the 72 hrs pre sample collection.

Imaging Results

The number of patients in the 'double negative' group whose chest imaging showed evidence of pneumonia was 25/35 (71.4%). Imaging used included chest X-rays and

chest CT-scans. These were either reported by radiologists (as routine practice) or reviewed by a clinician on ICU. Of those in the 'at least one positive' group, 91/110 (82.7%) had imaging suggestive of pneumonia. Note that patients who had no imaging report on the database (n=26) were excluded from this analysis.

Imaging was not a predictor of a whether a patient would have a positive or double negative result: there was no evidence of a difference in the proportion of patients with positive imaging (i.e., shows evidence of infection) between the 'double negative' and 'at least one positive' groups; difference 11.3%; 95% CI for difference: -5.3%, 27.9%.

Neutrophil Count and CRP Level

The median neutrophil counts and CRP levels in both the relevant groups are summarised in Table 3.5.

Table 3.5: Neutrophil count and CRP level in the ‘double negative’ and ‘at least one positive’ patient groups

	Double negative group - adults and paediatric (N=40)	**At least one positive group - adults and paediatric (N=131)**
Median neutrophil count (10^9 cells/l); IQR	8.00; 4.74-11.6	9.09; 5.8-14.1
Median CRP level (mg/dl); IQR	131.50; 26.63-223.03	132.00; 43-229.2

In the ‘double negative’ group missing CRP levels n=12, missing neutrophil counts n=3; and the ‘at least one positive’ group missing CRP levels n=35, missing neutrophil counts n=16. This table excludes these missing values.

Neither CRP nor neutrophil count helped predict whether the test result would be positive/ double negative: there was no evidence of a significant difference in either the median CRP level between the ‘double negative’ and ‘at least one positive’ groups (difference 0.5mg/dl, 95% CI -56.4 to 36.0, P value = 0.70 Mann Whitney test); or in the median neutrophil count between the 2 groups (difference 1.09x10^9cells/l, 95% CI -3.40 to 0.87, P value = 0.230 Mann Whitney test).

Antibiotic use in 72hr prior to sample collection

There was no evidence of a difference in proportion of patients on/off antibiotics in the 72hr prior to sample being taken between the ‘double negative’ and ‘at least one

positive' groups; difference 0.4%; 95% CI -7.2% to 8.0%. This is summarised in Table 3.6.

Table 3.6: Antibiotic use in the 72hr prior to sample collection in the 'double negative' and 'at least one positive' patient groups

	Double negative group - adults and paediatric (N=40)	**At least one positive group - adults and paediatric (N=131)**
Number of patients on antibiotics in 72hr pre sample taken (%)	38 (95.0%)	125 (95.4%)
Number of patients not on antibiotics in 72hr pre sample taken (%)	2 (5.0%)	6 (4.6%)

A binomial logistic regression model (Table 3.7) was run to describe the combined effects of neutrophil count, CRP, whether imaging reported pneumonia, and antibiotic use in the 72 hrs pre sample collection, on the results being either double negative or at least one of them being positive. These covariates did not statistically significantly predict the outcome (P>0.05 for all covariates). Therefore, the results were similar both unadjusted and after adjustment.

Table 3.7: Logistic Regression Model: Variables predicting a result being double negative or positive

Dependent covariate: Double negative or single positive result	**Odds Ratio**	**Standard Error**	**P value**	**95% CI**
Neutrophil count	0.987	0.391	0.749	(0.913, 1.067)
CRP	1.001	0.002	0.509	(0.997, 1.005)
Imaging report suggestive of pneumonia	1.926	1.037	0.224	(0.670, 5.535)
On antibiotics prior to sample collection	3.319	5.174	0.442	(0.156, 70.441)

3.4.8 Patients with negative FilmArray™ results and positive conventional diagnostic microbiology results

A total of six patients (4 adults, 2 children) had a negative FilmArray™ result and a positive conventional diagnostic microbiology result. Five patients were male and one female with a median age of 33.5 years. Cases where FilmArray™ was negative and conventional diagnostic microbiology reported *Candida spp.* were excluded (n=4). This is because the isolation of *Candida* spp. in respiratory tract

specimens almost invariably represents contamination, rather than infection so the decision was made to exclude these organisms.[103]

The pathogens reported by conventional diagnostic microbiology in these six patients were all different: *A. fumigatus* (n=1); *S. aureus* (n=1); *C. koseri* (n=1); *S. maltophilia* (n=1); RSV (n=1); the last case identified 2 pathogens Metapneumovirus (n=1) and Coronavirus NL63 (n=1) which the FilmArray™ did not detect. In total, three bacteria, one fungus, and three viruses were detected by routine laboratory methods and not by the FilmArray™ Pneumonia Panel Plus.

3.4.9 Characteristics of patients with positive and negative FilmArray™ results

Of the 148 patients (adult and paediatric) with results, 100 (67.6%) had positive results, and 48 (32.4%) had negative results. The median age of patients in the positive FilmArray™ group was 48 years, and in the negative FilmArray™ group was 54.5 years. The difference in the median age between the 2 groups was 6.5 years, P value (Mann Whitney test) = 0.65 (95% CI for median difference: -11 to 5). There was no evidence of a difference in the median age between the positive and negative FilmArray™ groups. There were 69/100 (69.0%) male patients in the positive FilmArray™ group, and 29/48 (60.4%) in the negative group. There was no evidence of a difference in proportion of male patients between the positive and negative FilmArray™ groups; difference 8.6%, 95% CI -7.9%, 25.1%. There was also no evidence of a difference in proportion of female patients between the positive and negative FilmArray™ groups; difference 0.6%, 95% CI -14.6%, 18.8%.

The following factors were examined to see whether they had an effect on a patient having a negative or positive FilmArray™ result.

Number of days in hospital prior to FilmArray™

In the positive FilmArray™ group, the median number of days a patient was in hospital prior to FilmArray™ test being performed was 7 days (IQR 4-17); and in the negative FilmArray™ group was 9 days (IQR 5-17). There was no evidence of a difference in the median number of days in hospital prior to the test between the positive and negative FilmArray™ groups; difference 2 days, P value (Mann Whitney test) = 0.399; 95% CI for median difference: -4.00 to 1.00.

Imaging results

In the positive FilmArray™ group, 70/85 (82.4%) of patients had imaging showing evidence of infection; and in the negative FilmArray™ group this was 30/41 (73.2%). There was no evidence of a difference in the proportion of patients with positive imaging between the positive and negative FilmArray™ groups; difference 9.2%; 95% CI -6.6%, 25.0%. Patients who had no imaging report on the database (n=22) were excluded from this analysis.

Patient temperature

The mean temperature in the positive FilmArray™ group (n=99) was 37.8C (SD 1.079), and in the negative FilmArray™ group (n=46) was also 37.8C (SD 1.511). There was no evidence of a difference in the mean patient temperature on the day the FilmArray™ was performed between the positive and negative FilmArray™ groups; difference 0C, P value (t- test) = 0.939. Patients who had no temperature recording on the database (n=3) were excluded from this analysis.

Antibiotic use in 72hr prior to sample collection

In the positive FilmArray™ group, 95/100 (95.0%) patients received antibiotics in the 72 hours prior to sample being taken for analysis; and in the negative FilmArray™ group this was 45/48 (93.8%). There was no evidence of a difference in proportion of patients on/off antibiotics in the 72hr prior to sample being taken between the positive and negative FilmArray™ groups; difference 1.2%, 95% CI -9.2%, 6.8%.

Neutrophil count and CRP Level

The median neutrophil count and CRP level in both the relevant groups are summarised in Table 3.8.

Table 3.8: Neutrophil count and CRP Level in the positive and negative FilmArray™ patient groups

	Positive FilmArray™ group - adults and paediatric (N=100)	**Negative FilmArray™ group - adults and paediatric (N=48)**
Median neut count (10^9 cells/l); IQR	9.2; 5.7-15.1	7.7; 5.7-11.2
Median CRP level (mg/dl); IQR	120.4; 43.5-228.8	89.5; 24.4-189.6

In the positive FilmArray™ group missing neutrophil counts n=14, missing CRP levels n=26; and in the negative FilmArray™ group missing neutrophil count n=3, missing CRP levels n=12. This table excludes these missing values.

Neither the neutrophil count nor CRP predicted whether the FilmArray™ result would be positive or negative: difference in the median neutrophil count between the two groups was 1.5x10^9cells/l, 95% CI: -0.74 to 3.54, P value = 0.183 (Mann Whitney test); and difference in the median CRP level between the two groups was 30.9 mg/dl, 95% CI: -19.60 to 64.60, P value = 0.375 (Mann Whitney test).

A binomial logistic regression model (Table 3.9) was run to describe the effects of the following covariates on whether the FilmArray™ results would be positive or negative: number of days in hospital pre FilmArray™ test being performed; neutrophil count; CRP; patient temperature; whether imaging report was suggestive of pneumonia; antibiotic use in the 72 hrs before sputum sample taken for FilmArray™ testing. These covariates did not statistically significantly predict the outcome (P>0.05 for all covariates). Therefore, the results were similar both unadjusted and after adjustment.

Table 3.9: Logistic Regression Model: Variables predicating a FilmArray™ result being negative or positive

Dependent covariate: Positive or negative FilmArray™ result	**Odds Ratio**	**Standard Error**	**P value**	**95% CI**
Number of days in hospital prior to FilmArray™	1.002	0.008	0.763	(0.988, 1.017)
Neutrophil count	0.970	0.361	0.413	(0.902, 1.043)
CRP	1.003	0.002	0.171	(0.999, 1.007)
Patient temperature	1.233	0.225	0.250	(0.862, 1.763)
Imaging report suggestive of pneumonia	1.341	0.723	0.588	(0.465, 3.868)
On antibiotics prior to sample collection	1.432	2.224	0.817	(0.682, 30.063)

3.4.10 Resistance Genes detected

Among all the FilmArray™ results, resistance genes were detected in three samples from three patients (one adult and two paediatric). This was the same gene for all three samples: MREJ-linked *mec*A/C, conferring methicillin resistance in *S. aureus.* These were not reported by the paired conventional diagnostic microbiology samples. No other resistance genes were detected by the FilmArray™.

3.5: DISCUSSION

3.5.1 Organisms as detected by conventional diagnostic microbiology

To date the organisms responsible for HAP/VAP, as isolated by conventional diagnostic microbiology, have been well reported.[72] Cultures, with susceptibility tests on relevant pathogens, take two to three days; the processing, interpretation and reporting of these results varies among laboratories.

From the 148 conventional diagnostic microbiology results amongst adult patients presented here, half (50.7%) yielded no organisms with a report of either 'no growth', 'no significant growth' or 'normal respiratory flora'. The upper airways and oropharynx are colonised with flora, resulting in the need to select out and report clinically relevant bacteria. The reporting of sputum results in the UK is done in accordance with the UK Standards for Microbiology Investigations, but this does not specify exactly what to report as 'no significant growth' or 'normal respiratory flora', so variation will occur from laboratory to laboratory.[3] Reports such as these are not an uncommon finding. The diagnostic yield of sputum samples in pneumonia has been much debated, there are two factors to consider: a) variations in reporting of sputum cultures, and b) sample quality. A study from the USA by Naidus et al. assessed the yield of non-invasive sputum culture in HAP patients over a 6-year period.[104] Of the 478 sputum samples, only 13.2% were positive. This study specifically used non-invasive sputum samples i.e., expectorated or induced, however almost two-thirds of the results presented in the present study are ETT exudates (61% of adults). In comparison, ETT samples are more invasive, however it could well be argued that an ETT sample is not invasive, when compared to e.g., a

BAL sample. What is significant is the quality of the sample and any risk of contamination. In terms of sample quality, BAL delivers a deep-lung specimen, and is widely performed in some countries such as France and the US, but is reserved for the more complex patients in the UK, being seen as invasive and carrying some risk.[105] European guidelines suggest obtaining distal quantitative samples with invasive techniques to improve the accuracy of results.[106] There is a possibility that ETAs may falsely suggest presence of bacteria, however they are easy to perform.[107]

A further retrospective observational study by Blasi et al. collecting data on the management of 2039 EU patients hospitalised with CAP, found that only 28.5% had a microbiological diagnosis.[20] One important factor relevant to this study is that common CAP pathogens, notably *Streptococcus pneumoniae* and *Haemophilus influenzae* are difficult to culture, leading to low recovery rates. In the study, sputum samples accounted for 45.2% of samples, and BALs for 8.7%, the remainder were urinary antigen tests, blood cultures and pleural fluid samples. When comparing this with the results from the current study: 26.6% were sputum samples (fewer than in the study by Blasi et al.), and 5.8% BAL samples, therefore sample quality differed. Both Blasi et al. and Naidus et al. published findings in line with the results presented here i.e., poor yield of results from culture.

Failure to grow a pathogen may reflect suppression of growth by antibiotic(s) already given to the patient, inappropriate culture technique, or a purely viral aetiology.[108] The present study's low microbiological yield from culture may be explained by the fact that when scanty or mixed opportunist organisms are grown from HAP and VAP patients, as is common with sputum and ETA samples, the healthcare scientist and medical/ clinical microbiologist must make a subjective

judgement of their significance, so reports will vary according to this. What is more, HAP and VAP are difficult to diagnose clinically in patients with multiple other pathologies.[109] Conventional diagnostic microbiology raises the issue of objectivity versus subjectivity when reporting results, and this highlights the importance of a reliable diagnostic test. For the purposes of INHALE, designed as a pragmatic real-world study, pneumonia was diagnosed by the ICU clinician and no trial criteria were given for pneumonia.

In the cases where a pathogen is identified, the agents of HAP and VAP are commonly: Enterobacterales, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, each accounting for around a quarter to a third of cases as described by Masterton et al.[72] A large multi-site study conducted in the USA by Kollef et al., described 4,543 culture-positive patients with pneumonia.[110] The most common pathogens in HAP were Methicillin-sensitive *Staphylococcus aureus* (MSSA) (26.2%) MRSA (22.9%) and *P. aeruginosa* (18.4%); and in VAP were MSSA (28.5%) and *P. aeruginosa* (21.2%) . *Streptococcus pneumoniae* and *Haemophilus* spp*.* were seen more frequently in CAP (16.6% and 16.6% respectively), but it is worth remembering that they can also be isolated from patients with HAP/VAP.

Of the 65 non-duplicate organisms reported by conventional diagnostic microbiology in this present study, *S. aureus* (21.5%) was the most common organism, followed by *P. aeruginosa (20.0%)* and *Enterobacterales* accounted for 41.5%*.* These results are in line with published literature, and aligns with Kollef et al. In the current study there were fewer organisms typical of CAP (as would be expected with this being a HAP/VAP study): nevertheless *H. influenzae* was detected in four patients (hospitalised between two to eight days) and *S. pneumoniae* in one patient (hospitalised for four days). *Candida* spp. or yeasts

accounted for 13 (8.8%) of all reported conventional diagnostic microbiology results. A study by Ewig et al. reported that *Candida* spp. isolates were associated with a delay in sample processing.[111] In the results from the current study, such organisms were not included amongst the pathogens thought to be causative for HAP/VAP.

In-house virology identified a total of three viral species out of four adult patients who were tested, Rhinovirus (n=1) and Coronavirus (n=2). The Coronaviruses referred to here were pre-COVID-19 pandemic. The low number of adults tested contributes to the number of viruses detected.

3.5.2 FilmArray™ Pneumonia Panel Plus detection compared with conventional diagnostic microbiology

Amongst adults, over a third (36.6%) of FilmArray™ reports were negative. Although still high, this is less than the 50.7% of conventional diagnostic microbiology results which yielded no organisms. This difference of 14.1% was significant: P=0.024, 95% CI for difference in proportions: 2.1%, 26.1%.

Of the 126 non-duplicate organisms identified by the positive FilmArray™ tests, the most common was *H. influenzae* (18.3%) followed by *S. aureus* (15.9%), and *P. aeruginosa* (14.3%). *H. influenzae* was not the most common pathogen identified by conventional diagnostic microbiology, in fact it accounted for just 6.2% of organisms. The most prevalent organism as detected by FilmArray™ in children was also *H. influenzae* (14%) *followed by S. aureus* (12%). Amongst children 25% of FilmArray™ results were negative. To date (June 2021) there is no literature comparing FilmArray™ pathogen detection in adults and children. It is important to note that there has been a limited comparison between adult and paediatric

microbiology results in the work presented here. There are two reasons for this; firstly, the sample types used for analysis differed because one of the paediatric centres used only BAL samples: overall, a third of paediatric samples were BAL and 5.8% of adult samples were BAL. Secondly, the two paediatric ICUs were tertiary referral units and therefore had a far more complex patient mix compared with the range of 10 adult ICUs in the trial from small district general hospitals to referral units.

H. influenzae is an organism typically associated with CAP, and on closer evaluation the patients who had *H. influenzae* detected by FilmArray™ had been in hospital from 2-58 days (median = 6 days). *H. influenzae* does appear in other HAP/VAP studies.[72] It was also detected by conventional microbiology in four patients in the present study, albeit the maximum duration they had been in hospital was eight days. A study by Cremet et al. evaluated utility of the FilmArray™ Pneumonia Panel Plus in 100 ICU patients with clinically suspected HAP using a total of 237 samples: 76 BALs and 161 ETAs.[107] The most common pathogens detected were *H. influenzae*, followed by *S. aureus*. *H. influenzae* was found in 40% of patients and *S. aureus* in 33%; and in terms of sample quality, they used a majority of ETA samples, as in our study. The organisms as reported by Cremet et al. support the findings of the current study.

Other explanations for the detection of *H. influenzae* in HAP/VAP patients include the FilmArray™ test detecting normal upper respiratory tract flora, i.e., the result could reflect upper respiratory tract contamination of the sample.[112] Secondly, an evaluation of PCR methods compared with routine culture by Enne et al. reported that for common HAP/VAP pathogens, FilmArray™ had sensitivity of 91.7-100.0% and conventional diagnostic microbiology had a lower sensitivity: 27.0% to 69.4%.[10]

This suggests the FilmArray™ has higher sensitivity for detecting low levels of nucleic acids from organisms that are difficult to culture or even detecting bacteria which are no longer alive.[113] The FilmArray™ may therefore not be detecting a high number of viable bacteria. Murphy et al. described a similar finding when evaluating the FilmArray™ Pneumonia Panel – reporting that the highest rates of false-positive detections were seen for the organisms most frequently detected: 163 total for both *S. aureus* and *H. influenzae.*[114] A study by Yoo et al. reported evaluation of the FilmArray™ Pneumonia Panel and conventional diagnostic microbiology results of 31 sputum and 69 ETA samples (similar proportions of sample type compared with the present study).[115] They too described a discrepancy between reporting of *H. influenzae* by FilmArray™ and conventional diagnostic microbiology. They suggested that this finding may represent the FilmArray's™ ability for detecting low levels of DNA from organisms that are difficult to culture or no longer alive. A combination of these reasons, i.e., upper respiratory tract contamination, detection of non-viable organisms, and higher sensitivity of the FilmArray™ is likely to account for the finding of *H. influenzae* presented in this study.

Studies have been performed to evaluate the utility of the FilmArray™ Pneumonia Panel in hospitalised patients, including those on ICU. One such study by Lee et al. tested ETAs and BALs obtained from 51 adult patients; sample breakdown was 40 ETA specimens, 13 BAL, and 6 bronchial washing specimens (some patients had more than one sample sent).[73] They reported a positivity rate of 55.9%, lower than ours which was 63.4%. The patient group described by Lee et al. was smaller than that in the current study. However, the cohort was similar to the current study in age (65 years versus 61.5 years) and the majority of patients were intubated. The most common pathogen detected by Lee et al. was *K. pneumoniae*

(16.9%) followed by *P. aeruginosa* (11.9%). In the current study, *K. pneumoniae* accounted for just 6.3% of FilmArray™ detections. This difference may be a reflection of sample type used: Lee et al's samples consisted of 22% BALs compared with 5.8% BALs in our study; or geographical location: the epidemiology of hospital acquired pathogens will differ in Taiwan and the UK. A paper by Murphy et al. describes studies that were conducted to evaluate performance of the Pneumonia Panel.[114] A total of 846 BAL and 836 sputum specimens, prospectively collected, were tested with the panel reporting at least one organism in 48.8% of BAL specimens and in 72% of sputum specimens. One possible interpretation of this is that the deeper the specimen, the less likely it is to be contaminated with upper respiratory tract flora. In the present study numbers of BAL samples were too low to perform an analysis with this level of granularity for adult patients. However, it was performed for paediatric FilmArray™ results, and there was no evidence of a difference in positivity of FilmArray™ results between BAL samples and more proximal samples (ETT/sputum samples) – CIs for difference in proportions included zero. The BAL sample size for children with an available FilmArray™ result was also small (n=10, 22.3%), therefore a true effect may not have been seen.

Webber et al. evaluated the FilmArray™ Pneumonia Panel in 200 lower respiratory tract samples, from patients in both A&E and ICU (important to note that the current study included only ICU patients).[116] BAL samples accounted for 35%, and the remainder were sputa. Almost half of the patients (47.5%) has respiratory symptoms within 48hr into hospital admission. The most common pathogen detected was *S. aureus* (22%), the second most common to be isolated in the present study.

A Cochrane Review by Jiang et al. reported that *S. aureus* and *E. coli* are both causes of HAP/VAP in children – *S. aureus* was the second most prevalent

organisms amongst children in the current study. However, adult patients in the present study had evidence of significantly more *E. coli* detections compared with children: 10% higher, 95% CI 3.55%, 16.75%. This may just be a reflection of the smaller number of children in the study.

There were seven instances of viral detections in adult patients by the FilmArray™ in the present study: Rhinovirus/Enterovirus, n=5; Coronavirus, n=1; RSV, n=1. This was more than the three detected by routine in-house virology (however only a total of four patients had in-house virology performed). It is somewhat surprising that overall more viruses were not detected given that the timeframe for recruitment of these patients was 05/07/2019 – 19/08/2020 i.e., spanning the winter respiratory virus season. One possible explanation is that due to COVID-19 the INHALE trial was on hold (16/03/2020 – 01/07/2020), this was specifically during the first peak of the pandemic. Furthermore, Public Health England reported low levels of influenza and RSV activity in the 2019-2020 season.[117] A reason for this could be the lockdowns in the UK, and measures such as social distancing and the wearing of face masks in public spaces. Such social restrictions would limit the spread of all respiratory viruses. The current study demonstrated that viruses were detected by FilmArray™ more frequently in children compared with adults: 33% higher in children; P<0.0001, 95% CI: 19.7%, 46.3%. Harris et al. describe in the British Thoracic Society Guidelines that viral infections are a frequent cause of pneumonia in children supporting the findings presented here.[118]

A systematic review by Huang et al. in 2018, including 20 studies, considered performance of the FilmArray™ Respiratory Panel, plus two other platforms for diagnosis of viral respiratory infections.[119] Although this is a different panel to that

used in the current study, the take-home messages are important. A total of 5510 upper and lower respiratory tract samples from children and adults were tested. These were compared with virus culture, direct fluorescent antibody tests and to commercial and local in-house real-time PCR (RT-PCR). The review concluded that these rapid systems helped early diagnosis of viral respiratory infections.

3.5.3 Repeat FilmArray™ Tests

Repeat FilmArray™ testing is not an aspect which has been extensively covered in the literature to date: there are data on use of the FilmArray™ Respiratory Panel in this setting, but not the FlimArray™ Pneumonia Panel. Repeat tests were permitted in the study provided the patient was randomised to the intervention arm, they were no less than 72 hours apart from the prior specimen and a specimen would have been taken regardless of trial participation. These were performed at the discretion of the ICU teams provided the criteria were met.

In the present study, 4.8% (seven) of adult patients had repeat tests performed; this represents 7.1% of adult FilmArray™ tests. The median number of days difference between the repeat tests was five days. A reason for there being such a small number of repeat tests could be the teams' unfamiliarity with a new test on the ICU. The FilmArray™ Pneumonia Panel had not previously been used as a POCT on any of the ICUs participating in the trial. A study by Qavi et al. reported results of repeat testing within seven days, using the FilmArray™ Respiratory panel.[120] They found that 10% of all FilmArray™ tests (n=12,536) were repeat tests, more than in this study (7.1%). Of the repeat tests, 6% identified new organisms, however the authors commented that these were not always clinically relevant and

that different specimen types had been used for the tests. No new organisms were identified on repeat testing in the current study, however the focus here is on bacteria rather than viruses but the study by Qavi et al. focuses on viruses. It could be argued that by a median of five days, as in the current study, appropriate antibiotic treatment would have had an effect which explains why in two cases the repeat FilmArray™ test reported only one of the two bacteria reported by the first test.

Repeat negative results occurred four times from four patients i.e., their first FilmArray™ test result was reported as negative as were their repeat tests. Clinicians may have thought it useful to repeat a test on a patient who remained clinically unwell after an initial negative test in the hope that a curable cause be found. Azadeh et al. performed a retrospective study on 86 patients who had the FilmArray™ Respiratory Panel performed on BAL specimens within seven days after a NP Respiratory Panel test.[121] They reported that in 20% of these patients, the BAL sample identified pathogens which were not detected by the NP sample, concluding that a BAL may be the way forward after a negative NP specimen. Of the repeated negative results from the current study, in one case the first test was performed on a BAL and the subsequent two on ETT samples (this is the patient who had two repeat tests performed); one repeat negative test was performed on an ETT sample (as was the first test): and the other repeat negative test was performed on a sputum sample (as was the first test). Had the repeat tests been BAL samples, perhaps the results would have differed in terms of organisms identified.

In four patients the same organism was found on repeat testing (Coronavirus n=1, *S. aureus* n=1 and *P. aeruginosa* n=2*).* In both cases where *P. aeruginosa* was detected on repeat testing (tests nine and five days apart), it had acquired resistance

to the antibiotics which the patient was on for treatment. *S. aureus* was detected on repeat FilmArray™ testing 20 days after the first test, and it is unclear why this occurred as the patient had received adequate antibiotics for treatment. Coronavirus detection on repeat testing was three days later, and could well be expected given that there was a short interval between tests.

When interpreting results from the current study a limitation is the sample size – only eight repeat tests were performed in seven patients. Both the studies described by Qavi et al. and Azadeh et al. did use the Respiratory Panel and not the Pneumonia Panel, but nevertheless the results are relevant and a useful comparator. They illustrate that repeat FilmArray™ tests are not a common occurrence, sample type is important and may provide differing results.

3.5.4 Whether any clinical variables result in a positive diagnostic test

The UK, European and IDSA guidelines on diagnosis of HAP/VAP reflect the complexities in diagnosis.[13,72,106] Imaging findings, oxygen requirements, fever and inflammatory markers all contribute to the clinical differential diagnosis.

Several clinical variables were analysed in this study to determine whether they had an effect on the outcome of whether patients had double negative results (i.e., a negative FilmArray™ and negative conventional diagnostic microbiology results) or one of these two results being positive. The thinking behind this was that a positive result (FilmArray™ or conventional diagnostic microbiology) would be suggestive of pneumonia. However, this approach must be cautious given that a positive result does not always mean infection. Reasons for this include detection of

non-pathogenic organisms, DNA of non-viable organisms, and organisms present in low density not always requiring treatment – as discussed in Chapter 3.5.2.

Variables analysed were whether imaging showed evidence of infection (95% CI for difference: -5.3%, 27.9%); neutrophil count (95% CI for difference: -3.40 to 0.87); CRP level (95% CI for difference: -56.4 to 36.0); whether the patient was on antibiotics in the 72 hours prior to test (95% CI for difference -7.2% to 8.0%). There was no evidence that any of these variables affected this outcome, this could be due to the sample size.

Clinicians chose to enrol patients whom they thought had a HAP/VAP; the trial design is pragmatic so there were no strict rules defining signs/symptoms the patient must have. Firstly, imaging can be difficult to interpret in the setting of HAP/VAP. It is well known that patients with HAP/VAP may have other causes for abnormalities on imaging. One systematic review found that new or worsening infiltrates had a specificity of 50-78%.[122] This is echoed in the UK guidelines for HAP which state that there is not enough evidence to assess the value of imaging.[72] They also remark that radiology is often more useful in patients who are not ventilated; in the current study 72.6% of all adults and 90.7% of all children were requiring invasive ventilation. Therefore, a reason why imaging did not affect the outcome could be due to a combination of the overwhelming majority of ventilated patients in the study, and difficulty in interpretation of imaging. Another factor to keep in mind is the lower sensitivity and specificity of portable chest X-rays, and given that all our patients were on ICU I would anticipate that most of the X-rays would have been portable, however we do not have the exact numbers.[13]

Secondly, the inflammatory markers (neutrophil count and CRP) also did not predict which of the two groups the patient would be in. The reason the neutrophil

count was used here and not the total WCC is because it is more reflective of infection than the total WCC. The European guidelines on management of HAP/VAP states that the WCC is part of the clinical bedside evaluation.[106] However they do not recommend the use of CRP to predict adverse outcomes in patients on antibiotics due to its elevation secondary to inflammatory disorders seen in ICU patients. An observational study of 148 patients who were mechanically ventilated reported that CRP levels were the same in patients with and without pneumonia.[123] The IDSA recommends decision of antibiotic initiation to be based on clinical criteria alone, i.e. without CRP as it cannot reliably distinguish patients with and without VAP.[13] This could explain why CRP as a variable, predicting whether the patient would be in the double negative group, was not significant – due to other reasons for its elevations. Both the neutrophil count and CRP level may well have been elevated due to patients having more than one infection.

Antibiotic use in the 72 hours prior to the sample taken for analysis was also not significant. In both groups the overwhelming majority of patients were on antibiotics, and this is not surprising given that the patients were all on ICU. A paper by Harris et al. examined 4678 patients with CAP to determine the influence of antibiotics on culture results (in this current analysis both conventional diagnostic microbiology and FilmArray™ tests were taken into account).[124] The paper concluded that there were significantly more bacterial detections ($P<0.01$) in sputum/ET cultures collected before antibiotics. Although these were patients with CAP, the same principle can be applied to those with HAP/VAP. The reason why these findings were not seen in the present study could be because firstly the vast majority of patients were on antibiotics, and secondly because a PCR test was also being evaluated alongside routine culture. The second study which warrants

mention is a case-controlled study of pneumonia in children in Africa and Asia (PERCH) which looked at the effect of antibiotic exposure on pathogens in induced sputum, NP/Oropharyngeal (OP) swabs, and blood in both hospitalised children with severe or very severe pneumonia, and controls who were from the community.[125] Out of 4223 children, antibiotic use was associated with a 20% reduction in induced sputum culture yield. Of the NP/OP specimens tested by PCR (Fast-track Diagnostics Respiratory Pathogens 33 multiplex PCR kit), the mean number of positive bacterial targets was higher in patients with antibiotic exposure compared to those without (P<0.001). The opposite was found in the control group – where patients with antibiotic exposure has fewer bacterial targets detected (P<0.001). The authors try to explain this by suggesting that there were lower bacterial densities in the control patients. Of course, the PCR test could be detecting non-viable bacteria, making it more difficult to determine whether antibiotics had an effect on whether results were positive or negative. It is probable that those patients with symptoms of infection were more likely to receive antibiotics.

A similar analysis was performed to assess how variables may influence whether the FilmArray™ test alone was positive or negative. This did not take conventional diagnostic microbiology results into account. To date (June 2021), there is no published data on the use of the variables analysed in the study as predictors of a positive or negative FilmArray ™Pneumonia Panel test. There was no evidence that any of these variables affected this outcome. Variables analysed were whether imaging showed evidence of infection (95% CI for difference -6.6%, 25.0%); neutrophil count (95% CI for difference: -0.74 to 3.54); CRP level (95% CI for difference: -19.60 to 64.60); on antibiotics in 72 hours prior to test (95% CI for difference: -9.3%, 6.8%); patient temperature (P=0.939); number of days in hospital

prior to FilmArray™ being performed (95% CI for median difference: -4.00 to 1.00); and reason for hospital admission (P=0.857).

The three categories for hospital admission were medical, surgical and trauma; with medical being the most common reason for admission (59.5%). Results showed that there was no evidence of an association between reason for hospital admission and whether or not the FilmArray test delivered a positive result (Chi squared test, P=0.857). A prospective study with 2436 patients from 27 ICUs across Europe, reported that trauma had a higher incidence of VAP.[126] This was not reflected in the current study, possibly because there were only a small number of trauma patients. Head trauma is thought to be a risk factor for developing a HAP/VAP.[127]

The patients' temperature was found to be insignificant with respect to the FilmArray™ result. The IDSA guidelines state that the Modified Clinical Pulmonary Infection Score (of which temperature is one component) should not be used to decide whether antibiotics are started for HAP/VAP.[13] This scoring system takes into account temperature, WCC, respiratory secretions, oxygen requirement and imaging findings. A meta-analysis of 13 studies reported that the pooled sensitivity of the score for diagnosing VAP was 65%, and specificity was 64%.[128] Reference standards used in this study were BAL fluid cultures and lung biopsy cultures. The IDSA recommends using clinical criteria alone to decide on antibiotic initiation. Therefore, temperature appears to be the subject of some debate, as it is part of the clinical criteria used to make a decision on antibiotic initiation, but the idea of having it as part of a scoring system was rejected. In the present study no scoring system was used, and temperature was used purely as a clinical factor. The European and UK guidelines also highlight the importance of temperature as part of clinical

evaluation.[72,106] A paper by Wunderink et al. reports that an infiltrate on imaging along with one feature of fever, raised WCC, purulent tracheal secretions has a high sensitivity for VAP but low specificity.[129] The author goes on to say that if all four criteria were required then the sensitivity would be very low (<50%). The present study agrees with this finding and looked at all these covariates together in a logistic regression model where they remained insignificant after adjustment. A possible reason why temperature was not found to be a predictor for a positive/ negative FilmArray™ result could be because some patients had another reason for the raised temperature e.g., another infection. Other suggestions are: haemodialysis in ICU patients will artificially reduce temperature, as will cooling post cardiac arrest. There is not detail enough in the database to examine how many patients this applied to.

The second variable used to determine whether the FilmArray™ would be positive or negative was the number of days in hospital prior to the test being performed. Again, this was not significant. Cook et al. performed a prospective cohort study on 1014 mechanically ventilated patients in 16 Canadian ICUs; reporting that the daily risk for pneumonia decreased as patient stay increased; with the highest risk being during the first 5 days of admission.[130] Therefore, the results presented by Cook et al. differ to those reported in the current study which found that the number of days in hospital did not affect the FilmArray™ result being positive. Cook et al. study classified pneumonia by either an adjudication committee, bedside clinician's diagnosis, Centres for Disease Control and Prevention (CDC) definition, Clinical Pulmonary Infection score, or positive culture from bronchoalveolar lavage or protected specimen brush (PCR testing was not used). To date (June 2021) there is no literature on FilmArray™ positivity rate according to length of stay. The published

literature relates to how such interventions can reduce length of stay rather than length of stay being a predictor of a positive FilmArray™ test.

In summary, this detailed discussion on variables, which are typically associated with pneumonia, are just as likely to represent negative FilmArray™ and conventional diagnostic microbiology results. This highlights the need to examine whether all positive results are representative of pneumonia and warrant treatment. Further work to help evaluate this includes determining whether these clinical variables have any relationship to the density or colony forming unit (CFU)/ml or organism identified. This seeks to examine whether a higher density of organism present is more likely to represent infection. Evaluating patient outcomes in accordance with this would be valuable.

3.5.5 Negative FilmArray™ with positive conventional diagnostic microbiology results

There were instances in this study where conventional diagnostic microbiology detected organisms which the FilmArray™ did not: three bacteria (*S. aureus, C. koseri, S. maltophilia,)* one fungus (*A. fumigatus)*, and three viruses (RSV, Metapneumovirus and Coronavirus NL63). *C. koseri,* and *S. maltophilia* are not on the Pneumonia Panel which explains why they were not detected.

A general limitation of PCR systems is that they can only detect targets for which they have PCR primers. The organisms represented on the BioFire FilmArray™ Pneumonia Panel cause around 90-95% of pneumonia cases.[13] It would be difficult to expand this proportion to 99% due to limitations on the number of primers that can be multiplexed. However, *S. maltophilia* is a notable omission from the FilmArray™ Pneumonia Panel, accounting for around 1-6% of VAP

cases.[29,30] This specific organism was not included in the panel by bioMérieux due to the high false positive rate the company encountered as it is a common contaminant. With respect to *S. aureus,* a recent review highlighted that in the manufacturers' dataset *S. aureus* has a negative percent agreement (this is the specificity of a test when compared to a non-reference standard) of 91% (below the ideal of 95%).[131] Webber et al. report a negative percent agreement for *S. aureus* of below 90%.[116] This shows that others have observed higher false positive rate for *S. aureus* detection. However, a study by Buchan at al. described a similar scenario to the present study, but the specimen type was BAL (in the current study it was ETT exudate). i.e., *S. aureus* detected by conventional diagnostic microbiology but not by FilmArray.[74]

Webber et al., in their evaluation of the Pneumonia Panel, commented on the absence of fungal targets and *S. maltophilia*.[116] They evaluated the diagnostic yield and accuracy of the Pneumonia Panel utilising 200 specimens. Fungi were detected in 26 of the specimens, and *S. maltophilia* in 4. The fungi included: 21 yeasts, 2 *Aspergillus* spp., 1 *Blastomyces dermatitidis/B. gilchristii* isolate, 1 *Paecilomyces* species, and 1 *Pneumocystis jirovecii* isolate. In the present study, *Candida spp.* was omitted because it was not thought to be clinically significant in pneumonia.[132] This is because the isolation of *Candida* spp. in respiratory tract specimens almost invariably represents contamination, rather than infection so the decision was made by the medical microbiologists and scientists on the trial team to exclude these organisms.[103] However, there was one case of *A. fumigatus* identified by the local laboratory. This was included in the results as the patient presented to hospital with septic shock on a background of Hodgkin's Lymphoma. Therefore the *A. fumigatus* may have been significant, and without further details (not recorded in

the database) it is difficult to know. In the immunocompromised host it will be especially important for clinicians to be aware of the limitations of such PCR panels, so as to aid diagnosis. For example, should the Pneumonia Panel be used on a HIV positive patient, *Pneumocystis jirovecii* is absent and must be remembered in the differential diagnosis.

The remaining discordant cases relate to viruses, with the FilmArray™ failing to detect RSV, Metapneumovirus and Coronavirus NL63. These results belong to two paediatric patients from the same hospital. A possible explanation is described in a study by Renaud et al. comparing the FilmArray™ Respiratory Panel with a laboratory developed real-time PCR.[133] They prepared samples by mixing respiratory specimens (including nasal washes, nasal swabs, bronchoalveolar lavages, sputum, and tracheal aspirates). These samples were known to be positive by the laboratory developed test, and viral cultures. They reported that the FilmArray™ identified 90% of the viruses (n=80), and six of the eight viruses (these six included RSV, Metapneumovirus and Coronavirus, as in the current study) not detected had a PCR cycle threshold values >35. This could account for our findings i.e., the FilmArray™ not detecting viruses with a high cycle threshold. Another reason could be that the samples used differed: nasopharyngeal swabs were used for in-house virology tests, whereas in the current study a sputum/ETT sample was used.

3.5.6 Antimicrobial Resistance genes

A paper by Anand et al. highlights that the pathogens causing HAP/VAP are more likely to be MDR.[134] In the current study, three patients (one adult and two

paediatric) had FilmArray™ results which reported a resistance gene. In all cases the gene reported was MREJ-linked *mec*A/C, conferring methicillin resistance in *S. aureus.* A total of 148 patients had FilmArray™ results available to analyse, indicating an MRSA prevalence of 2.0%. In the UK, around 4.7% of *S. aureus* isolated from LRTIs have the *mec-A* gene (according to BSAC surveillance data (2018-2019), http://www.bsacsurv.org).[15] This figure was as high as 43.4% in 2008-2009.[15] It is important to note that the FilmArray™ only detects a limited number of resistance genes, so others may have been present but not detected.

A retrospective cohort study in the USA consisting of 4543 patients by Kollef et al. examined the epidemiology of pneumonia.[110] They reported that MRSA accounted for 22.9% of HAP (n=835) and 14.6% of VAP (n=499). In a review by the IDSA, the pooled overall prevalence of MRSA pneumonia was reported to be 10%; and for VAP specifically it was 8%.[135] These two papers do differ in figures, but nonetheless they are higher than what we currently see in the UK (4.7%). The epidemiology of pneumonia differs from country to country and the prevalence of 2.0% in this present study is encouraging, and if anything a little lower than the 4.7% in the UK as reported by BSAC. Risk factors for an MRSA related HAP/VAP include age>65 years, length of stay, and acute renal failure.[106] Typically, late-onset VAP (i.e. at or after day five of admission) is related to MDR pathogens e.g. MRSA.[136] Interestingly, in the current study the three patients were in hospital for 10, 15, and 58 days; which supports this theory of association with late-onset VAP.

MRSA was reported by none of the conventional diagnostic microbiology samples highlighting the potential value of molecular testing. From the three FilmArray™ reports, *S. aureus* was reported at a density of >10*7 twice, and at 10*6 for the third sample. Conventional diagnostic microbiology reported *S. aureus* in two

out of three cases and the instance where it did not report it, the result was ‘normal respiratory flora’ whereas the FilmArray™ detection was >10*7 *S. aureus.* Evaluation of the FilmArray™ Pneumonia Panel by Webber et al. also reported a similar finding: four cases occurred where the FilmArray™ identified MRSA but culture did not.[116] They suggested possibilities for why conventional diagnostic microbiology did not report MRSA: the detected *S. aureus* had an empty SCC*mec* cassette, or the MRSA was below the threshold for reporting, however in the present study this is made less plausible with such high densities of *S. aureus* reported. Other possibilities include both MRSA and MSSA being present and routine culture failing to detect the MRSA (possibly in lower numbers) in a mixed specimen.

3.5.7 Summary of Key Findings

Organisms as detected by FilmArray™ and conventional diagnostic microbiology in HAP/VAP patients differed. The most common organism identified by FilmArray™ was *H. influenzae,* whereas conventional diagnostic microbiology reported *S. aureus* to be the most common. Significantly more negative conventional diagnostic microbiology results were reported when compared with negative FilmArray™ results (95% CI for difference in proportions: 2.1%, 26.1%). Viruses were an uncommon finding (especially amongst adults), as were antimicrobial resistance genes. Clinical variables which are often associated with pneumonia had no correlation with positive or negative FilmArray™ results or conventional diagnostic microbiology results.

CHAPTER 4

MICROBIOLOGY OF ICU HAP/VAP PATIENTS WITH COVID-19

4.1: INTRODUCTION

Research into bacterial co-infection in COVID-19 has grown since the start of the pandemic. Increasing numbers of studies emphasise the importance of Gram-negative organisms in this patient group.[60] Few studies have deployed the use of the FilmArray™ Pneumonia Panel Plus[99] in COVID-19 patients, utilising this to both aid diagnosis of HAP/VAP and direct antimicrobial treatment.

Recruitment to INHALE was halted due to the pandemic in 2020 (16/03/2020 – 01/07/2020) which resulted in the development of a COVID-19 sub-study. This Chapter focuses on the COVID-19 patients participating in the sub-study during the 2020 pandemic, and comparison is made with the adult patients from the INHALE trial. Five of the twelve ICUs participated, with the aim of describing the organisms responsible for HAP/VAP, as detected by the FilmArray™ and conventional diagnostic microbiology in this novel patient group. When the sub-study was performed there was little literature describing bacterial co-infections in COVID-19.

The work presented examines bacterial respiratory co-infections in COVID-19 patients, all in ICU. Patients were included based on the ICU physicians' suspicion of bacterial infection. Findings from the COVID-19 sub-study are compared with those from the INHALE adult patients, pre-pandemic (Chapter 3).

4.2: AIMS

1. To describe the organisms causing HAP/VAP in COVID-19 patients as detected by the FilmArray™ Pneumonia Panel Plus and conventional diagnostic microbiology.

2. To compare these organisms with those detected in patients without COVID-19.

4.3: METHODS

4.3.1: Contribution

Data was collected by the research teams at the sites (Appendix 4). I worked with the Norwich Clinical Trial Unit to check and clean the data prior to analysis. Queries were raised with sites on any spurious datapoints. Once data input was complete the data pages were locked so no further changes could be made. I developed the statistical analysis plan, and performed all analyses in this chapter.

4.3.2: Patient Recruitment

Five of the ten adult ICUs participated in the COVID-19 sub-study: Aintree University Hospital (part of Liverpool University Hospitals NHS Foundation Trust), Chelsea and Westminster Hospital NHS Foundation Trust, Royal Free London NHS Foundation

Trust, University College London NHS Foundation Trust and Watford General Hospital (part of West Hertfordshire NHS Trust). The two paediatric hospitals did not take part, therefore the data described represents adults only. The sub-study used the infrastructure in place for the INHALE trial. Patients were recruited to this sub-study from 03/04/2020 – 23/06/2020.

The COVID-19 sub-study was not randomised, unlike the main INHALE trial which is an RCT. In order to be eligible for the COVID-19 sub-study, patients had to be in-patients in a participating ICU and to have clinically-diagnosed or PCR-proven COVID-19, with clinical features compatible with a suspected secondary bacterial pneumonia over and above those expected for COVID-19 viral pneumonia alone. As was the case for the INHALE trial: patients also needed to have sufficient surplus lower respiratory tract sample (200 μl sputum/ bronchoalveolar lavage (BAL) or endotracheal tube aspirate [ETA]) for the FilmArray™ test. A second FilmArray™ test ≥ 5 days from the first test was permitted if a new or continuing bacterial pneumonia was suspected. In parallel, a respiratory sample was sent to the hospital laboratory for routine microbiological investigation, performed according to the Standard UK Laboratories Operating Procedures.[3]

This group of patients was compared with the INHALE trial patients who did not have COVID-19.

4.3.3: Data Collection

Data collected consisted of: baseline data including age, sex, comorbidities, date of COVID-19 diagnosis, admission to hospital, and ICU admission. FilmArray™ test results, clinical microbiology results, antibiotics administered to the patient

from seven days prior to seven days after the FilmArray™ tests were recorded. A bespoke REDCap database was used for data collection and storage.[100]

The COVID-19 patient data was compared with the adult data from the INHALE trial. The trials team granted permission to download the relevant data from the REDCap database into an Excel spreadsheet.

4.3.4: Statistical analysis plan

Stata v.16 was used for statistical analyses. Patient demographics, sample type, number and type of bacteria, viruses and resistance genes identified using FilmArray™ and by conventional diagnostic microbiology were described using frequency (%), mean (standard deviation) or median (interquartile range), as appropriate. Bacterial species were counted once per patient for any method, even if they were detected repeatedly.

When comparing the proportion of each organism detected by FilmArray™, 95% CI and P-values from Chi-squared tests or Fishers exact tests were used. The same statistical methods were used when comparing conventional diagnostic microbiology results, sample types used, and ventilation status in the two groups. When comparing the number of days of hospital admission prior to a positive or negative FilmArray™ test result, medians were compared using a Mann-Whitney test and the associated 95% CI. P-values from Mann-Whitney test, and 95% CI were also used to compare the median number of days of hospital admission prior to a positive FilmArray™ test. Any missing data has been accounted for in the analyses presented.

4.4: RESULTS

4.4.1 Sample types used and ventilation status of COVID-19 patients; comparison with non-COVID-19 patients

All patients recruited to the COVID-19 sub-study were included in this analysis. The recruitment timeframe for these 126 patients was 03/04/2020 – 23/06/2020. Across the five participating sites recruitment varied from 12 to 52 patients per site; no paediatric sites participated. The proportion of male patients recruited was 85/126 (67.5%). This demographic data was compared with the adult non-COVID-19 patients (Table 4.1).

Table 4.1: Comparison of demographics between COVID-19 and non-COVID-19 patient groups

	Non-COVID-19 (N=146)	**COVID-19 (N=126)**	**Difference in proportions (95% CI)**
Male (%)	107 (73.3)	85 (67.5)	5.8% (-5.1%,16.7%)
Female (%)	39 (26.7)	41 (32.5)	-
Median Age (years); IQR	66; 52-73	59; 50-65.75	7 (2,9)

The difference in the proportion of male patients in the two groups was 5.8%, P value (Chi squared test) = 0.293 (95% CI for difference in proportions: -5.1%, 16.7%). There was no evidence of a difference in the proportion of males between

the COVID-19 and non-COVID-19 groups. The difference in the median age between the two groups was seven years, P value (Mann Whitney test) = 0.0015 (95%CI for median difference: 2.9). Therefore, the COVID-19 patients were significantly younger than those without COVID-19.

The sample types used in the COVID-19 patients, and comparison with the non-COVID-19 patients are summarised in Table 4.2; all samples (including repeat samples) were included.

Table 4.2: Sample types used for analysis in both COVID-19 and non-COVID-19 patients

Sample Type	**Adult samples non-COVID-19 (%) (N=154)**	**Adult samples COVID-19 (%) (N=157)**	**Difference in proportions (95% CI)**
BAL	9 (5.8)	2 (1.3)	4.5% (0.5, 8.7%)
ND BAL	4 (2.6)	6 (3.8)	-1.2% (-5.1%, 2.7%)
ETT exudate	94 (61.0)	141 (89.8)	-28.8% (-37.8%, -19.8%)
Sputum	41 (26.6)	5 (3.2)	23.4% (15.9%, 30.9%)
Tracheal aspirate	3 (2.0)	2 (1.3)	0.7% (-2.1%, 3.5%)
Tracheostomy	3 (2.0)	1 (0.6)	1.4% (-1.1%, 3.8%)

There was an association between sample type and COVID-19 versus non-COVID-19: Fishers exact test P<0.0001. The proportion of BAL samples amongst the non-COVID-19 group was 4.5% higher than for non-COVID-19 patients; 95% CI for difference in proportions: (non COVID-COVID): 0.4%, 8.7%. Similarly, the proportion of sputum samples amongst the non-COVID-19 group was 23.4% higher than for the COVID-19 patients; 95% CI for difference in proportions: (non COVID-COVID): P<0.0001, 15.9%, 30.9%. However, the proportion of ETT exudate samples amongst the COVID-19 patients was 28.8% higher than for non-COVID-19 patients; 95% CI for difference in proportions (COVID – non COVID): P<0.0001, 19.8%, 37.8%. The remainder of sample types were not significantly different between the two groups (CIs for proportions included zero).

The ventilation status for both groups, which is linked to the sample type, e.g., invasively ventilated patients will have an ETT sample, is presented in Table 4.3.

Table 4.3: Ventilation status in both COVID-19 and non-COVID-19 patients

Ventilation Status	**Adult patients non-COVID (%) (N=146)**	**Adult patients COVID (%) (N=126)**	**Difference in proportions (95% CI)**
Invasive ventilation	106 (72.6)	119 (94.4)	-21.8% (-30.0%, -13.5%)
NIV	4 (2.7)	2 (1.6)	1.1% (-2.3%, 4.6%)
Not ventilated	36 (24.7)	2 (1.6)	23.1 (15.7%, 30.4%)
Trache mask	0	2 (1.6)	1.6 (-3.8%, 0.6%)
Optiflow	0	1 (0.8)	0.8 (-2.3%, 0.8%)

There was an association between ventilation status and COVID-19 versus non-COVID-19: Fishers exact test P<0.0001. The proportion of invasively ventilated patients amongst the COVID-19 group was 21.8% higher than for non-COVID patients; P<0.001 (Chi Squared), 95% CI for difference in proportions (COVID-non COVID): 13.5%, 30.0%. The proportion of patients not requiring any ventilation amongst the non-COVID-19 patients was 23.1% higher than for the COVID-19 patients; P<0.001 (Fishers exact), 95% CI for difference in proportions (non COVID-COVID): 15.7%, 30.4%). The remainder of ventilation types were not significantly different between the two groups (CIs for difference in proportions included zero).

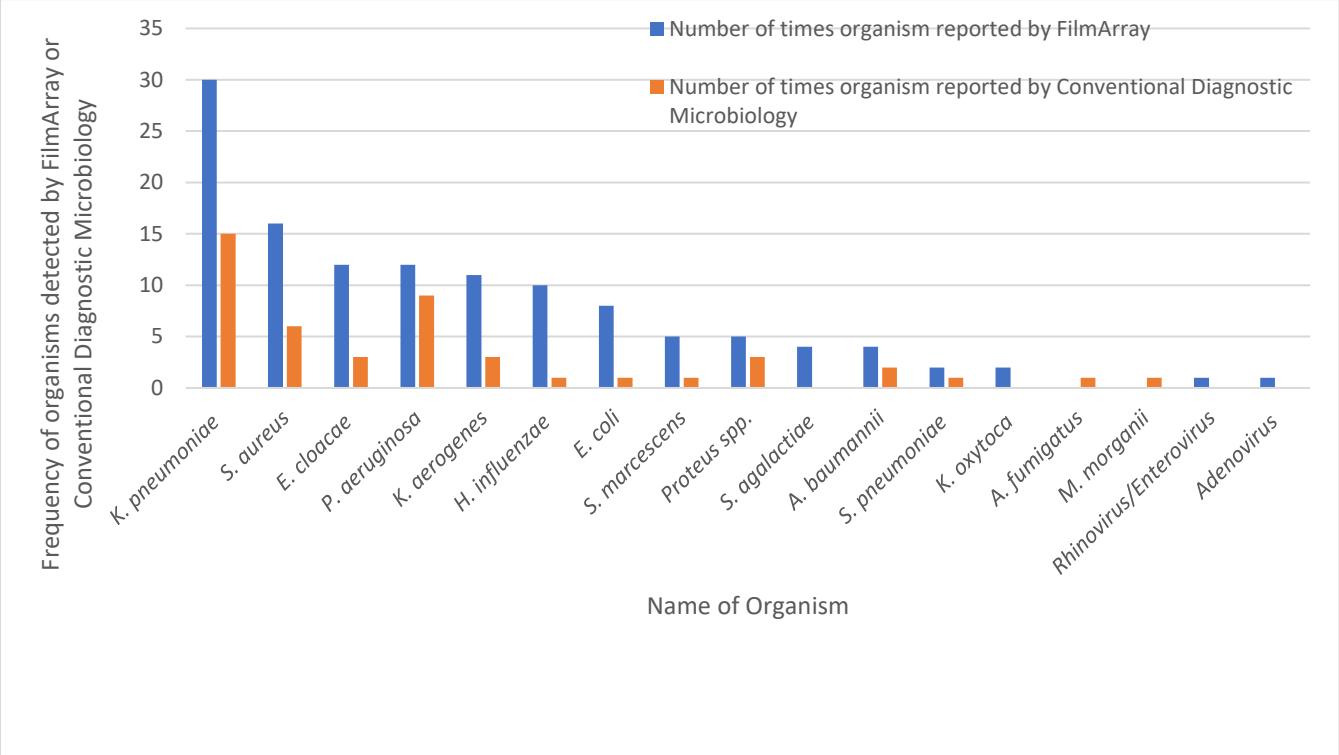
4.4.2 Microbiology of HAP/VAP in COVID-19 patients, as determined by the FilmArray™ Pneumonia Panel and conventional diagnostic microbiology

4.4.2.1 FilmArray™ Results

Of the COVID-19 patient group (n=126), 125 patients had a valid first FilmArray™ test result, with one test failed; 32/126 (25.4%) patients had repeat valid FilmArray™ tests; one patient was planned for a repeat test but deteriorated and died prior to the test, resulting in 157 valid tests in total. There were no missing FilmArray™ results in this patient group. Of the 157 FilmArray™ results across the five sites, 86 (54.8%) were positive and 71 (45.2%) were negative. Of the 32 second tests, 14 (43.8%) were negative. In eleven cases, both the first and second tests were negative; in 6 cases the first and second tests detected the same organisms; in five cases the first and second tests were both positive but the organisms detected were not identical; in nine cases one result was positive and one negative; and in one case the first FilmArray™ test had failed but the second was negative.

A total of 123 non-duplicate organisms were identified by the FilmArray™ tests, as shown in Figure 4.1.

Figure 4.1: Organisms reported by the FilmArray™ Pneumonia Panel Plus and conventional diagnostic Microbiology across all samples from COVID-19 patients (excluding multiple instances of the same species from a single patient and negative results).



The most prevalent organism as detected by the FilmArray™ was *K. pneumoniae,* followed by *S. aureus, E. cloacae,* and *P. aeruginosa.* There was one detection of Rhinovirus/Enterovirus, and one of Adenovirus.

4.4.2.2 Conventional Diagnostic Microbiology Results

A total of 146 conventional diagnostic microbiology results were available for analysis for the 126 COVID-19 sub-study patients. The 146 results account for 13 samples with no results: eight of these were from first test performed and five from repeat tests. Of the 146 results, 79 (54.1%) were reported as no growth, no significant growth or normal respiratory flora; 17 (11.6%) were reported as *Candida spp.* or yeasts; one (0.7%) was reported as *Enterococcus faecium*; and there were no positive in-house virology results. This left 49 specimens with a total of 47 non-duplicate potentially significant organisms (some specimens reported more than one organism). *Candida* spp. and *E. faecium* were thought to be clinically insignificant and were therefore not included in the 47 non-duplicate organisms demonstrated in Figure 4.1. There were 46 non-duplicate bacteria and one fungus (*A. fumigatus).* Among the bacteria identified, the most prevalent was *K. pneumoniae* followed by *P. aeruginosa.*

4.4.3 Comparison of microbiology between COVID-19 and non-COVID-19 patients

4.4.3.1 Comparison of FilmArray™ Results in COVID-19 and non-COVID-19 patients

The FilmArray™ results are summarised in Table 4.4. There was evidence of a difference in the proportion of *P. aeruginosa, E. coli,* and *H. influenzae* FilmArray™ results between the COVID-19 and non-COVID-19 patients. The proportion of these three organisms, as detected by FilmArray™, was significantly higher in the patients who did not have COVID-19. The difference in the proportion of *P. aeruginosa* was

8.5% higher amongst the non-COVID-19 patients than for the COVID-19 patients; 95% CI for difference in proportions (non COVID-COVID): 0.5%, 16.5%; the difference in proportion of *H. influenzae* was 14.1% higher amongst the non-COVID-19 patients; P=0.001 (Chi Squared), 95% CI for difference in proportions (non COVID-COVID): 5.7%, 22.5%. *E. coli* was 8.3% higher amongst the non-COVID-19 patients; 95% CI for difference in proportions (non COVID-COVID): 1.1%, 15.5%

The proportion of *K. pneumoniae,* and *K. aerogenes*, as detected by FilmArray™, was significantly higher in the COVID-19 patients. The difference in proportion of *K. pneumoniae* FilmArray™ results was 12.0% higher amongst the COVID-19 patients then for the non-COVID-19 patients; P=0.005 (Chi Squared), 95% CI for difference in proportions (COVID- non COVID): 4.2%, 19.8%; the difference in proportion of *K. aerogenes* was 6.1% higher in the COVID-19 patients; 95% CI for difference in proportions (COVID- non COVID): 1.8%, 10.5%. The remainder of the organisms detected by FilmArray™ were not significantly different between the COVID-19 and non-COVID-19 patient groups (CIs for proportions included zero).

Table 4.4: Comparison of the frequency of organisms detected by the FilmArray™ in the COVID-19 and non-COVID-19 patients

Name of Organism	**Non-COVID FilmArray™ results* (%) (N=112)**	**COVID FilmArray™ results* (%) (N=157)**	**Difference in proportions (95% CI)**
S. aureus (inc MRSA)	21 (18.8)	16 (10.2)	8.6% (0.0%, 17.2%)
K. pneumoniae	8 (7.1)	30 (19.1)	-12.0% (-19.8%, -4.2%)
K. aerogenes	1 (0.9)	11 (7.0)	-6.1% (-10.5%, -1.8%)
K. oxytoca	5 (4.5)	2 (1.3)	3.2% (-1.0%, 7.4%)
P. aeruginosa	18 (16.1)	12 (7.6)	8.5% (0.5%, 16.5%)
H. Influenzae	23 (20.5)	10 (6.4)	14.1% (5.7%, 22.5%)
E. coli	15 (13.4)	8 (5.1)	8.3% (1.1.%, 15.5%)
E. cloacae	13 (11.6)	12 (7.6)	4.0% (-3.2%, 11.2%)
A. baumannii	0 (0)	4 (2.5)	-2.5% (-5.0%, 0.1%)

S. pneumoniae	4 (3.6)	2 (1.3)	2.3% (-1.6%, 6.2%)
S. marcescens	4 (3.6)	5 (3.2)	0.4% (-4.0%, 4.8%)
Proteus spp.	2 (1.8)	5 (3.2)	-1.4% (-5.1%, 2.3%)
S. agalactiae	2 (1.8)	4 (2.5)	-0.7% (-4.2%, 2.7%)
M. catarrhalis	1 (0.9)	0 (0)	0.9% (-0.8%, 2.6%)
M. pneumoniae	1 (0.9)	0 (0)	0.9% (-0.8%, 2.6%)
S. pyogenes	1 (0.9)	0 (0)	0.9% (-0.8%, 2.6%)
Coronavirus	1 (0.9)	0 (0)	0.9% (-0.8%, 2.6%)
Rhino/enterovirus	5 (4.5)	1 (0.6)	3.9% (-0.1%, 7.9%)
RSV	1 (0.9)	0 (0)	0.9% (-0.8%, 2.6%)
Adenovirus	0 (0)	1 (0.6)	-0.6% (-1.9%, 0.6%)

* excluding multiple instances of same species from single patient

There was no evidence of a difference in the proportion of negative FilmArray™ results between the COVID-19 and non-COVID-19 groups (difference 8.6%, P value (Chi squared test) = 0.158; 95% CI for difference in proportions: -3.2%, 20.4%).

4.4.3.2 Comparison of Conventional Diagnostic Microbiology Results in COVID-19 and non-COVID-19 patients

The conventional diagnostic microbiology results are summarised in Table 4.5. The proportion of *E. coli* and *C. koseri* was significantly higher in the patients who did not have COVID-19. The difference in the proportion of *E. coli* conventional diagnostic microbiology results was 4.0% higher amongst the non-COVID-19 patients than for the COVID-19 patients; 95% CI for difference in proportions (non COVID-COVID): 0.4%, 7.7%. The difference in the proportion of *C. koseri* as reported by conventional diagnostic microbiology was 3.4% higher in the non-COVID-19 patients; 95% CI for difference in proportions (non COVID-COVID): 0.5%, 6.3%.

The proportion of *K. pneumoniae,* as reported by conventional diagnostic microbiology, was significantly higher in the COVID-19 patients; 6.9% higher, 95% CI for difference in proportions: (COVID-non COVID): 1.2%, 12.6%. There was no difference in the proportion of the rest of the organisms as reported by conventional diagnostic microbiology between the COVID-19 and non-COVID-19 patient groups (CIs for proportions included zero).

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Table 4.5: Comparison of the organisms reported by the conventional diagnostic microbiology in the COVID-19 and non-COVID-19 patients

Name of Organism	**Non-COVID conventional diagnostic microbiology results* (%) (N=148)**	**COVID conventional diagnostic microbiology results* (%) (N=146)**	**Difference in proportions (95% CI)**
S. aureus (inc MRSA)	14 (9.5)	6 (4.1)	5.4% (-0.3%,11.1%)
K. pneumoniae	5 (3.4)	15 (10.3)	-6.9% (-12.6%, -1.2%)
K. aerogenes	1 (0.7)	3 (2.1)	-1.4% (-4.0%, 1.3%)
K. oxytoca	3 (2.0)	0 (0)	2.0% (-0.2%, 4.3%)
P. aeruginosa	13 (8.8)	9 (6.2)	2.6% (-3.4%, 8.6%)
H. Influenzae	4 (2.7)	1 (0.7)	2.0% (-0.9%, 5.0%)
E. coli	7 (4.7)	1 (0.7)	4.0% (0.4%, 7.7%)
E. cloacae	3 (2.0)	3 (2.1)	- 0.1% (-3.3%, 3.2%)
Enterobacter sp	1 (0.7)	0 (0)	0.7% (-0.6%, 2.0%)
Acinetobacter spp	0 (0)	2 (1.4)	-1.4%

			(-3.3%, 0.5%)
S. pneumoniae	1 (0.7)	1 (0.7)	0.0% (-1.9%, 1.9%)
S. marcescens	2 (1.4)	1 (0.7)	0.7% (-1.6%, 3.0%)
P. mirabilis	1 (0.7)	3 (2.1)	-1.4% (-4.0%, 1.3%)
M. catarrhalis	1 (0.7)	0 (0)	0.7% (-0.6%, 2.0%)
M. morganii	0 (0)	1 (0.7)	-0.7% (-2.0%, 0.7%)
R. ornitholytica	2 (1.4)	0 (0)	1.4% (-0.5%, 3.2%)
C. koseri	5 (3.4)	0 (0)	3.4% (0.5%, 6.3%)
S. maltophilia	1 (0.7)	0 (0)	0.7% (-0.6%, 2.0%)
Coronavirus	2 (1.4)	0 (0)	1.4% (-0.5%, 3.2%)
Rhino/enterovirus	1 (0.7)	0 (0)	0.7% (-0.6%, 2.0%)
A. fumigatus	1 (0.7)	1 (0.7)	0.0% (-1.9%, 1.9%)

* excluding multiple instances of same species from single patient

The difference in the proportion of negative conventional diagnostic microbiology results in the two groups was 3.4%, P value (Chi squared test) = 0.560 (95% CI for difference in proportions: (-14.8%, 8.0%). There was no evidence of a difference in the proportion of negative conventional diagnostic microbiology results between the COVID-19 and non-COVID-19 groups. Negative results included those reported as ‘no growth’, ‘no significant growth’ and ‘normal respiratory flora’.

4.4.4 Length of hospital stay and FilmArray™ test outcome

The median length of ICU stay for COVID-19 patients was 29 days (IQR 16-44), and in the non-COVID-19 patients it was 19 days (IQR 10-31). There was evidence of a significant difference between the two groups; 10 days higher in those with COVID-19, P=0.0001 (Mann Whitney test), 95% CI for difference in medians (COVID-non COVID): 4.0, 13.0.

The overall length of stay prior to a FilmArray™ test was a median of 15 days for the COVID-19 patients (IQR 8-27) and in the non-COVID-19 groups this was a median of 8.5 days (IQR 5-17). The median number of days in hospital prior to a FilmArray™ test was 6.5 days higher amongst the COVID-19 patients than for the non-COVID-19 patients; P <0.0001 (Mann Whitney test), 95% CI for difference in medians (COVID-non COVID): 3.0, 7.0). The COVID-19 patients were in hospital for a median of 15.5 days (IQR 9.25-27) prior to a positive FilmArray™ result, and for a median of 14 days (IQR 8-26) prior to a negative FilmArrray™ result. There was no evidence of a difference in the median number of days in hospital prior to the test between the positive and negative FilmArray™ groups (median 1.5 days, 95% CI: -2.0, 5.0).

The patients without COVID-19 were in hospital for a median of 7 days prior to a positive FilmArray™ result (IQR 4-17), as reported in Chapter 3; and those with COVID-19 for a median of 15.5 days (IQR 9.25-27). Amongst those with a positive FilmArray™ test, COVID-19 patients were in hospital for significantly more days prior to the test result than non-COVID-19; difference of 8.5 days, P<0.0001 (Mann Whitney), 95% CI for median difference (COVID-non COVID): 4, 11.

4.4.5 Patients with negative FilmArray™ results and positive conventional diagnostic microbiology results

There were two cases where a patient had a negative FilmArray™ result and positive conventional diagnostic microbiology. Cases where FilmArray™ was negative and conventional diagnostic microbiology reported *Candida spp.* were excluded as they were not thought to be clinically relevant.

The two pathogens identified from these two patients were an ESBL-producing *K. pneumoniae* and a *M. morganii.* In the non-COVID-19 adult patients, 3 bacteria (*S. aureus, C. koseri, S. maltophilia*), no resistance genes and 1 fungus *(A. fumigatus)* were reported by conventional diagnostic microbiology where the FilmArray™ remained negative. The bacteria reported by conventional diagnostic microbiology, with a negative FilmArray™ result, in the non-COVID-19 groups were different to those detected in the COVID-19 patients.

4.4.6 Resistance Genes detected

Resistance genes were detected by FilmArray™ in a total of 10 samples representing eight patients; two of these were in repeat tests. Of the eight patients, seven were male and one was female; their median age was 61.5 years (IQR: 56.5-68.65).

MREJ-linked *mec*A/C, which confers methicillin resistance in *S. aureus,* was found in five samples from four patients at three ICUs. *bla*$_{\text{CTX-M}}$ genes, encoding ESBLs, were detected in five samples, from four patients; three of these four patients were on the same ICU. Three of these samples were positive only for *K. pneumoniae*, one for both *K. pneumoniae* and *E. coli,* and the last for *P. aeruginosa*.

In the non-COVID-19 adult patients, MREJ-linked *mec*A/C was the only resistance gene detected and this was detected in one patient. More resistance genes were therefore identified in the COVID-19 group. There was an MRSA prevalence of 0.96% in the non-COVID-19 adults, and of 3.17% in the COVID-19 patients. There was no significant difference in the proportion of *mec*A/C genes between the COVID-19 and non-COVID-19 patient groups (difference 2.21%; 95% CI: -1.4%, 5.8%). The prevalence of *bla*$_{\text{CTX-M}}$ in the COVID-19 group was 3.17%, and it was not detected in the non-COVID-19 adults. The difference in the proportion of *bla*$_{\text{CTX-M}}$ genes, as detected by FilmArray™, was 3.17% higher amongst the COVID-19 group when compared with the non-COVID-19 patients; 95% CI for difference in proportions (COVID-non COVID): 0.1%, 6.2%.

There was one case where conventional diagnostic microbiology reported one *K. oxytoca* isolate with a phenotype suggesting hyper-production of K1 chromosomal

β-lactamase, and FilmArray™ detected the *K. oxytoca* but not the mutation (which is not included in the panel).

4.5: DISCUSSION

4.5.1 Demographics, Sample type and Ventilation status

The age, and sex profiles of these patients are in keeping with those widely reported by other studies in severe COVID-19 disease.[137] The Intensive Care National Audit and Research Centre (ICNARC) report dated up until August 2020 (similar time period to the current study), states that 70% of critically-ill patients with COVID-19 were male.[137] Similarly, in the current study 67.5% of patients were male. The proportion of men/ women in both the COVID-19 and non-COVID-19 groups were not significantly different (P=0.293; 95% CI -5.1%, 16.7%); however, the COVID-19 patients were significantly younger, 59 years versus 66 years, a difference of 7 years (P=0.0015; 95% CI 2, 9). This difference in age is a reflection of the impact of COVID-19. The significant difference seen in median ages may reflect the pressure on the NHS due to the pandemic, with younger patients more likely to survive from COVID-19 and therefore priority for an ICU bed. Emanuel et al. discuss, in detail, the allocation of resources in COVID-19.[138] They make reference to the Italian guidelines which potentially assigned younger patients as a higher priority for intensive care than elderly patients. Certainly, the median age of COVID-19 patients in the current study (59 years) is similar to that reported by ICNARC (60 years).[137] A

UK based study investigating co-infection in COVID-19 patients on ICU also reported their median age as 59 years.[60]

Sample types differed between the two groups, with the non-COVID-19 patients having significantly more BAL samples than the COVID-19 group (4.5% difference; 95% CI for difference in proportions: 0.4%, 8.7%); and significantly more sputum samples for analysis (23.4% difference; P<0.0001; 95% CI 15.9%, 30.9%). However, the COVID-19 patients had significantly more ETT exudate samples than the non-COVID-19 patients (28.8% difference, P<0.0001; 95%CI (19.8%, 37.8%). This is consistent with the ventilation status; with the COVID-19 patient groups comprising of significantly more invasively ventilated patients (21.8% difference, P<0.0001, 95%CI 13.5%, 30.0%). Similarly, significantly more of the non-COVID-19 patients required no ventilation (23.1% difference, P<0.0001; 95%CI 15.7%, 30.4%). Given that COVID-19 is a respiratory disease it would be expected that more of them would require invasive ventilation; whereas the patients without COVID-19 would have been on ICU for various reasons – not just respiratory. It is not surprising that the COVID-19 patients had significantly more ETT exudate samples (and the non-COVID-19 patients had significantly more sputum samples sent) given that 94.4% were invasively ventilated. The non-COVID-19 group may have had higher numbers of BALs because they were clinically more stable and the ICU staff had more time to perform such procedures; furthermore, staff may have been wary of infection risk in COVID-19 patients with procedures performed related to practises in the ICUs that participated.

4.5.2 Organisms as detected by FilmArray™ and comparison with non-COVID-19 patients

Thirty-two repeat FilmArray™ tests were performed (from a total of 157 tests) in the COVID-19 patients. A second test was permitted if it was carried out ≥ 5 days from the first test provided a new or continuing bacterial pneumonia was suspected. One study by Camelena et al. reports the use of repeat FilmArray™ Pneumonia Panel testing in COVID-19 patients.[66] They reported that of the 96 samples tested 67.7% were negative, and of the repeat tests, 60% were negative. In the current study, 43.8% of second tests were negative, however Camelena et al. used BAL samples which represent a superior sample quality compared with the ETT exudate samples used here. Sample quality was discussed in Chapter 3, (3.5.1).

Results of the repeat tests were identical in 17/32 patients. In Chapter 3 there were no instances of new organisms identified on repeat testing (albeit only eight repeat tests were performed), however in nine COVID-19 patients new organisms were identified on repeat testing. The median length of ICU stay for COVID-19 patients was significantly longer (median difference 10 days) compared to those without COVID-19 (P=0.0001, 95% CI: 4.0, 13.0); the median length of stay pre FilmArray™ test in the COVID-19 patients was also significantly longer: 15 days versus 8.5 days (p<0.0001; 95% CI: 3.0, 7.0). A longer ICU stay puts patients at increased risk of developing a HAP/VAP, which could explain the new organisms identified on repeat testing.[106] Of the nine patients where new organisms were detected on second tests, seven had negative first tests and the remaining two second tests detected one additional organism (*E. coli* and *P. aeruginosa*). The median number of days between the two tests in these nine patients was 9 days

(IQR 6-16), supporting the previous statement of length of stay increasing likelihood of HAP/VAP.

Among 157 FilmArray™ tests, representing 126 patients, 55% recorded bacteria or, in two cases, a second virus. The microbiology was typical of HAP/VAP, in being dominated by Enterobacterales.[72] The most prevalent organism being *K. pneumoniae* (24.4%), followed by *S. aureus* (13.0%), and *P. aeruginosa* (9.8%). Organisms typically associated with CAP were much less prominent: nevertheless *H. influenzae* was detected in 10 patients and *S. pneumoniae* in two, with the *H. influenzae* detections being for patients who had been hospitalised anywhere from 1 day to several weeks, and for 16 and 22 days in the case of the *S. pneumoniae* patients. There was no evidence of a difference in the proportion of negative FilmArray™ results between the COVID-19 and non-COVID-19 groups (difference of 8.6%, P=0.158, 95% CI -3.2%, 20.4%).

Despite the dominance of pathogens typically associated with HAP/VAP, the species distribution differed from that seen in the adult patients without COVID-19. Species that showed an increase in the COVID-19 population compared with adult patients without COVID-19 were *K. pneumoniae* (12.0% higher, 95% CI: 4.2%, 19.8%, P=0.005); and *K. aerogenes* (6.1% higher, 95% CI: 1.8%, 10.5%). Those that were less prevalent among COVID-19 patients were *H. influenzae* (14.1% lower, 95% CI: 5.7%, 22.5%; P=0.001); *E. coli* (8.3% lower, 95% CI: 1.1%, 15.5%), and *P. aeruginosa* (8.5% lower, 95% CI: 0.5%, 16.5%). This suggests that as yet unidentified features of the COVID-19 pandemic response or SARS-CoV-2 infection may influence the aetiology of secondary pneumonia in critically ill COVID-19 patients. Possible factors that may play a role include the particular lung pathology/ host defence systems associated with SARS-CoV-2, or differing antimicrobial use

and infection control measures during the COVID-19 pandemic.[139] For example, the impact of alcohol on impaired host defence against *K. pneumoniae* has been reported.[140] Factors may impair the host defence against *K. pneumoniae* in COVID-19. Impaired immune cell function and the damage to the alveolar membrane, is particularly noticeable in COVID-19 patients which could provide increased opportunity for secondary pneumonias with bacteria e.g., *K. pneumoniae* to occur.[141] Baccolini et al., describe the impact of COVID-19 on healthcare-associated infections in a retrospective cohort study.[142] They report an increased incidence of such infections (including VAP), and state that reasons for this include staff being concerned about contracting COVID-19 and therefore reducing the compliance to hygiene precautions and increasing the risk of cross-contamination; poor supply of personal protective equipment; working with a large number of patients and decreased number of staff giving rise to less effective infection control procedures which would ordinarily help limit healthcare-associated infections.[142] These factors may have given rise to the increased incidence of specific Gram-negative HAP/VAP in the current study. Empirical use of antibiotics used to treat Gram-positive co-infections e.g., *S.* aureus (as seen in influenza), or other community-acquired organisms could also explain the numbers of *K. pneumoniae* reported - the following Chapter will focus on antibiotics. The distribution of bacteria seen also differed markedly from that typically seen following influenza, which is dominated by community-acquired pathogens such as *S. pneumoniae* and *H. influenzae,* with *S. aureus* also prominent.[48]

A recent UK-based study by Baskaran et al. reviewed routine laboratory results for 254 COVID-19 patients across seven ICUs for evidence of secondary infections at a range of body sites.[60] Early (<48h after hospital admission) and late-

onset (>48h after hospital admission) secondary infections were distinguished. As in the current study, the organisms responsible for the late onset infections were mainly Gram-negative bacteria including *K. pneumoniae.* This study by Baskaran et al. reported *E. coli* to be the second most common organism responsible for late-onset secondary infection in COVID-19 patients – contrary to the current study.[60] Another study of HAP/VAP in COVID-19, by Camelena et al., also using the FilmArray™ Pneumonia Panel, reported *P. aeruginosa* (14.5%) to be the most prevalent bacteria followed by *S. aureus* (11.5%).[66] This study was single-centre (43 patients) and in France which may explain why *P. aeruginosa* and not *K. pneumoniae* was the most prevalent organism detected. In their small patient group, a true representation of organisms may not have been seen, and the distribution of organisms causing HAP/VAP in the French hospital may have differed to the five UK hospitals. Two further studies from France, and one from China, primarily sampled COVID-19 patients at admission; these predominantly found *S. aureus, Moraxella catarrhalis*, Streptococci and *H. influenzae*.[64,65,143] Kolenda et al. (also reporting use of the FilmArray™ in COVID-19 patients) comment that the sensitivity of the FilmArray™ was 100%, however 60.5% of bacterial targets reported positive using this assay were not found in culture.[67] The issue of differentiating colonisation versus true infection is also acknowledged by the authors, and a similar question is posed by the results of our study.

A large European wide study, consisting of 1050 patients, by Rouze et al. compared prevalence of bacterial infection in ventilated patients with COVID-19 and influenza.[144] Samples used to identify a positive bacterial culture were ETAs, BALs, blood cultures, and pneumococcal or legionella urinary antigen tests. Rouze et al. reported that 58% of patients with COVID-19, and 72% with influenza group had

bacterial infections caused by Gram-positive bacteria, mainly *S. aureus* and *S. pneumoniae.* They also commented that there was significantly less early bacterial infection in COVID-19 patients compared with influenza patients, suggesting that antibiotic use should be carefully evaluated in COVID-19. It is important to note that Rouze et al. looked specifically at early bacterial infections i.e., within 48 hours after intubation, whereas the overall length of stay prior to a FilmArray™ test in the current study was a median of 15 days; this accounts for the differing organism distribution.

Also of note, only two of our 126 patients (1.6%) had an additional respiratory virus whereas 6.7% (7/104) of non-COVID-19 adult patients were positive for respiratory viruses. This last result contrasts with data presented by Zhu et al. and Stanford University USA, where 22.6 - 31.5% of COVID-19 patients had co-infection with other viruses.[143,145] The key difference may be that we specifically examined ICU patients, many of whom had been hospitalised for prolonged periods, whereas other authors examined broader groups of COVID-19 patients with more recent community residency. These studies were conducted up until March 2020, overlapping with the winter respiratory season when more viruses would have been circulating, whereas we recruited later, starting in April 2020. Lockdown measures, social distancing and wearing of face coverings, would also have impacted the viruses reported in our study.

A key finding from this sub-study is the organism distribution as detected by FilmArray™ is different from HAP/VAP in patients without COVID-19, with *K. pneumoniae* and *K. aerogenes* more prominent and *E. coli, H. influenzae* and *P. aeruginosa* less so. Severe COVID-19 patients do not appear to progress to secondary bacterial infection in the same way as do severe influenza patients and do not have the same pathogens.

4.5.3 Organisms as detected by conventional diagnostic microbiology and comparison with non-COVID-19 patients

Of the 146 conventional diagnostic microbiology results, 79 (54.1%) were reported as no growth, no significant growth or normal respiratory flora; similar to the non-COVID-19 patients (50.7%). There was no significant difference in the proportion of these 'negative' results between the two groups (difference 3.4% P=0.560, 95% CI: -14.8%, 8.0%).

Conventional diagnostic microbiology detected 46 non-duplicate bacteria and one fungus (*A. fumigatus).* Among the bacteria identified, the most prevalent was *K. pneumoniae* (31.9%) followed by *P. aeruginosa* (19.1%). As with the non-COVID-19 patients, *Candida spp.* was considered clinically insignificant and was not counted; *E. faecium* was also considered clinically insignificant. *Candida* spp. isolated from the respiratory tract almost invariably represents contamination, rather than infection so the decision was made to exclude these organisms.[103] *E. faecium* is not usually associated with pneumonia and to date there have been 24 case reports describing it, for this reason it was excluded.[146]

The most prevalent organism reported by both conventional diagnostic microbiology and FilmArray™ was *K. pneumoniae.* A UK observational cohort study on co-infection in COVID-19 patients by Baskaran et al. reported that the most common organism identified by culture after 48 hours of hospital admission was *Klebsiella spp.*[60] They suggest that the predominance of these organisms and other Gram-negative organisms reflects nosocomial infection after antibiotic use and prolonged ICU stays; these findings support those of the current study, where the median length of stay for patients was 29 days. Antibiotic use will be discussed in

Chapter 5. Interestingly, a study of late-onset HAP/VAP in COVID-19 patients by Dudoignon et al., likewise predominantly found Gram-negative organisms, as reported by culture, though mostly non-fermenters (57%) not Enterobacterales (28%).[147]

In contrast, a study by Kolenda et al. also reported results on use of the FilmArray™ Pneumonia Panel and conventional diagnostic microbiology in COVID-19 patients where samples had been taken within 48 hours of intubation or in the absence of intubation.[67] They examined 99 low respiratory tract samples, comprising of 38 ETAs, 12 bronchial aspirates, 13 BALs, and 36 mini-BALs. Culture identified 17 bacteria in 15 of 99 samples (15.1%), the most prevalent was *S. aureus* (n=7), followed by *H. influenzae* (n = 4). However, this study represents early infection in hospital stay, whereas the current study analyses patients who have been in hospital beyond 48 hours.

As with the FilmArray™ reports, differences were noted in the organisms reported by conventional diagnostic microbiology between the COVID-19 and non-COVID-19 patients. Species that showed increases in the COVID-19 population compared with adult patients without COVID-19 were *K. pneumoniae* (6.9% higher, 95% CI: 1.2%, 12.6%). Species that showed a decrease in the COVID-19 population compared with non-COVID-19 patients were *E. coli* (4.0% lower, 95% CI: 0.4%, 7.7%); and *C. koseri* (3.4% lower, 95% CI: 0.5%, 6.3%).

As seen with the conventional diagnostic microbiology findings, the FilmArray™ also detected a significant difference in the proportion of *E. coli* and *K. pneumoniae* between the two patient groups. *C. koseri* is not on the FilmArray™ panel, therefore this is reported only by conventional diagnostic microbiology, so no comparisons can be drawn.

When comparing the findings, as reported by FilmArray™ and conventional diagnostic microbiology, in the COVID-19 patient group with those who did not have COVID-19 we must consider sample type and sensitivity of FilmArray™ versus conventional diagnostic microbiology. This was discussed in Chapter 3 (3.5.1). A further important analysis to perform when comparing these groups would be to match for sample types used. A study by Camelena et al. utilising the same panel in COVID-19 patients concludes that it is unclear how long bacterial loads remain detectable after the initiation of appropriate antibiotic treatment in COVID-19 patients on ICU.[66] They suggest that monitoring of bacterial load by molecular tests would be useful in patients with suspected VAP. This needs to be considered when comparing PCR with conventional microbiology.

Anecdotal reports from the participating hospitals in the trial, suggest that ICU clinicians used this new diagnostic platform as a POCT to aid rapid detection, or exclusion, of bacteria in deteriorating patients' lower respiratory tracts, and as a guide to treatment. A caution is that the greater diagnostic yield, compared with culture, may prompt overtreatment of patients who merely carry colonising bacteria. The significance of organisms detected at low population densities (10^4 to 10^5 CFU/ml) remains debatable. More generally, the clinical context must be taken into account and detection of an organism does not necessarily prove that it is causing infection. Balancing these factors will need careful liaison between ICUs, microbiology, and other antimicrobial stewards.

4.5.4 Length of hospital stay pre FilmArray™ test

The ICNARC dataset reports that patients who survived on ICU with confirmed COVID-19 admitted up to 31 August 2020, were on ICU for a median of 22 days (IQR 12, 35).[137] This is longer than the ICNARC reported median stay of 2.4 days (IQR 1.1, 5.0) for patients who survived on ICU pre-pandemic.[148] The study by Camelena et al. also utilising the FilmArray™ Pneumonia Panel in COVID-19 patients, reported a median length of stay on ICU of 11 days (IQR 8, 13) which lies between these two figures.[66] The current study reported the median length of ICU stay for COVID-19 patients was 29 days (IQR 16-44), and in the non-COVID-19 patients it was 19 days (IQR 10-31); it was significantly longer in the COVID-19 patients - P=0.0001, 95% CI: 4.0, 13.0. The present study reports a longer ICU stay (29 days) compared with Camelena et al. (11 days), however the latter study was performed in one centre with a cohort of 43 patients. The ICNARC data reporting a longer median ICU stay of 22 days supports the results of the present study. Long hospital stay leads to increased exposure and risk of hospital-associated infections. COVID-19 was a new disease when this sub-study took place, therefore it is expected that these patients had a longer duration of stay – with uncertainties in how to manage a novel infectious disease in a critically unwell patient group.

Furthermore, there was a significant difference in the median number of days in hospital prior to a FilmArray™ result being positive in the COVID-19 and non-COVID-19 patient groups. The difference was 8.5 days longer in the COVID-19 patients; P<0.0001 95% CI: 4, 11. The COVID-19 patients were in hospital for a longer period of time before a positive FilmArray™ test result, this could be because their overall ICU stay was longer prior to having a test performed, however it should

be remembered that ICU clinicians chose when to perform the FilmArray™ test on COVID-19 patients. Therefore, timing of tests according to clinicians, and study opening dates also need to be taken into account.

There was no significant difference in the median number of days a COVID-19 patient was in hospital prior to a positive or negative FilmArray™ test; difference of 1.5 days, 95% CI: -2.0, 5.0. We know from published literature that the longer a patient is on ICU the higher chance they have of developing a HAP/VAP.[106] This specific finding from the current study does not support this, and there is no other data to my knowledge analysing the length of hospital stay prior to FilmArray™ use, or the length of stay as a predictor of positivity in either COVID-19 or non-COVID-19 patients.

4.5.5 Positive conventional diagnostic microbiology results and negative FilmArray™ test results

There were cases where the FilmArray™ test was negative but organisms were reported by conventional diagnostic microbiology. This occurred in two patients and the two bacteria were ESBL-producing *K. pneumoniae* and *M. morganii.* The ESBL-producing *K. pneumoniae* was reported within 24 hours of another patient who had a FilmArray™ detection of *bla*$_{\text{CTX-M}}$ together with *K. pneumoniae* (with negative conventional diagnostic microbiology) giving rise to the possibility that the samples were confused, of course this cannot be confirmed. The finding of *M. morganii* by conventional diagnostic microbiology is more easily explained because it is not a target on the FilmArray™ Pneumonia Panel Plus. *M. morganii*, although described as an opportunistic pathogen which can be difficult to treat, is not a common cause

of pneumonia so it is absent from the panel.[149] The instances in the non-COVID-19 patients where FilmArray™ was negative and conventional diagnostic microbiology positive were associated with different organisms (*S. aureus, C. koseri, S. maltophilia, A. fumigatus*, RSV, Metapneumovirus and Coronavirus NL63).

On a similar note, there was one instance where conventional diagnostic microbiology identified a *K. oxytoca* isolate with a phenotype suggesting hyper-production of K1 chromosomal β-lactamase; FilmArray™ detected *K. oxytoca* in the sample but does not seek the mutations that cause hyper-production of this enzyme. Therefore, the organism was identified by the FilmArray™ but the mutation was not.

4.5.6 Resistance genes

A total of eight resistance gene sequences were identified by FilmArray™ (excluding duplicate tests).

MREJ-linked *mec*A/C, which confers methicillin resistance in *S. aureus,* was found in five samples from four patients at three ICUs. Conventional diagnostic microbiology reported MRSA in three of the five the samples where FilmArray™ detected it. The other two samples were reported as 'no growth'. *bla*$_{\text{CTX-M}}$ genes, encoding ESBLs, were detected in five samples, from four patients; three of these four patients were on the same ICU. Three of these samples were positive only for *K. pneumoniae*, a frequent host of CTX-M ESBLs, one for both *K. pneumoniae* and *E. coli,* and the last for *P. aeruginosa*, an unlikely host of *bla*$_{\text{CTX-M.}}$ Culture did not detect ESBL-producing organisms in any of the four samples where FilmArray™ found *bla*$_{\text{CTX-M.}}$ together with *K. pneumoniae*. Conventional diagnostic microbiology

did not detect any ESBL-producing organism in a fifth sample, where FilmArray™ found a *bla*$_{\text{CTX-M.}}$ gene and *P. aeruginosa.*

The case where FilmArray™ reported a *P. aeruginosa* and *bla*$_{\textit{CTX-M.}}$ gene, conventional diagnostic microbiology reported *P. aeruginosa* susceptible to ceftazidime and piperacillin-tazobactam. From this susceptibility pattern it is likely that another *bla*$_{\text{CTX-M}}$-positive organism was present, likely at low concentration, and not detected by FilmArray™; *bla*$_{\text{CTX-M}}$ is very rarely seen in *P. aeruginosa* as described by Mushtaq et al.[150]

Amongst the non-COVID-19 adult patients analysed in Chapter 3, MREJ-linked *mec*A/C was the only resistance gene detected and this was detected in one patient. When directly comparing the resistance genes as detected by FilmArray™ in the COVID-19 and non-COVID-19 groups: the MRSA prevalence in the non-COVID-19 adults was 0.96%, and 3.17% in the COVID-19 patients. The difference in the proportion of mecA/C genes, as detected by FilmArray™ was not significant (difference of 2.21%, 95% CI: -1.4%, 5.8%). The prevalence of *bla*$_{\text{CTX-M}}$ in the COVID-19 group was 3.17%, and it was not detected in the non-COVID-19 adults (difference 3.17%; 95% CI: 0.1%, 6.2%). The numbers are low here due to overall low prevalence of these genes and a larger sample size would be needed to definitively determine if there is a significant difference between the two groups.

The study by Kolenda et al., reported no resistance genes, among their 99 COVID-19 patients, detected by FilmArray™ or routine culture.[67] Reasons for this may be the smaller sample size or different location (France instead of England). Furthermore, the timing of these samples in terms of hospital stay is not known. Therefore, if they were in hospital <48 hours the prevalence of resistance genes would be less as it is know that the longer they stay the more likely it is to isolate a

MDR pathogen.[136] Patients in the current study fit into this group of patients who have a longer ICU stay.

The study by Camelena et al. does however report the finding of resistance genes in their group of 43 COVID-19 patients on ICU.[66] They report the FilmArray™ detected two 2 *bla*$_{CTX-M}$ genes, one *bla*$_{VIM}$ carbapenemase, and two *S. aureus* methicillin-resistance genes. Conventional diagnostic microbiology (i.e., disc diffusion or VITEK 2) did not always report these genes (as in the current study); detecting one of the *bla*$_{CTX-M}$ genes and both MRSA isolates. However, culture did not detect the other resistance genes: one *bla* $_{CTX-M}$ and one *bla* $_{VIM}$. The authors state that this helps to highlight the limitation of molecular tests to predict phenotypic susceptibility. In addition, it is important to note that culture may have missed resistant organisms. These findings are similar to those reported from the current study.

4.5.7 Summary of Key Findings

The organism distribution in critically ill COVID-19 patients with a suspected HAP/VAP as detected by FilmArray™ was different from HAP/VAP patients without COVID-19, with *K. pneumoniae* and *K. aerogenes* more prominent and *P. aeruginosa, H. influenzae* and *E. coli* less so. Additional viral infections were rare. Conventional diagnostic microbiology supported the finding of a significant increase in *K. pneumoniae* amongst COVID-19 patients when compared with non-COVID-19 patients. The length of stay amongst COVID-19 patients prior to having a FilmArray™ test performed was significantly longer compared with non-COVID-19

patients. Resistance genes were reported more frequently in the COVID-19 patients.

CHAPTER 5

ANTIBIOTIC PRESCRIBING AND PRESCRIBING ALGORITHM ADHERENCE IN ICU HAP/VAP PATIENTS

5.1: INTRODUCTION

Antibiotic prescribing for HAP/VAP is often empiric and broad spectrum.[70] For example, NICE (2019) recommends that patients with suspected HAP who have severe symptoms/ higher risk of resistant organisms, are started on intra-venous antibiotics, listing the following antibiotics as options: piperacillin/ tazobactam, ceftazidime, ceftriaxone, cefuroxime, meropenem, ceftazidime-avibactam, or levofloxacin.[70] NICE define 'higher risk of resistance' to include 'symptoms or signs starting more than five days after hospital admission, relevant comorbidity such as severe lung disease or immunosuppression, recent use of broad-spectrum antibiotics, colonisation with multidrug-resistant bacteria, and recent contact with a health or social care setting before current admission'.

Patients with COVID-19 often receive antibiotics in the absence of bacterial co-infection as reported by Lansbury et al. in a systematic review and meta-analysis.[83] They included 30 studies published between 01/01/20 to 17/04/20, with 3834 patients in total, reporting that a low proportion of COVID-19 patients had a bacterial co-infection: overall 7% of patients in hospital, increasing to 14% when looking at ICU patients only. In terms of antibiotic use, they report that in 10 studies, over 90% of patients received empirical antibiotics. A meta-analysis published in 2021 by Langford et al., describing antibiotic prescribing during the first six months of the pandemic, reported that amongst 30,623 patients the prevalence of antibiotic

prescribing was 74.6%.[151] Furthermore they reported the co-infection rate for patients was 8.6%, highlighting the overuse of antibiotics in this patient group.

This chapter examines antibiotic prescribing in both the COVID-19 sub-study patients, and the patients without COVID-19 from the INHALE trial, comparing the two groups in terms of broad and narrow spectrum antibiotics usage. A key part of this chapter describes how conventional diagnostic microbiology results and FilmArray™ Pneumonia Panel Plus results translate in antibiotic prescribing. With this, we can set out to answer the question of whether the microbiological results lead to a change in the antibiotics prescribed for a HAP/VAP. A prescribing algorithm was specifically written for the trial to help clinicians translate FilmArray™ results into antibiotic treatment decisions. This bespoke algorithm and adherence to it in both patient groups is evaluated in this chapter.

5.2: AIMS

1. To describe, antibiotic usage for HAP/VAP in COVID-19 patients in comparison with non-COVID-19 patients, and the effect of the FilmArray™ system on prescribing.

2. To describe adherence to the prescribing algorithm in the COVID-19 cohort in comparison with the non-COVID-19 cohort.

5.3: METHODS

5.3.1: Contribution

Data was collected by the research teams at the sites (Appendix 4). I contributed to writing and design of the prescribing algorithm, and led the algorithm negotiations with sites. I worked with the Norwich Clinical Trial Unit to check and clean the data prior to analysis. Queries were raised with sites on any spurious datapoints. Once data input was complete, the data pages were locked so no further changes could be made. I developed the statistical analysis plan, and performed all analyses in this chapter.

5.3.2: Design and development of draft 'Master Prescribing Algorithm'

A prescribing algorithm was developed for INHALE (Appendix 3). The FilmArray™ results can be complex and given that the platform was used at the bedside by clinicians, often with little or no microbiological input, the INHALE team thought it prudent to write a prescribing algorithm to aid treatment decisions in real time.

Firstly, the 18 bacteria and 9 viruses detected by the FilmArray™ Pneumonia Panel Plus (Table 2.1) were listed and this was extended to include all possible combinations of organism groups. The next step was to identify the narrowest-spectrum antibiotics, with HAP and VAP indications, able to cover these pathogens and combinations. This was further extended to include cases where the FilmArray™ detected particular resistance genes, identifying agents that evaded

these mechanisms. Since the FilmArray™ seeks only a narrow range of resistance genes the extent to which preferred narrow-spectrum agents were compromised by resistance was considered, as reflected by publications and the BSAC national surveillance data for lower respiratory tract infections, avoiding combinations of agents and pathogens where a >15% resistance rate was recorded.[15] Owing to their good efficacy and low toxicity β-lactam antibiotics were preferred in general but, for each pathogen, or combination of pathogens and resistances, alternatives were identified for patients with mild/ moderate and severe penicillin allergy. Cephalosporins, carbapenems and monobactams were allowed in the former instance, but not the latter.

Key factors requiring consideration by the prescribing physician were noted on the front page of the 'Master Prescribing Algorithm' (Appendix 3). These included: (i) taking into account the patient's renal function and adapting HAP/VAP treatment accordingly; (ii) considering if the patient had an infection at some other site necessitating other antibiotics e.g., meningitis or intra-abdominal infection; (iii) pointing out that the FilmArray™ does not detect several relevant pathogens, most notably *Stenotrophomonas maltophilia* but also *Citrobacter* spp.

This Master Prescribing Algorithm was used as a starting point for discussion at the 12 INHALE trial sites, which led to negotiation of local adaptations (Appendix 6). Consultation involved face to face meetings and telephone discussions with site medical microbiologists, ICU pharmacists, and ICU clinicians. Once the algorithm was agreed at each site, it was open to local interpretation so other treatment options were feasible and we did not insist that sites adhere rigidly to their local version; this is because its purpose was as a guide especially on those ICUs without daily microbiology input.

Once algorithm variants had been agreed with senior site ICU physicians and medical microbiologists, we provided training to a wider group of site ICU staff, including both physicians and research nurses on its use. This was done face-to-face, using example FilmArray™ reports, and with group teaching on how to interpret the algorithm, and which antibiotics to use. A teaching PowerPoint with further examples was sent by email to each site (Appendix 7).

A laminated copy of the algorithm was kept beside each installed platform for quick reference, with a copy to be handed to the prescribing clinician with every FilmArray™ result. Research nurses were taught to help interpret it, if needed. A copy of the FilmArray™ report was either uploaded to the electronic patient notes or written in the notes.

5.3.3: Data Collection

The terms ‘broad-spectrum’ and ‘narrow-spectrum’ were used to record antibiotics prescribed. The definitions used to describe these terms are those from the INHALE trial, decided upon by the medical microbiologists and scientists in the trial team. Broad-spectrum antimicrobials were defined as *an antibiotic that has inherent activity against wild-type strains (i.e., those without acquired resistance) of at least three of the following groups: i)* Streptococci, inc. *S. pneumoniae; ii) S. aureus*, where beta-lactamase production is now taken as the wild phenotype, being so ubiquitous*; iii)* Enterobacteriales, including *Enterobacter* spp. AND *Klebsiella* spp.*; iv)* Non fermenters, including *P. aeruginosa.* Any antimicrobial that targets two groups or less was classified as narrow spectrum. Where a combination was prescribed, broad versus narrow-spectrum was judged for the combination rather than single

agents. Cases where at least one of the antimicrobials prescribed in a combination is a broad-spectrum were classified as broad spectrum. Combinations of two or more narrow-spectrum agents were considered individually. Appendix 8 lists the individual agents and common combinations as broad or narrow-spectrum.

Antibiotic courses prescribed were classified as broad or narrow-spectrum as both individual agents and as combination courses. Individual agents were defined as the single antibiotic agent prescribed, and the spectrum of that agent was recorded. Individual agents often overlapped with each other and were prescribed in combination – hence the spectrum of the combination was also recorded and analysed.

Antibiotic decisions after the FilmArray™ was performed were divided into started antibiotics = patient was off antibiotics pre FilmArray™, but antibiotics were started after result was available; stopped antibiotics = patient was on antibiotics pre FilmArray™, but they were stopped after result was available; continued the same antibiotics pre and post FilmArray™; and stayed off antibiotics = patient was off antibiotics pre FilmArray™ and remained off after result was available. The other two categories were escalated antibiotics, and de-escalated antibiotics. The definition of escalated antibiotics included instances where the spectrum was changed from narrow to broad; another agent was added to the combination; the spectrum of the new antibiotic was wider e.g., switching from piperacillin-tazobactam to meropenem. The definition of de-escalated antibiotics included instances where the spectrum was changed from broad to narrow; one agent was removed from the combination; the spectrum of the new antibiotic was narrower e.g., switching from meropenem to piperacillin-tazobactam.

The total number of antibiotic courses prescribed for each patient in the seven days prior to the sputum sample being taken for analysis were recorded, and these were categorised into broad or narrow-spectrum. Patients could be on a mixture of broad and narrow-spectrum antibiotics/ purely on broad-spectrum agents/ purely on narrow-spectrum agents in this seven day period. The number of each course of broad and narrow-spectrum agents were counted and recorded. The spectrum of antibiotic the patient was on patient was on i.e., broad or narrow-spectrum, at the time the sputum sample was taken and at 24 hours after the FilmArray™ was performed, were recorded. A 24 hours timepoint was selected to enable any microbiological input to take place post FilmArray™ test, e.g., ward rounds/ consults.

Adherence to the prescribing algorithm in the COVID-19 patients and the non-COVID-19 patients was captured by reviewing the antibiotic decisions 24 hours post FilmArray™ test and comparing those to the suggested antimicrobial in the site specific algorithm. FilmArray™ instances were then divided up into those where the antibiotics adhered to the algorithm and those which did not, and they were further examined. This included analysis of any other infections the patients had recorded (identified by conventional diagnostic microbiology), and indication for the antibiotic prescription (aside from pneumonia). An antibiotic regimen was considered adherent if it was exactly the same as that specified on the site specific algorithm.

Data collected included: baseline data; microbiology/FilmArray™ results; in addition to the prescribing data (categorised after collection into broad and narrow-spectrum antibiotics); adherence to the prescribing algorithm; other infections recorded, and indications for antibiotics. Data was analysed for both the non-COVID-19 group and the COVID-19 sub-study patients, to enable comparison

between these two groups. A bespoke REDCap database was used for data collection and storage.[100]

The INHALE trials team granted permission to download the relevant data from the REDCap database into an Excel spreadsheet.

5.3.4: Statistical analysis plan

Stata v.16 was used for statistical analyses. Patient demographics, and initial antibiotics prescribed (this included any antibiotics prescribed in the seven days prior to the respiratory tract specimen being taken for FilmArray™ analysis): categorised into either not on antibiotics, broad or narrow-spectrum, were described using frequency (%). The mean number of broad-spectrum and narrow-spectrum antibiotics prescribed in the seven day period described were compared using a T-test. P-values from Chi-squared or Fishers exact tests, and 95% CI were used to compare the proportion of patients not on antibiotics and those on broad or narrow-spectrum antibiotics, across the ten adult sites. McNemar's test was used to compare the antibiotics prescribed for the patient at the point when the specimen was taken for analysis and at 24 hours after the FilmArray™ was performed.

Adherence to the prescribing algorithm was defined as an exact match to the site specific algorithm, and was described using frequency (%) in the COVID-19 patients and the non-COVID-19 patients. These proportions for the two groups were compared using P-values from Chi-squared or Fishers exact tests, and 95% CI. Analysis of patients who had other infections recorded (identified by routine microbiology), and those who had another indication for the antibiotic prescription

(i.e., not pneumonia) were also described using frequency (%) and proportions compared using P-values from Chi-squared or Fishers exact tests, and 95% CI.

Prescribing decisions following FilmArray™ results (i.e., whether antibiotics were stopped/ escalated/ de-escalated/ started (if off antibiotics pre FilmArray™)/ continued/ remained off antibiotics) were described using frequency (%). These prescribing decisions at 24 hours after the FilmArray™ test amongst COVID-19 patients was described using frequency (%). These proportions for the two groups were compared using P-values from Chi-squared or Fishers exact tests, and 95% CI. The same tests were used to compare prescribing decisions at 24 hours after the FilmArray™ tests in COVID-19 patients with negative and positive FilmArray™ test results; and to compare prescribing decisions at 24 hours after the FilmArray™ tests in both COVID-19 and non-COVID-19 patients with positive and negative FilmArray™ test results.

For any prescribing decisions made, patients included in the non-COVID-19 group were only those randomised to the intervention arm as only these patients had a FilmArray™ result on which the clinicians acted. All patients in the COVID-19 sub-study had a FilmArray™ performed as part of the intervention.

Any missing data has been accounted for in the analyses presented.

5.4: RESULTS

5.4.1 Antibiotics prescribed for HAP/VAP in COVID-19 patients, and comparison with those used in non-COVID-19 patients

All patients recruited to the COVID-19 sub-study (n=146) and adults without COVID-19 from the INHALE trial (n=126) were included in these analyses. Demographic data for these two groups is compared in Table 4.1 (Chapter 4).

A summary of the individual/ combination antibiotics prescribed (and their spectrum) in the seven days prior to the respiratory tract specimen being taken for FilmArray™ analysis, for COVID-19 patients, is shown in Table 5.1. Antibiotics prescribed prior to repeat tests are also included in this table.

Table 5.1: Antibiotic courses prescribed in the seven days prior to specimen taken for analysis in COVID-19 patients

	Individual Antibiotic Courses* (N=328)		**Combination Antibiotic Courses* (N=205)**	
	Broad Spectrum (%)	**Narrow Spectrum (%)**	**Broad Spectrum (%)**	**Narrow Spectrum (%)**
Total number of antibiotic courses	198 (60.4)	130 (39.6)	152 (74.1)	53 (25.9)
Mean number of antibiotic courses per patient	1.96	1.67	1.54	1.08
Standard deviation	1.08	0.75	0.76	0.28

* Excludes missing antibiotic data pre specimen for 3 patients (1 patient had missing data for repeat testing too). Therefore, represents 153 patient results, not 157. Includes repeat tests.

The maximum number of individual antibiotic courses prescribed for a patient in this seven day period prior to sputum sample being taken for analysis was six, and the minimum was zero. The difference in mean number of individual broad versus narrow-spectrum individual antibiotic courses prescribed in the seven days pre specimen being taken was 0.29, this was statistically significant; P=0.045 (t-test); 95% CI 0.072, 0.573; with the mean number of broad-spectrum antibiotic courses prescribed being higher than the mean number of narrow-spectrum antibiotic courses. When reviewing the spectrum of the combination of antibiotics prescribed in a course (as opposed to the spectrum of the individual agents): a total of 205

combination courses were prescribed, of which 74.1% were broad-spectrum courses and 25.9% were narrow-spectrum. The difference in mean number of broad versus narrow-spectrum combined antibiotic courses prescribed in the seven days pre specimen being taken was 0.46, this was statistically significant; P=0.0001 (t-test); 95% CI 0.238, 0.682.

A comparison of the spectrum of antibiotic combinations prescribed at the point when the specimen was taken for analysis and at 24 hours after the FilmArray™ was performed, for COVID-19 patients, and is shown in Table 5.2. There was no evidence of a difference in the proportion of patients not on antibiotics, on narrow-spectrum antibiotics, or on broad-spectrum antibiotics between the two timepoints (McNemar's test: CIs for proportions included zero).

Table 5.2: Comparison of antibiotics prescribed pre and post FilmArray™ in COVID-19 patients

	When sample was taken (N=142)* (%)	**24 hrs after FilmArray™ was performed (N=142)* (%)**	**Difference in proportions (95% CI)**
Number of instances when patients not on antibiotics	42 (29.6)	45 (31.7)	-2.1% (-7.8%, 12.1%)
Number instances when patients on narrow spectrum antibiotics	12 (8.5)	15 (10.6)	-2.1% (-3.9%, 8.2%)
Number of instances when patients on broad spectrum antibiotics	88 (61.9)	82 (57.7)	4.2% (-14.5%, 6.0%)

*Excludes missing antibiotic data pre specimen for 4 episodes, and an additional 10 missing antibiotic data points post FilmArray™; 1 patient RIP after FilmArray™, so no antibiotics recorded. Includes repeat tests. Paired data analysed resulting in a total of 142 instances.

The same analyses were also performed for non-COVID-19 patients. Table 5.3 summarises the antibiotic courses prescribed in the seven days prior to the respiratory tract specimen being taken for FilmArray™ analysis.

Table 5.3: Antibiotic courses prescribed in the seven days prior to specimen taken for analysis in non-COVID-19 patients

	Individual Antibiotic Courses* (N=351)		**Combination Antibiotic Courses* (N=230)**	
	Broad Spectrum (%)	**Narrow Spectrum (%)**	**Broad Spectrum (%)**	**Narrow Spectrum (%)**
Total number of antibiotic courses	186 (53.0)	165 (47.0)	169 (73.5)	61 (26.5)
Mean number of antibiotic courses per patient	3.44	3.11	1.40	1.15
Standard deviation	0.91	0.74	0.69	0.36

The maximum number of individual antibiotic courses prescribed in this seven day period was seven, and the minimum was zero. The difference in mean number of broad versus narrow-spectrum individual antibiotic courses prescribed in the seven days pre specimen being taken was 0.33, this was statistically significant; P=0.0186 (t-test); 95% CI -0.604, -0.056. When reviewing the spectrum of the combination of antibiotics prescribed in a course (as opposed to the spectrum of the individual agents): a total of 230 combination courses were prescribed, of which 73.5% were broad-spectrum courses and 26.5% were narrow-spectrum. The difference in mean number of broad versus narrow-spectrum combined antibiotic courses prescribed in the 7 days pre specimen being taken was 0.25, this was statistically significant; P= 0.0137 (t-test); 95% CI 0.052, 0.448. There was no significant difference when

comparing the proportion of combination broad-spectrum antibiotic courses prescribed (in the 7 days pre specimen being taken) between the COVID-19 and non-COVID-19 patients: difference 0.6%, 95% CI -7.68%, 8.88 %.

A comparison of the spectrum of the combination of antibiotics prescribed at the point when the specimen was taken for analysis and at 24 hours after the FilmArray™ was performed for patients without COVID-19, is shown in Table 5.4. There was no evidence of a difference in the proportion of patients not on antibiotics, on narrow-spectrum antibiotics, or on broad-spectrum antibiotics between the two timepoints (McNemar's test: CIs for proportions included zero).

Table 5.4: Comparison of antibiotics prescribed pre and post FilmArray™ in non-COVID-19 patients

	When sample was taken (N=79)* (%)	**24 hrs after FilmArray™ was performed (N=79)* (%)**	**Difference in proportions (95% CI)**
Number of instances when patients not on antibiotics	9 (11.4)	6 (7.6)	3.8% (-12.5%, 4.9%)
Number of instances when patients on Narrow spectrum	9 (11.4)	6 (7.6)	3.8% (-11.6%, 4.0%)
Number of instances when patients on Broad spectrum	61 (77.2)	67 (84.8)	-7.6% (-2.8%, 18.0%)

*Includes patients randomised to the FilmArray™ arm only to enable a comparison to be made with the COVID-19 patients, all of whom had a FilmArray™ test performed. Repeat tests included. Paired data analysed resulting in a total of 79 instances.

Antibiotic prescribing (where spectrum is shown it refers to the spectrum of the combination of antibiotics) was compared between the COVID-19 and non-COVID-19 patients, both at the point when the specimen was taken for analysis and at 24 hours after the FilmArray™ was performed. This is shown in Tables 5.5 and 5.6.

Table 5.5: Comparison of antibiotics prescribed between non-COVID-19 and COVID-19 patients at time when samples were taken

	When sample was taken (non-COVID-19 patients) (N=153)* (%)	**When sample was taken (COVID-19 patients) (N=153)^ (%)**	**Difference in proportions (95%CI)**	**P value**
Not on Antibiotics	13 (8.5)	52 (34.0)	-25.5% (-34.2%, -16.8%)	<0.0001
On Narrow spectrum	20 (13.1)	12 (7.8)	5.3% (-1.5%, 12.2%)	0.130
On Broad spectrum	120 (78.4)	89 (58.2)	20.2% (10.0%, 30.4%)	0.0001

* Repeat tests included.

^ Excludes missing antibiotic data pre specimen for 4 episodes. Repeat tests included.

Table 5.6: Comparison of antibiotics prescribed between non-COVID-19 and COVID-19 patients at 24 hours after FilmArray™ was performed

	24 hr after FilmArray™ was performed (non-COVID-19 patients) (N=79)^ (%)	**24 hr after FilmArray™ was performed (COVID-19 patients) (N=142)* (%)**	**Difference in proportions (95% CI)**	**P value**
Not on Antibiotics	6 (7.6)	45 (31.7)	-24.1 % (-33.7%, -14.5%)	<0.0001
On Narrow spectrum	6 (7.6)	15 (10.6)	-3.0% (-10.7%, 4.7%)	0.467
On Broad spectrum	67 (84.8)	82 (57.7)	27.1% (15.8%, 38.4%)	<0.0001

^ Includes patients randomised to the FilmArray™ arm only to enable a comparison to be made with the COVID-19 patients, all of whom had a FilmArray™ test performed. Repeat tests included.

* Excludes 14 missing antibiotic data points; 1 patient RIP after FilmArray™. Repeat tests included.

Overall, there was evidence of an association between both the antibiotics prescribed (i.e., not on antibiotics, narrow-spectrum antibiotics, broad-spectrum antibiotics) at the time when samples were taken and COVID-19 versus non-COVID-19 groups: Chi squared test P<0.0001; and between antibiotics prescribed at 24

hours after the FilmArray™ was performed and COVID-19 versus non-COVID-19: Chi squared test P<0.0001.

When evaluating this association in more detail: significantly more COVID-19 patients were not on antibiotics compared with non-COVID-19 patients as the time of sampling (difference in proportion of 25.5%, 95% CI 16.8%, 34.2%; P<0.0001 Chi squared test). Significantly more non-COVID-19 patients were on broad-spectrum antibiotics at the time of sampling (difference in proportion of 20.2%; 95% CI: 10.0%, 30.4%; P = 0.0001 Chi squared test). There was no evidence of a difference in the proportion of patients on narrow-spectrum antibiotics between the two groups (CIs for proportions included zero). Significantly more of the COVID-19 patients were less likely to receive treatment at the 24 hour point after the FilmArray™ test was performed (difference in proportion of 24.1%; 95% CI 14.5%, 33.7%; P<0.0001 Chi squared test). Additionally, more of the non-COVID-19 patients received treatment with broad-spectrum antibiotics at 24 hours after the FilmArray™ test had been performed (difference in proportion of 27.1%; 95% CI 15.8%, 38.4%; P<0.0001 Chi squared test). There was no evidence of a difference in the proportion of patients on narrow-spectrum antibiotics between the two groups (CIs for proportions included zero).

5.4.2 Adherence to the prescribing algorithm in COVID-19 and non-COVID-19 patients

Amongst the COVID-19 patients, the prescribing algorithm was adhered to in 35.9% of FilmArray™ instances (represents 51/142 FilmArray™ instances). Amongst the non-COVID-19 patients, the prescribing algorithm was adhered to in

35.5% of FilmArray™ instances (represents 27/76 FilmArray™ instances). This data is summarised in Table 5.7, along with a comparison of the two groups. There was no evidence of a difference in the proportion of FilmArray™ instances with adherence to the algorithm between the two groups (CIs for proportions included zero).

Table 5.7: Comparison of algorithm adherence between COVID-19 and non-COVID-19 patients

	COVID-19 (N=142)* (%)	**Non-COVID-19 (N=76)^ (%)**	**Difference in proportions (95% CI)**	**P value**
Number of FilmArray™ instances which adhered to algorithm	51 (35.9)	27 (35.5)	0.4% (-13.6%, 13.0%)	0.965

* Excludes 14 missing antibiotic data points; 1 patient RIP after FilmArray™. Repeat tests included.

^ Excludes 3 instances where FilmArray™ report not uploaded to database.

To examine adherence to the prescribing algorithm amongst the non-COVID-19 patients, two further analyses were performed. Firstly, any other infections the patients had recorded (i.e., details of pathogens identified by routine microbiology) were evaluated and compared for the group who adhered to the algorithm (n=49) and the group who did not adhere (n=27). This additional infection data was not recorded for the COVID-19 patients as shown in Table 2.2. There was no significant difference in the proportion of non-COVID-19 patients who had other infections listed

between the two groups (CI for proportions included zero): 11.5% who adhered to the algorithm and 14.6% who did not adhere to the algorithm had other infections listed (difference of 3.1%).

Secondly, indication for antibiotic prescription (aside from pneumonia) which may explain non-adherence to the algorithm was evaluated. Again, this data was not recorded for the COVID-19 patients as shown in Table 2.2. There was no significant difference in the proportion of non-COVID-19 patients who had other indications listed for antibiotics between the two groups (CI for proportions included zero): 44.4% who adhered to the algorithm and 44.9% who did not adhere to the algorithm had other infections listed (difference of 0.5%).

5.4.3 Effect of the FilmArray™ results on prescribing decisions in COVID-19 and non-COVID-19 patients

Antibiotic decisions at 24 hours after the FilmArray™ was performed were compared in the COVID-19 and non-COVID-19 groups. Decisions were recorded as: started antibiotics (if the patient was not on them before the FilmArray™ was performed); escalated antibiotics; de-escalated antibiotics; stopped antibiotics; continued same antibiotics; and stayed off antibiotics. Table 5.8 shows these results.

Table 5.8: Comparison of antibiotic decisions in COVID-19 and non-COVID-19 patients, 24 hours after FilmArray™ performed

	Number of decisions in COVID-19 group N=142* (%)	**Number of decisions in Non-COVID-19 group N=79^ (%)**	**Difference in proportions (95% CI)**
Started Antibiotics	20 (14.1)	7 (8.9)	5.2% (-3.3%, 13.7%)
Escalated Antibiotics	16 (11.3)	16 (20.2)	-8.9% (-19.2%, 13.7%)
De-escalated Antibiotics	7 (4.9)	8 (10.1)	-5.2% (-12.7%, 2.3%)
Stopped Antibiotics	24 (16.9)	3 (3.8)	13.1% (5.6%, 20.6%)
Continued same Antibiotics	54 (38.0)	42 (53.2)	-15.2% (-28.8%, -1.6%)
Stayed off Antibiotics	21 (14.8)	3 (3.8)	11% (3.8%, 18.2%)

* Excludes 14 missing antibiotic data points; 1 patient RIP after FilmArray™. Repeat tests included.

^ Includes patients randomised to the FilmArray™ arm only to enable a comparison to be made with the COVID-19 patients, all of whom had a FilmArray™ test performed. Repeat tests included.

There was an association between antibiotic decisions and COVID-19 versus non-COVID-19 groups: Fishers exact test P<0.0001. Significantly more of the non-COVID-19 patients continued the same antibiotics post FilmArray™ result when compared with COVID-19 patients (difference in proportion of 15.2%; 95% CI 1.6%,

28.8%). The FilmArray™ results led to both, significantly more of the COVID-19 patients stopping antibiotics (difference in proportion of 13.1%; 95% CI 5.6%, 20.6%); and significantly more of the COVID-19 patients remaining off antibiotics (difference in proportion of 11.0%; 95% CI 3.8%, 18.2%). The remainder of the antibiotic decisions were not significantly different between the COVID-19 and non-COVID-19 groups (CIs for difference in proportions included zero).

5.4.4 Effect of negative and positive FilmArray™ results on prescribing decisions in non-COVID-19 and COVID-19 patients

The effects of a negative or positive FilmArray™ result on antibiotic prescribing at 24 hours after the FilmArray™ was performed were compared in non-COVID-19 and COVID-19 patients. Results are summarised in Tables 5.9 and 5.10.

Table 5.9: Comparison of antibiotic decisions in COVID-19 and non-COVID-19 patients with a negative FilmArray™ result at 24 hours after FilmArray™ performed

	Number of decisions in COVID-19 groups with negative FilmArray™ results (%) N=65*	**Number of decisions in non-COVID-19 groups with negative FilmArray™ results (%) N=29^**	**Difference in proportions (95% CI)**
Started Antibiotics	5 (7.7)	2 (6.9)	0.8% (-10.5%, 12.1%)
Escalated Antibiotics	4 (6.2)	2 (6.9)	-0.7% (-11.6%, 10.2%)
De-escalated Antibiotics	5 (7.7)	2 (6.9)	0.8% (-10.5%, 12.1%)
Stopped Antibiotics	14 (21.5)	2 (6.9)	14.6% (1.0%, 28.2%)
Continued same Antibiotics	21 (32.3)	20 (69.0)	-36.7% (-57.0%, -16.4%)
Stayed off Antibiotics	16 (24.6)	1 (3.4)	21.2% (8.8%, 33.6%)

^ Intervention arm patients only. Excludes 3 instances where FilmArray™ report not uploaded to database. * Excludes 9 FilmArray™ results which have no associated antibiotic data recorded. Repeat tests included

Table 5.10: Comparison of antibiotic decisions in COVID-19 and non-COVID-19 patients with a positive FilmArray™ result at 24 hours after FilmArray™ performed

	Number of decisions in COVID-19 groups with positive FilmArray™ results N=77 (%)*	**Number of decisions in non-COVID-19 groups with positive FilmArray™ results N=47 (%)^**	**Difference in proportions (95% CI)**
Started Antibiotics	15 (19.4)	5 (10.6)	8.8% (-3.7%, 21.3%)
Escalated Antibiotics	12 (15.6)	14 (29.8)	-14.2% (-29.6%, 1.2%)
De-escalated Antibiotics	2 (2.6)	6 (12.8)	-10.2% (-20.3%, 0.0%)
Stopped Antibiotics	10 (13.0)	1 (2.1)	10.9% (2.3%, 19.4%)
Continued same Antibiotics	33 (42.9)	19 (40.4)	2.5% (-15.4%, 20.4%)
Stayed off Antibiotics	5 (6.5)	2 (4.3)	1.9% (-5.7%, 10.2%)

* Excludes 9 FilmArray™ results which have no associated antibiotic data recorded. Repeat tests included

^ Intervention arm patients only. Excludes 3 instances where FilmArray™ report not uploaded to database.

There was an overall association between antibiotic decisions in negative FilmArray™ results and COVID-19 versus non-COVID-19: Fishers exact test P= 0.010; and also between positive FilmArray™ results and COVID-19 versus non-COVID-19: Fishers exact test P= 0.022.

A negative FilmArray™ result led to significantly more of the non-COVID-19 patients being continued on the same antibiotics (difference in proportions 36.7%; 95%CI 16.4%, 57.0%; P=0.001). However, amongst the COVID-19 patients a negative FilmArray™ result led to significantly more antibiotics being stopped (difference in proportions 14.6%; 95% CI 1.0%, 28.2%); and also significantly more patients remaining off antibiotics (difference in proportions 21.2%; 95% CI 8.8%, 33.6%), when compared with the non-COVID-19 patients. A positive FilmArray™ result also led to significantly more of the COVID-19 patients stopping antibiotics (difference in proportions 10.9%; 95% CI 2.3%, 19.4%). The remainder of the antibiotic decisions in the negative and positive FilmArray™ groups were not significantly different between the COVID-19 and non-COVID-19 patients (CIs for difference in proportions included zero).

When analysing the COVID-19 patients alone, significantly more patients were started on antibiotics following a positive FilmArray™ test result compared with a negative FilmArray™ test result (difference in proportion of 11.7%; 95% CI 0.7%, 22.7%); and significantly more patients remained off antibiotics following a negative FilmArray™ test result (difference in proportion of 18.1%; 95% CI 6.3%, 29.9%; P=0.003). The remainder of the antibiotic decisions were not significantly different between the positive and negative FilmArray™ groups amongst the COVID-19 patients (CIs for difference in proportions included zero).

5.5: DISCUSSION

5.5.1 Antibiotics prescribed pre-FilmArray™ test in ICU patients

Amongst the COVID-19 patients, there was evidence of significantly more broad-spectrum combination antibiotic prescriptions in the seven days pre specimen taken compared with narrow-spectrum combination agents: P=0.0001; 95% CI 0.238, 0.682. Literature to date suggests there is high antibiotic use in COVID-19 patients. One key paper is by Lansbury et al., describing in a systematic review and meta-analysis looking at co-infections in COVID-19 patients, relevant studies published between 1 January 2020 to 17 April 2020.[83] They included 30 studies, with 3834 patients in total, reporting that a low proportion of COVID-19 patients had a bacterial co-infection: overall 7% of patients in hospital, increasing to 14% when looking at ICU patients only. In terms of antibiotic use, they report that in 10 studies, over 90% of patients received empirical antibiotics. Goncalves et al. report similar high antimicrobial usage is reported in a smaller single-centre retrospective study of 242 patients : they found bacterial co-infection in 19% of patients, but 67% of patients received antibiotics.[88] Goncalves et al. go on to state that COVID-19 can present as a systemic inflammatory response syndrome, therefore empirical broad-spectrum antibiotics may be used.[88] Findings from these two papers by Lansbury et al. and Goncalves et al. support the findings of the present study – the use of significantly more broad-spectrum agents in COVID-19 patients.

Given the use of broad-spectrum agents in the current study, the hypothesis of a change in antibiotic prescriptions in COVID-19 which may have selected out for *K. pneumoniae* (more prevalent in COVID-19 patients as described in Chapter 4) is

not supported. Were narrow spectrum agents e.g., amoxicillin, or teicoplanin used then the hypothesis would have been supported. However, the broad-spectrum agents prescribed would have treated *K. pneumoniae*.

Khurana et al. offer an explanation for the use of more broad-spectrum agents in the COVID-19 patient group.[152] They reported secondary infections amongst COVID-19 patients in a tertiary hospital in India: 56/375 (15%) ICU patients and 95/804 (12%) non-ICU patients developed secondary infections. An interesting observation was made in their paper: they stated that invasive tests used to help diagnose secondary infections were restricted in COVID-19 patients due to infection control policies. Therefore, clinicians used more broad-spectrum agents empirically which in turn selected for MDR pathogens. If appropriate samples were taken for culture then antibiotics may have been de-escalated and narrow spectrum agents could have been favoured. They go on to highlight the importance of antimicrobial stewardship programmes to aid appropriate antimicrobial use in this patient group.

In the current study, there was a difference when analysing the prescribing of broad versus narrow-spectrum antibiotics at the point when the FilmArray™ test was performed between the COVID-19 and non-COVID-19 patient groups. More of the non-COVID-19 patients were on broad spectrum antibiotics when the sample was taken: 20.2% higher; P=0.0001; 95% CI for difference in proportions: 10.0%, 30.4%. However, more of the COVID-19 patients were not on antibiotics when the sample was taken: 25.5% higher, 95% CI for difference in proportions (COVID- non COVID) 16.8%, 34.2%; P<0.0001. One possible explanation for this is that patients without COVID-19 had a variety of reasons for ICU admission and some of these reasons e.g., post-operative, sepsis, will have necessitated ongoing antibiotics. The reason for hospital admission in the non-COVID-19 patients is summarised in Table 3.3,

demonstrating that 41.1% were either surgical or trauma cases, hence likely prescribed broad spectrum post-operative combination antibiotics. However, the COVID-19 patients will have had fewer of these indications for antibiotics.

Secondly, the COVID-19 patients had longer ICU stays than those without COVID-19; the present study reports that the overall length of ICU stay of the COVID-19 patients prior to having a FilmArray™ test performed was significantly longer: 6.5 days, P<0.0001, 95% CI: 3.0, 7.0. This translates into more time for microbiological cultures to be sent to the laboratory and to return results, which translates into stopping/ switching to narrow-spectrum agents prior to when the FilmArray™ test was performed. This is an interesting observation because it would be expected that a longer stay in ICU (as seen amongst the COVID-19 patients) is a risk factor for hospital associated infections. However, more of the COVID-19 patients were not on antibiotics at the time of sampling.

The third reason, which is key and also helps explain why significantly more of the COVID-19 patients were not on antibiotics at the time of sampling, is familiarity with the FilmArray™ platform and a better understanding of its results. By the time the COVID-19 sub-study started, the FilmArray™ platform had been on ICUs as part of INHALE for almost a year. ICU consultants have reported anecdotally to the INHALE team that they were more familiar with using the FilmArray™ and therefore if they suspected a COVID-19 patient of having a HAP/VAP they were more inclined to perform the FilmArray™ before initiating antibiotics in the knowledge that a result would be available in approximately 75 mins. Trusting the results from a new platform is also key, and having had use of the platforms for a period of time pre COVID-19, the ICU staff will have had time for familiarisation. These behavioural issues are discussed in detail by Pandolfo et al., also working on the INHALE trial.[153]

5.5.2 Adherence to the prescribing algorithm in ICU patients

In the current study, amongst the patients with and without COVID-19, there was no evidence of a difference in the proportion of FilmArray™ instances with adherence to the algorithm between the two groups. In the COVID-19 group, the prescribing algorithm was adhered to in 35.9% of FilmArray™ instances, and in the non-COVID-19 patients it was adhered to in 35.5% of FilmArray™ instances. These results show that the prescribing algorithm was adhered to in approximately one third of patients in both groups. An explanation for the low adherence is that hospitals have different local guidance in respect of which empirical antibiotics to use for HAP/VAP. Accordingly, there was a degree of site-to-site variation in preferences based on FilmArray™ results, and sites retained the spirit of preferring narrow-spectrum agents and promoting antimicrobial stewardship where possible. The algorithm was a guide and alterative antibiotic options remained available if preferred or clinically indicated. For example, if *P. aeruginosa* was reported by the FilmArray™, the algorithm would have suggested treatment with Ceftazidime, however the site may have preferred to use another agent such as piperacillin/tazobactam.

A paper by Westblade et al. reviewed publications which evaluated bacterial co-infections in patients with COVID-19, and wrote an algorithm for suggested antibiotic use.[154] They reviewed a total of 10 studies with a minimum of 100 patients assessing co-infection on admission; they reported that less than 4% of patients had bacterial co-infection when admitted to hospital. When they reviewed the nine studies assessing hospital-acquired infections in COVID-19 patients, they reported that they occurred in 3.7-21.9% of patients. It appears that bacterial co-infection is

more of a problem in patients who have a longer hospital stay, and they suggest that mechanical ventilation could be a risk factor. Westblade et al. have designed an algorithm to help decide on antibiotic use upon hospital admission. They recommend that antimicrobials should be started only in patients who are 'critically ill, severely immunocompromised, or have radiographic or multiple laboratory findings suggestive of bacterial coinfection'.[154] Results of adherence to this algorithm would be valuable.

The non-COVID-19 FilmArray™ instances were divided into those which adhered to the algorithm and those which did not, and further analyses were performed on these two groups to examine factors which may have contributed to non-adherence. It was hypothesised that there would be more instances of other infections recorded amongst the group which did not adhere to the algorithm. However, results showed that there was no significant difference between the two groups. The second analysis performed to examine why patients' antibiotics did not adhere to the prescribing algorithm was to look at indications for antibiotic prescriptions (i.e., where the indication was not pneumonia). These indications included another source of sepsis (e.g., intra-abdominal), and post-operative antibiotics. Again, there was no evidence to suggest that cases which did not adhere to the algorithm were more likely to have other indication for antibiotics.

A further reason for non-adherence could simply be the treating clinicians own preference for antibiotic choice. As a comparator, a study by Rossio et al. reported adherence to antibiotic treatment guidelines in 317 patients with pneumonia.[155] Guidelines used in this study were those of the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS). They found that 38.8% of them received an empirical antibiotic regimen that was adherent to

guidelines. This proportion of 38.8% is very similar to that in the current study: 35.5.% and 35.9%. A second study by Deptula et al. investigated whether data from the European Centre for Disease Prevention and Control point prevalence survey of healthcare-associated infections and antimicrobial use could evaluate adherence to national guidelines for the treatment of CAP.[156] They reported that amongst 153 patients, 22.8% were treated according to the guidelines; lower than in the current study. A paper by Sakaguchi et al. specifically investigated adherence to VAP guidelines amongst 95 patients.[157] Using ATS/IDSA criteria, 19% of patients received therapy complaint with guidelines; and using Japanese Respiratory Society criteria, 28% of patients received treatment adherent to the guideline. Again, lower compared with the current study.

These comparative studies highlight that adherence to guidelines is not anywhere near 100%, supporting the low adherence figures to the prescribing algorithm in the current study. One important difference is the current study compares prescribing with an actual result to a guideline rather than empirical therapy compared to a guideline. Going forward, behavioural work assessing non adherence to the algorithm would provide valuable results to help identify specific reasons why adherence was poor and how it could be improved.

5.5.3 Prescribing decisions in conjunction with FilmArray™ results in ICU patients

Antibiotic prescribing decisions at 24 hours after the FilmArray™ was performed were compared between patients with and without COVID-19. From the results presented, when analysing the current data in terms of broad versus narrow-

spectrum agents utilised and directly comparing the patients with and without COVID-19, more of the non-COVID-19 patients were on broad-spectrum antibiotics at 24 hours after the FilmArray™ test had been performed (27.1% higher; P<0.0001 [Chi squared test]; 95% CI: 15.8%, 38.4%). There was evidence of a significant difference in the proportion of patients not on antibiotics between the two groups: more of the COVID-19 patients were not on antibiotics at the 24 hour point; 24.1% difference, P<0.0001; 95% CI: 14.5%, 33.7%). This could be because the patients without COVID-19 had other infections/ were post-surgical patients necessitating broad spectrum agents, and clinicians chose to continue with these antimicrobials.

These findings suggest that there was less usage of antibiotics amongst the COVID-19 patients: a larger proportion of them remained off antibiotics, had antibiotics stopped, and fewer of them were on broad-spectrum agents at 24 hours after the FilmArray™ was performed when compared with the non-COVID-19 patients. One limitation of the current study is that the COVID-19 and non-COVID-19 groups were not matched, therefore other factors, e.g., severity of disease, may have influenced the outcomes.

However, there was no evidence of a difference in the proportion of patients not on antibiotics, on narrow-spectrum antibiotics, or on broad-spectrum antibiotics between the time when the sample was taken for analysis and at 24 hours after the FilmArray™ was performed. This was true for patients with and without COVID-19. These results suggest that the FilmArray™ results had little or no effect on the spectrum of antibiotic prescribed.

A study by Furukawa et al. investigated the use of the FilmArray™ Pneumonia Panel as an antimicrobial stewardship tool.[158] This was a small study: reporting results for 17 BAL clinical samples (patients did not have COVID-19). All

the cases led to de-escalation or prevention of unnecessary antibiotic escalation. The most common intervention was discontinuation of anti-pseudomonal antibiotics (8 cases, 47.1%), followed by discontinuation of anti-MRSA antibiotics, (7 cases, 41.2%). These results also suggest that the FilmArray™ makes for a promising stewardship tool (supporting the findings of the current study), however the numbers were small so the effect seen may not have been true.

Maataoui et al. evaluated the performance and the impact of the FilmArray™ Pneumonia Panel on 112 respiratory samples from 67 COVID-19 ICU patients suspected of co-infections.[97] Samples included: 77 mini-BAL, 28 BAL, 4 sputa, and 3 ETA. Overall, the FilmArray™ led to antibiotic changes in 38/112 (34%) episodes: 16 withdrawal of antibiotics, 13 initiations, 3 adaptations, 5 de-escalations, and one change resulting in inadequacy. The authors concluded that in patients with a suspected HAP/VAP who had a negative FilmArray™ result, 19% had antibiotics discontinued and 24% remained antibiotic-free, however they did not make a comparison with non-COVID-19 patients. In the current study, when all FilmArray™ results were taken into account; 16.9% of COVID-19 patients had antibiotics stopped, and 14.8% remained off antibiotics. A similar proportion of patients had antibiotics stopped, however in the present study fewer remained antibiotic free when compared with Maataoui's work. It should be noted that Maataoui's results referred to here report negative FilmArray™ results, therefore it is not unexpected that fewer patients in the current study (i.e., those with positive and negative FilmArray™ results) remained off antibiotics.

Overall, from the present study, the COVID-19 patients had evidence of FilmArray™ results leading to a significantly higher proportion of antibiotics being stopped (13.1% higher; 95% CI: 5.6%, 20.6%) when compared with those without

COVID-19. There was also evidence of the FilmArray™ results leading to a significantly higher proportion of patients remaining off antibiotics (11.0% higher; 95% CI: 3.8%, 18.2%) compared to the non-COVID-19 patients. The reasons for this are the same as those discussed in 5.5.1: familiarity with the FilmArray™ and patients without COVID-19 would have been on ICU for several reasons, some of which may have required antibiotics, whereas the COVID-19 patients would have mainly been on ICU for respiratory support. Therefore, stopping/ keeping patients without COVID-19 off antibiotics would have been more challenging due to the reasons for their ICU admission.

A limitation of this work is that adherence to the algorithm was not taken into account when evaluating these prescribing decisions. Reasons why these antibiotic decisions were made after the FilmArray™ results are multifactorial, with the algorithm being one factor - depending on whether or not it was followed.

5.5.4 How prescribing decisions are affected by positive and negative FilmArray™ results in ICU patients

There was evidence of a significant difference (11.7%) in the proportion of antibiotics started, at 24 hours after the FilmArray™ was performed, between the positive and negative FilmArray™ groups in patients with COVID-19; significantly more antibiotics were started in the positive FilmArray™ group: 95% CI: 0.7%, 22.7%. This would be expected given that a positive result would likely be acted on and treated. Amongst the 112 COVID-19 patients in the study by Maataoui et al., 104 were suspected of having a HAP/VAP and the remainder a CAP.[97] Of the 104 HAP/VAP patients, 36 FilmArray™ results were positive and 68 were negative. Of the 36 positive results:

36% (13/36) had antibiotic initiation, 8% (3/36) led to antibiotic therapy adaptation, and 4 (11%) to de-escalation. In the current study, 19.4% of patients had antibiotics started and 2.6% had antibiotics de-escalated at 24 hours after a positive FilmArray™ result, these are lower proportions compared with those reported by Maataoui et al. (36% and 11% respectively). A possible reason for this could be sample type (discussed in Chapter 3; 3.5.1): with Maataoui et al. using mainly mini-BAL / BAL samples (93.8% of samples), whereas the majority of samples in the current study were ETA samples (89.8%).

In the current study, there was also evidence that significantly more COVID-19 patients remained off antibiotics in the negative FilmArray™ group when compared with the positive FilmArray™ group, at 24 hours after the FilmArray™ was performed: difference of 18.1%, P= 0.003; 95% CI: 6.3%, 29.9%. The study by Maataoui et al. reported that of the 68 negative FilmArray results: 24% (16/68) remained antibiotic-free and 13 (19%) led to antibiotic withdrawal. In 57% (39/68) of episodes, antibiotics were continued due to: severe sepsis (n = 20), infection from another site (n = 9), continuation of previous treatment (n = 7), or severely immunocompromised patients (n = 3).[97] In the current study, 24.6% of COVID-19 patients remained antibiotic free at 24 hours and 21.5% had antibiotic stopped after a negative FilmArray™ result, very similar figures to what was reported by Maataoui et al. (24% and 19% respectively).

When comparing patients in the present study with and without COVID-19, the non-COVID-19 patients had significantly more of the same antibiotics continued after a negative FilmArray™ result: 36.7% higher than for COVID-19 patients; 95% CI (non COVID-COVID): 16.4%, 57.0%; P=0.001. A reason for this is that those patients without COVID-19 may have been admitted to ICU for several reasons –

some of which necessitated antimicrobials; also, COVID-19 itself does not require antibiotic treatment.

Those patients with COVID-19 had evidence of significantly more antibiotics being stopped after a negative FilmArray™ result: 14.6% higher than for non-COVID-19 patients; 95% CI (COVID-non COVID): 1.0%, 28.2%. There was also evidence that significantly more COVID-19 patients stayed off antibiotics after a negative FilmArray™ result: 21.2% higher than for non-COVID-19 patients; 95% CI (COVID-non COVID): 8.8%, 33.6%. Furthermore, there was evidence that the COVID-19 patients had significantly more antibiotics stopped after a positive FilmArray™ result: 10.9% higher than for non-COVID-19 patients; 95% CI (COVID-non COVID): 2.3%, 19.4%. The latter result could be due to clinicians deciding that COVID-19 alone was responsible for the patient's symptoms and the FilmArray™ result did not require treatment. These results are all encouraging and support the theory that the FilmArray™ has a place as a stewardship tool amongst COVID-19 patients whether the result is positive or negative, as Maataoui et al. suggest in their study. In addition, results suggest that for non-COVID-19 patients there are more alternative explanations that might prey on the clinicians' minds when making prescribing decisions. In the COVID-19 patients a negative FilmArray™ result will provide evidence of absence of a bacterial co-infection in the chest, whereas the patients without COVID-19 have a variety of reasons for ICU admission and other potential sites of infection making antimicrobial prescribing more spectrum. Further work needs to be performed on a larger scale to add to the dataset.

5.5.5 Summary of Key Findings

The results presented indicate that antibiotic usage in COVID-19 patients in the participating centres is more conservative than the literature reports, and use of the FilmArray™ in this patient group has potential to promote good antimicrobial stewardship. However, adherence to the prescribing algorithm was less pronounced, at just over a third of cases adhering to it, similar to what is reported in the literature with respect to other prescribing guidelines. When examining factors (i.e., other infections the patients had and indications for antibiotics) which may have led to deviation from the algorithm, no significant differences were seen in the group that adhered to the algorithm and the group that did not adhere.

The more conservative approach to antimicrobial usage in the COVID-19 patients is evidenced by the prescription of more broad-spectrum antibiotics in the non-COVID-19 patients when the FilmArray™ sample was taken: P=0.0001; 95% CI: 10.0%, 30.4%; and more of the COVID-19 patients were not on any antibiotics at sampling: P<0.0001, 95% CI: 16.8%, 34.2%.

Patients with COVID-19 had evidence of significantly more antibiotics being stopped after a negative FilmArray™ result, when compared to those without COVID-19: 95% CI: 1.0%, 28.2%. Furthermore, more COVID-19 patients stayed off antibiotics after a negative FilmArray™ result: 95% CI: 8.8%, 33.6%; and the COVID-19 patients had significantly more antibiotics stopped after a positive FilmArray™ result: 95% CI: 2.3%, 19.4%. These results suggest that the use of the FilmArray™ has an impact antimicrobial prescribing in COVID-19 patients.

CHAPTER 6

FINAL CONCLUSIONS AND FUTURE DIRECTIONS

6.1: Summary and Conclusions

This thesis has outlined the potential, with respect to diagnosis, treatment and antimicrobial stewardship, of the FilmArray™ Pneumonia Panel Plus rapid molecular test when used as a POCT, in twelve ICUs across the UK. Both patients with and without COVID-19 have been included in the analyses.

Differences were noted between organisms detected by FilmArray™ and conventional diagnostic microbiology. The most common organism identified by FilmArray™ was *H. influenzae* and by conventional diagnostic microbiology was *S. aureus*. Significantly more negative conventional diagnostic microbiology results were reported when compared with negative FilmArray™ results (95% CI for difference in proportions: 2.1%, 26.1%). The FilmArray™ is more sensitive than conventional diagnostic microbiology, is more likely to detect non-viable organisms, and organisms which are non pathogenic will be detected and reported too.[114] This makes interpretation of results challenging and the current study has highlighted the importance of the clinical picture in this context.

The FilmArray™ rapidly identified organisms associated with HAP/VAP in ICU patients. The organism distribution in COVID-19 patients was different from those without COVID-19, with *K. pneumoniae* and *K. aerogenes* more prominent and *H. influenzae*, *P. aeruginosa,* and *E. coli* less so. Conventional diagnostic microbiology supported the finding of a significant increase in *K. pneumoniae* amongst COVID-19 patients when compared with non-COVID-19 patients. Reasons for this difference in organisms is likely to be multifactorial and remains unclear: impact of COVID-19 on

lung pathology and the immune response; antibiotic use in the local ICUs and the effect of this on the hospital-acquired organisms, are all possible reasons.

There are suggestions that use of the FilmArray™ in ICU patients aids antimicrobial stewardship. Results from this work showed that when directly comparing the patients with and without COVID-19, more of the non-COVID-19 patients were on broad-spectrum antibiotics at 24 hours after the FilmArray™ test had been performed: P<0.0001, 95% CI: 15.8%, 38.4%. Furthermore, patients with COVID-19 had significantly more antibiotics stopped after a negative FilmArray™ result: 95% CI: 1.0%, 28.2%; and more COVID-19 patients stayed off antibiotics after a negative FilmArray™ result: 95% CI: 8.8%, 33.6%. These are encouraging and support the use of the FilmArray™ as an antimicrobial stewardship tool.

When interpreting these results which compare both groups, it must be noted that the two patient groups i.e., COVID-19 and non COVID-19 were clinically different. The COVID-19 patients on ICU were extremely unwell with a novel respiratory disease; whereas the non COVID-19 patients included a far wider variety of patients such as elective post-operative patients where antibiotic use would have followed a protocol.

6.2: Limitations

When comparing the COVID-19 and non COVID-19 patient groups in the present study it is important to remember that the groups were not matched. The non COVID-19 group was more diverse including surgical, medical and trauma patients. This will have impacted on length of stay in ICU and also their antimicrobial treatment choices. Furthermore, the dataset collected for the COVID-19 patients

was less comprehensive than that collected for the INHALE patients (Table 2.2). The reason for this was during the pandemic staff had been reallocated from research to work on ICU and we did not want to burden them with large quantities of data collection. This also impacted on the comparison of the two groups, and the analysis performed on the COVID-19 patients. Stratification of non COVID-19 groups was not performed, however if patients were stratified according to underlying disease or comorbidities and analysed in those groups, it may provide further insight into which specific groups the FilmArray™ is most valuable.

The hospitals which participated in this study warrant mention as consideration needs to be taken when extrapolating these results to the UK. The two paediatric centres were both large referral hospitals with children referred from the UK or other countries. These children had complex, often rare, pathologies. The antibiotic exposure history of these patients and antimicrobial use in these units is not representative of paediatric centres across the UK. Tertiary hospitals were also over-represented amongst the adult patients, again the microbiology in these units is not necessarily applicable to the rest of the country. Therefore, there is an element of bias when reading the results and this must be remembered.

The FilmArray™ reports the number of gene copies of organisms detected, however this was not examined. It would be useful to anaylse this especially correlating it with antibiotic decisions, to determine whether clinicians use it to help differentiate whether an organism is a pathogen or a colonising bacteria.

The COVID-19 results presented are from data collected during the first peak of the pandemic. Treatment of the disease has changed rapidly since this time with agents including dexamethasone and Tocilizumab now being used as standard of care. It could be argued that such agents increase the risk of co-infection in COVID-

19 patients; if the FilmArray™ were to be used on this group today we may see a different picture to that presented in this thesis.

6.3: PCR tests deployed as a POCT

Several questions must be asked prior to deploying molecular tests on a large scale. One question is where will the test will be done? If the answer is 'In the laboratory', then it is likely that delays will be incurred i.e., transport to the laboratory, booking the samples in, with these things partially negating the advantages of these rapid tests. Such issues will be heightened where laboratories are divorced from their hospitals – a common occurrence in the UK. We often see centralised laboratories serving multiple hospitals, with the aim of saving money. A recent review by Vandenberg et al. discusses this issue in greater detail.[159] A possible remedy to the laboratories being centralised it to have a small 'hot' laboratory at every hospital for urgent tests, however this would add significant cost.

The FilmArray™ provides a potential solution to this – a rapid test available as a POCT by the bedside. This would come with its own challenges including, ward staff needing to be trained in the use of such tests, meaning that the tests must not require specialist laboratory expertise, and that their results and interpretation cannot be operator dependent (Chapter 1.1.5). The test used in the current study reports the organisms and resistance genes detected, removing the variability associated with reporting conventional microbiology results. However, how best to interpret these results must be understood by ward staff give that the test is not in the laboratory. Alternatively, communication systems must facilitate swift microbiology advice when needed. In the present study, interpretation of FilmArray™ results

required training (performed by the INHALE team) alongside use of the prescribing algorithm. This procedure sometimes required repeated site visits as interpretation of complex results could be challenging. If this platform were to be used as a POCT for routine care, detailed planning and thought must go into training staff members on the interpretation and treatment of complex microbiological results.

Quality control must also be considered, as ward-based POCTs may longer be under the remit of laboratory accreditation, unless managed by the laboratory. Further factors to consider are platform maintenance and interfacing with hospital computer systems. The steps to implement a POCT are described in Table 1.1, with clinical governance being integral. For the INHALE RCT, the molecular platforms used were provided by the trial for trial use only. The trial team provided training, wrote a user manual, were the lead for quality assurance, dealt with any platform failures, and provided the reagents. If these platforms were to be implemented as part of routine care, then a POCT team and manager would be necessary to take on the responsibility for such roles. The trial demonstrated the considerable work necessary when deploying a new test. Once teams had been trained on its use and interpretation of results, having run the first few samples on the platform, confidence in its use increased.

6.4: Interpretation of PCR tests when used as a POCT

Crucial to test interpretation is the issue of distinguishing colonising organisms from pathogens, as highlighted in a study by Jahn et al., who tested 35 diagnostic BAL specimens using a rapid broad-range PCR and microarray-based nucleic acid amplification test called Prove-it Sepsis Assay.[160] The authors concluded that the

clinical relevance of the results was uncertain, and that colonising organisms may be difficult to differentiate from pathogens. This is important to note if tests are deployed as a POCT, with no medical or clinical microbiologist to interpret/ offer advice, you could end up in a situation where every organism reported is treated, thereby increasing antibiotic use. Results from the current study show that amongst the COVID-19 patients, a positive FilmArray™ result actually led to significantly more patients stopping antibiotics (difference in proportions 10.9%; 95% CI 2.3%, 19.4%). This can be explained by the COVID-19 patients having an alternative diagnosis for their respiratory symptoms. Therefore, a positive test actually led to a reduction in antibiotic prescribing rather than clinicians opting to treat all organisms, in this specific patient group.

It is prudent to remember that laboratory culture of sputum is also limited in that it cannot distinguish colonisation from infection; this issue of which organisms are pathogens exists for both PCR and culture. However, the microbiology laboratory has a key role to play in reporting out sputum culture results. These reports are decided upon by the healthcare scientists and medical/ clinical microbiologists and therefore the likelihood of clinicians treating potentially colonising organisms is reduced. Medical and clinical microbiologists bridge the gap between the laboratory and the patient – helping clinicians decide what needs treating and how. The FilmArray™ Pneumonia Panel discussed in this thesis is quantitative giving rise to the possibility of helping determine the differentiation between colonist and pathogen. In future, biomarkers e.g., procalcitonin may help with this decision too.

Although POCTs have greatest potential as bedside tools, their use for pneumonia patients will need strong microbiology and/or infectious disease advice if

their often-complex findings are to be best-translated into treatment advice and antimicrobial stewardship. Results from this thesis show that 21.5% of patients with COVID-19 had antibiotics stopped after a negative FilmArray™ result versus 6.9% of patients without COVID-19. These results suggest potential use of the FilmArray™ as a stewardship tool, especially amongst the COVID-19 patient group. However, it must be remembered that the outcomes of these patients in terms of whether the decisions made relating to antibiotics were correct were not recorded as part of data collection for the COVID-19 patients.

Differentiating between HAP/VAP and COVID-19 versus COVID-19 alone can be difficult. The utility of a test like the FilmArray™ in such a scenario is useful as it helps provide clinicians with the confidence to stop antibiotics in a patient group where the COVID-19 diagnosis explains the symptoms. Such a test, if negative, would help move bacterial infection as a cause lower of fever/ increased oxygen requirements down the differential diagnosis list, leading to a reduction in antibiotic use.

A prescribing algorithm was written for the INHALE trial to translate output into prescriptions. Despite only a third of cases adhering to the algorithm, research nurses did anecdotally report it to be a useful tool. If such an algorithm was not in use, then there would be a case for microbiology input for each result – in helping decide the most appropriate narrow-spectrum agent. This however would prove difficult in practice for ICUs where daily microbiology input was not possible – one of the reasons behind writing a prescribing algorithm for the INHALE trial.

Ginocchi et al. performed a large study including fifty-two laboratories from 13 European countries and Israel testing a total of 2476 adult and paediatric samples using both the FilmArray™ Pneumonia Panel and standard care.[23] Details of the

patient groups are not published so whether they were exclusively patients with HAP/VAP or also CAP is unknown. They reported that the FilmArray™ panel identified significantly more positive specimens (76.13%) than standard of care (56.03%) (P≤ 0.0001) and more potential pathogens than standard of care (P≤ 0.0001) independent of specimen type. This study by Ginocchi et al. is the largest performed to date, however unlike the current study, they did not look at the impact of the FilmArray™ on antibiotic use – despite this they did conclude that the FilmArray™ had the potential to improve antimicrobial stewardship and patient outcomes.

6.5: Costs associated with PCR tests

Molecular tests are considerably more expensive than bacterial culture, costing anywhere per test from £100-400 *vs.* c. £15-25.[33] A timely example of the cost of PCR tests has been a common feature in the media of late – quoting high-street companies such as Boots charging £120 for a COVID-19 PCR test. This would be charged to the individual and is to most a considerable sum of money; however, the costs to a health service would be far more complex than a one off payment for a test.

A comprehensive health economic analysis is required to establish whether swifter refinement of patients' treatment translates into cost savings – this is being done in conjunction with the INHALE trial. Moreover, unless tests are comprehensive – which is unlikely, rapid tests for pathogens and resistances will be in addition to conventional testing, not a replacement. Gains in stewardship and

patient management may recoup some additional costs, but work needs to be done to determine this.

Moreover, efficiency gains are notoriously difficult to translate into cash savings in socialised healthcare, such as the UK NHS, operating at near-full capacity. A patient may be discharged earlier, giving a notional 'saving,' but their bed is immediately filled by a new patient, whose new costs are likely to exceed those of an extra day's stay by the original patient. Cost savings may be more obviously realisable in settings where the patient, or their insurer, pays directly for example in the USA. However, the value to the patient is important – fewer days in hospital if discharged sooner is of paramount benefit for the patient, both physically and mentally.

6.6: Behavioural aspects

Last, and most subtle: behavioral aspects are crucial, and are apt to vary with place and human culture. People and tradition may well be big barriers to change, and deployment of these tests will demand significant changes to ways of working both in the clinic and in the microbiology department. ICU decisions relating to antibiotic prescribing in particular are multifactorial and complex, as outlined in systematic reviews, by Warreman et al. and Krockow et al.[34,35] Key factors include fear of adverse outcomes and the personal experience of the clinician. Such factors may have impacted the use of the prescribing algorithm in the current study – clinicians' having their own preferences for antibiotics based on past experiences impacting current choices.

Work published by Pandolfo et al., as part of INHALE, characterised prescribers' beliefs about molecular diagnostics and barriers to using such results for antibiotic prescribing for pneumonia patients, using vignette based interviews conducted in 2018 (prior to commencement of the RCT).[153] Of note, clinicians reported that they wanted more information about the molecular test, including sensitivity and specificity; they also stated that they worried about non-pathogenic bacteria being reported by the test which could lead to unnecessary antibiotic prescriptions; regarding negative molecular results clinicians reported that they would not be reassuring enough to withhold or stop antibiotics because they were not sure whether the test could detect all respiratory pathogens of concern.[153]

This study shows that, amongst the non-COVID-19 patients, utilising molecular tests which detect more organisms and resistance genes than laboratory culture may prompt polypharmacy rather than better stewardship. It is unclear how much clinicians will trust these novel tests and how this will change if the platform sits in the ICU rather than remotely in the laboratory. The INHALE trial is exploring these behavioural aspects as part of the RCT.

6.7: Future Directions

The sample size of the INHALE RCT work presented is smaller than anticipated due to the pause in recruitment secondary to COVID-19. The trial aims to recruit a total of 552 patients – the analyses performed in this thesis would have benefitted from this larger sample size. However, the COVID-19 dataset presented in this thesis represents the complete patient cohort recruited.

This thesis describes the utility of the FilmArray™ Pneumonia Panel as a POCT in the ICU setting. Future work on the use of this panel in A&E or on hospital wards, alongside impact on antibiotic prescribing, would be beneficial. Having such a test available in A&E could provide clinicians with additional information when deciding on whether to admit or discharge a patient and what treatment to start should they have a pneumonia. The FilmArray™ could also prove a useful diagnostic tool to support disease surveillance by linking it with organisations such as Public Health England. This would give them real-time data enabling earlier identification of outbreaks. A country-specific FilmArray™ panel may also be beneficial as organisms and their resistance genes differ in ICUs across the globe. In future it might be that each country has it's own tailor-made panel.

Although the multiplex PCR panel offers a faster result availability, the correlation of a positive result and clinical likelihood of infection remains unclear as seen from the data presented. As the FilmArray™ results provide bin values i.e., number of gene copies of the organism detected, it would be useful for future work to correlate this with prescribing decisions. This would give further insight into whether clinicians chose mainly to treat organisms where more bin copies were detected. A longitudinal study examining serial ETT samples of patients daily over a duration of their stay would provide information into how long the FilmArray™ results remain positive. This could be compared with conventional diagnostic microbiology. It is likely that in future, collective approaches including PCR, sequencing, and biomarkers, will facilitate a major shift in the management of respiratory infection.

One such approach is the use of transcriptome biomarkers. The FilmArray™ pneumonia panel provides clinicians with the identification of organisms present in sputum samples, as well as resistance markers including bla_{IMP} $bla_{CTX\text{-}M}$, bla_{KPC},

bla$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *mecA/mecC*. It may not be clear whether these organisms are playing a pathogenic role in the sputa, however in conjunction with the blood biomarker results, it is hypothesised that this question of pathogenicity will be easier to answer. With the help of Norwich Clinical Trials Unit, I have designed a sub-study to measure procalcitonin and transcriptome biomarkers from blood at enrolment to the INHALE trial. The aim is to evaluate whether this combination approach (either with routine culture, or FilmArray™) will aid HAP/VAP diagnostics, and guide antimicrobial treatment.

This sub-study is currently recruiting and includes participants at University College London Hospital (UCLH), Royal Free London NHS Foundation Trust and Watford General Hospital sites. The transcriptome biomarker panel being used is the HostDx panel by Inflammatix, based in the USA.[161] The HostDx panel uses a blood sample from the patient to identify the presence, type (bacterial/viral), and severity of an acute infection in 30 minutes. It seeks 29-mRNAs, produced by white cells in peripheral blood, that may have their expression modulated by infection. Likelihood ratios are calculated using proprietary bioinformatics data at the Inflammatix laboratory. Specific details of the gene set have not been released and the test requires clinical evaluation. To perform these tests, an additional blood sample (10ml) is taken for the sub-study at onset of pneumonia (enrolment). This blood is collected from the participant's standard care cannula or central line at the same time as routine blood collection, within 24 hours of the qualifying sputum sample being collected for INHALE. Results are currently awaited.

Novel biomarkers like these could provide a multistep diagnostic approach. An algorithm could be devised whereby a patient with a suspected HAP/VAP has a blood biomarker test performed, and if this is positive they go on to have a

FilmArray™ test. Therefore the question pertaining to the FilmArray™ result representing a pathogen or colonising organism would be more easily answered and acted upon, with the patient already having a positive biomarker result suggestive of bacterial infection.

In closing, rapid diagnostics have the potential to be at the cornerstone of future patient care, both for patient diagnosis and treatment, as well as an antimicrobial stewardship tool. This thesis has highlighted the value of the FilmArray™ amongst the COVID-19 patient group; a group of patients who have single organ pathology with a low pre-test probability of a secondary bacterial infection. These are the situations in which the FilmArray™ is most valuable.

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APPENDICIES

Appendix 1: Norwich Clinical Trials Unit Team

Ann Marie Swart – Clinical Trials Unit Director

Juliet High – Trial Manager

Kerry Dresser – Trial Assistant

Antony Colles – Data Manager

Sue Stirling – Statistician

Appendix 2: INHALE Team

Virve Enne, University College London: Programme Manager

Vanya Gant, University College London Hospital: Chief Investigator

David Livermore, University of East Anglia: Co-chief Investigator

Charlotte Russell, University of East Anglia: Senior Research Associate

Julie Barber, University College London: Lead Statistician

Justin O'Grady, Quadram Institute Bioscience: Co-applicant

Robert Horne, University College London: Behavioural Science Lead and Co-applicant

Alyssa Pandolfo, University College London: Behavioural Science Research Assistant

David Turner, University of East Anglia: Health Economics Lead and Co-applicant

Appendix 3: Master Prescribing Algorithm

INHALE WP3: Antibiotic Prescribing Guidance for use with FilmArray™ Result

CONSIDERATIONS

1. Adjust dosages for renal function as per Manufacturer's SPC
2. For patients already on antimicrobial treatment for infections necessitating specific regimens e.g. infective endocarditis/ meningitis - please discuss with Microbiology how best to adapt their treatment for organism(s) found by FilmArray.
3. Pregnant and paediatric patients: Please note general recommendations regarding use of fluoroquinolones, tetracyclines, colistin, temocillin and ceftazidime-avibactam. See https://www.medicines.org.uk/emc/ for specifics.
4. Please be aware that Biofire FilmArray does not detect *Stenotrophomonas maltophilia.* If *S. maltophilia* infection is suspected please adjust therapy accordingly in at risk populations.

Key

No known allergy to antibiotics
Mild allergy to β–lactams i.e. rash
Severe allergy to β–lactams, i.e. anaphylaxis
Not applicable

Table 1. To be used when ONE Organism is Detected by FilmArray

First ***What organism was found & it the patient allergic to β-lactams***		***Second*** ***If NO resistance genes found, this is the advised R_x***	***Third*** ***If resistance genes found, this is your advised R_x***			
			Resistance Marker			
		None	CTX-M	KPC or OXA-48	IMP, NDM or VIM	*mecA* or *mecC*
	No organisms found	Antibiotics should be stopped unless there is clear evidence for probable or proven bacterial infection severe enough to warrant them				
	Any virus	Co-amoxiclav +antiviral if appropriate				
		Cefuroxime +antiviral if appropriate				
		Levofloxacin + antiviral if appropriate				

		First ***What organism was found & it the patient allergic to β-lactams***	***Second*** ***If NO resistance genes found, this is the advised R_x***	***Third*** ***If resistance genes found, this is your advised R_x***			
			None	CTX-M	KPC or OXA-48	IMP, NDM or VIM	*mecA* or *mecC*
	Any virus + 1 or more bacteria		Treat as indicated for bacterial infection and add antiviral treatment where appropriate				
Organism	*A. baumannii*		Meropenem[1]				
			Meropenem[1]				
			Colistin alone or in combination[2] if clinically appropriate				
	E. aerogenes, E. cloacae, E. coli, K. pneumoniae or K. oxytoca		Temocillin (2g TDS)	Temocillin (2g TDS)	Ceftazidime-avibactam[3]	Colistin alone or in combination[2] if clinically appropriate	
			Ceftriaxone (*Klebsiella* spp. & *E. coli*) OR Meropenem for *Enterobacter* spp.	Meropenem	Ceftazidime-avibactam[3]	Colistin alone or in combination[2] if clinically appropriate	
			Levofloxacin or Ciprofloxacin	Colistin alone or in combination[2] if clinically appropriate	Colistin alone or in combination[2] if clinically appropriate	Colistin alone or in combination[2] if clinically appropriate	
	Proteus spp. or *S. marcescens*		Piperacillin-tazobactam for Serratia sp. OR Temocillin 2g TDS for Proteus sp.	Temocillin 2g TDS for Proteus sp. OR Meropenem for Serratia sp.	Ceftazidime-avibactam[3]	Fosfomycin[4]	
			Ceftriaxone	Meropenem	Ceftazidime-avibactam[3]	Fosfomycin[4]	
			Fosfomycin[4]	Fosfomycin[4]	Fosfomycin[4]	Fosfomycin[4]	
	H. influenzae		Co-amoxiclav				
			Cefuroxime				
			Doxycycline OR Levofloxacin or Ciprofloxacin				
	M. catarrhalis		Co-amoxiclav				
			Cefuroxime				
			Doxycycline OR Levofloxacin or Ciprofloxacin				
	P. aeruginosa		Ceftazidime (2g TDS)	Meropenem	Ceftazidime-avibactam[3]	Colistin alone or in combination[2] if clinically appropriate	
			Ceftazidime (2g TDS)	Meropenem	Ceftazidime-avibactam[3]	Colistin alone or in combination[2] if clinically appropriate	
			Colistin alone or in combination[2] if clinically appropriate	Colistin alone or in combination[2] if clinically appropriate	Colistin alone or in combination[2] if clinically appropriate	Colistin alone or in combination[2] if clinically appropriate	

	S. aureus	Flucloxacillin[5]				Glycopeptide[6] or Linezolid
		Cefuroxime				Glycopeptide[6] or Linezolid
		Glycopeptide[6] or Linezolid				Glycopeptide[6] or Linezolid
	S. agalactiae, S. pneumoniae or *S. pyogenes*	Amoxicillin				
		Cefuroxime				
		Glycopeptide[6] or Linezolid				
	C. pneumoniae, L. pneumophila, M. pneumoniae	Macrolide[7] OR Levofloxacin or Ciprofloxacin				
		Macrolide[7] OR Levofloxacin or Ciprofloxacin				
		Macrolide[7] OR Levofloxacin or Ciprofloxacin				

Footnotes

1. In units with high rates of carbapenem resistance, or if experiencing outbreak of carbapenem-resistant *A. baumannii* follow same recommendations as for treatment in case of allergy
2. Colistin can be combined with an appropriate second antimicrobial such as rifampicin or tigecycline. The choice is left open according to local preference.
3. Please discuss with microbiologist before prescribing
4. Consider adding colistin as metallo β-lactamase likely to be present in undetected host organism
5. If clinical picture suggests PVL-positive *S. aureus* consider ordering PVL test and switching to linezolid
6. Vancomycin or teicoplanin
7. Clarithromycin or azithromycin

Key

No known allergy to antibiotics

Mild allergy to β–lactams i.e. rash

Severe allergy to β–lactams, i.e. anaphylaxis

Not applicable

Table 2. Recommended treatment for combination of* TWO *or more organisms are detected by FilmArray

PLEASE READ THIS TABLE FROM LEFT TO RIGHT; Coloured boxes refer to allergy status as in Table 1.

***Key**: + organism present, - organism absent,* ± either present or absent

First, What combination of bacteria have been found?						**Second: Therapy if no resistance genes**	**Third: if resistance genes found**			
A. baumannii	*Enterobacterales: E. aerogenes, E. cloacae, E. coli, K. pneumoniae ,K. oxytoca, Proteus* sp., *S. marcescens*	*P. aeruginosa*	*H. influenzae/M. catarrhalis*	*S. aureus*	*S. agalactiae, S. pneumoniae* or *S. pyogenes*		*mecA/C* found	CTX-M found	*C. pneumoniae, L. pneumophila* OR *M. pneumoniae*	Carbapen-emase found
Does the mixture include *Acinetobacter*? If YES ; stay with this block; if NO, go to next block										
+	Any one or more second organism found					Meropenem[8]	Add Glycopeptide [10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
+	± Any one or more second organism found					Meropenem[8]	Add Glycopeptide [10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
+	Add Levofloxacin or Ciprofloxacin [9]	Add Levofloxacin or Ciprofloxacin [9]	Add Levofloxacin or Ciprofloxacin [9]	Add Glycopeptide[10] OR Linezolid	Add Glycopeptide[10] OR Linezolid	Colistin Combination	Add Glycopeptide [10] OR Linezolid	Discuss with Micro-biology	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology

First, What combination of bacteria have been found?						Second: Therapy if no resistance genes	Third: if resistance genes found			
A. baumannii	*E. aerogenes, E. cloacae, E. coli, K. pneumoniae ,K. oxytoca, Proteus* sp., *S. marcescens*	*P. aeruginosa*	*H. influenzae/M. catarrhalis*	*S. aureus*	*S. agalactiae, S. pneumoniae* or *S. pyogenes*		*mecA/C* found	CTX-M found	*C. pneumoniae, L. pneumophila* OR *M. pneumoniae*	Carbap en-emase found
If NO *Acinetobacter*: but ≥1 *Pseudomonas*/Enterobacteriales found start here										
-	+ (at least one)		±	±	±	Piperacillin/Tazobactam	Add Glycopeptide [10] OR Linezolid	Escalate to Meropenem	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
-	+ (at least one)		±	±	±	Meropenem	Add Glycopeptide [10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
-	Add Levofloxacin or Ciprofloxacin [9]	Add Levofloxacin or Ciprofloxacin[9]	Add Levofloxacin or Ciprofloxacin[9]	Add Glycopeptide[10] OR Linezolid	Add Glycopeptide[10] OR Linezolid	Colistin Combination as indicated	Add Glycopeptide [10] OR Linezolid	Discuss with Micro-biology	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
If NO *Acinetobacter* NO *Pseudomonas* & NO Enterobacteriaceae start here										
None of these			Any 2 or more of these			Co-amoxiclav	Add Glycopeptide [10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	
None of these			Any 2 or more of these			Levofloxacin	Add Glycopeptide [10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	
None of these			Any 2 or more of these			Levofloxacin	Add Glycopeptide [10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	

Footnotes

8. Add colistin in in areas of high carbapenem-resistance among *A. baumanii.*
9. Consider adding tigecycline of fosfomycin if fluoroquinolone resistance locally prevalent
10. Vancomycin or teicoplanin
11. Clarithromycin or azithromycin

Appendix 4: Research nurses at sites

Site	Research Nurses
Aintree University Hospital	Amie Reddy Colette Criddle-Jones Ian Turner-Bone Laura Wilding
Birmingham Women's and Childrens' Hospital	Helen Winmill Carly Tooke Sarah Fox Samantha Owen Roxanne Williams Harriet Payne
BUPA Cromwell	Eleanor Tudtud Zoran Aman
Chelsea and Westminster Hospital	Rhian Bull Jaime Carungcong Laura Gomes de Almeida Martins Patricia Correia Da Costa Kribashnie Nundlall
Dudley Hospital	Elena Anastasescu Karen Reid
Great Ormond Street Hospital	Lauran O'Neill Eugenia Abaleke Ana Luisa Tomas Helen Vander-Johnson Holly Belfield

	Tara McHugh Gbenga Akinkugbe
James Paget Hospital	Julie North Siobhan Parslow-Williams
Royal Free Hospital	Helder Filipe Amitaa Maharajh Sara Mingo Glykeria Pakou Margaret McNeil
Royal Liverpool Hospital	Karen Williams Jaime Fernandez Roman Victoria Waugh Dave Shaw
Royal Stoke Hospital	Minnie Gellamucho Gwen Keay Resti Varquez Ibraar Hussain
University College London Hospital	Deborah Smyth Georgia Bercades Jung Ryu Ingrid Hass
Watford General Hospital	Xiaobei Zhao

Appendix 5: Principal Investigators at sites

Site	Principal Investigators
Aintree University Hospital	Robert Parker
Birmingham Women's and Childrens Hospital	Jane Cassidy
BUPA Cromwell	Jeronimo Cuesta
Chelsea and Westminster Hospital	Suveer Singh
Dudley Hospital	Julian Sonksen
Great Ormond Street Hospital	Mark Peters Nigel Klein
James Paget Hospital	Michael Karlikowski
Royal Free Hospital	Mark DaNeef Dan Martin
Royal Liverpool Hospital	Ingeborg Welters
Royal Stoke Hospital	Nehal Patel
University College London Hospital	David Brealey
Watford General Hospital	Hala Kandil Valerie Page

Appendix 6: Site specific exceptions to the prescribing algorithm

Site	Changes to Algorithm for patients not allergic to β-lactams	Changes to Algorithm for β-lactam allergic patients
Adult 1	Ceftazidime or meropenem preferred over temocillin for Enterobacterales.	None.
Adult 2	None.	None.
Adult 3	Colistin alone or in combination when *A. baumannii* detected.	None.
Adult 4	Piperacillin/tazobactam preferred over ceftazidime as an antipseudomonal.	None.
Adult 5	Ertapenem preferred over meropenem (except if antipseudomonal required). Meropenem instead of piperacillin/tazobactam for AmpC β-lactamase producing organisms.	None.
Adult 6	None.	None.
Adult 7	Ceftazidime or meropenem preferred over temocillin for Enterobacterales.	None.
Adult 8	None.	Fluoroquinolones preferred over cephalosporins in mild allergy. Glycopeptide or linezolid preferred over cefuroxime if *S. aureus* detected in mild allergy

Adult 9	None.	Fluoroquinolones preferred over cephalosporins in mild allergy.
Adult 10	None.	None.
Paediatric 1	Alternatives to temocillin, which lacks a paediatric licence (generally piperacillin/tazobactam or meropenem). Addition of aminoglycoside (with Enterobacterales detected) if patient severely ill. Add aminoglycoside if using piperacillin/tazobactam *versus* AmpC β-lactamase producing organisms.	None.
Paediatric 2	Alternatives to temocillin, which lacks a paediatric licence (generally piperacillin/tazobactam or meropenem). Addition of clindamycin if *S. pyogenes* isolated or PVL *S. aureus* suspected. Add aminoglycoside if using piperacillin/tazobactam *versus* AmpC β-lactamase producing organisms.	None.

67 Appendix 7: Algorithm teaching slides

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Patient has no penicillin allergy

FilmArray
LRTI Panel - IUO

BIOFIRE
www.BioFireDx.com

Run Information			
Sample ID:	RS092	Run Date:	21 Feb 2018 10:14 AM
Pouch:	LRTI Panel v2.0	Controls:	Passed
Run Status:	Completed	Protocol:	SPUTUM v3.3
Serial No.:	10856270	Operator:	INHALE INHALE (INHALE)
Lot No.:	637217	Instrument:	2FA01854

Detection Summary		
Bacteria		
Detected:		None
Antimicrobial Resistance Genes		
Detected:		None
	⚠	**Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility to the resistance drug class. Culture is required for species identification and susceptibility testing of isolates.
Atypical Bacteria		
Detected:		None
Viruses		
Detected:	✓	Influenza A

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Answer

- No penicillin allergy (in red on top of screen), therefore answer will be in a green line
- Only Influenza A detected

Go to page 2 of algorithm

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Prescribing Algorithm

Key

No known allergy to antibiotics
Mild allergy to β–lactams i.e. rash
Severe allergy to β–lactams, i.e. anaphylaxis
Not applicable

Table 1. To be used when ONE Organism is Detected by FilmArray

First ***What organism was found & it the patient allergic to β-lactams***	***Second*** ***If NO resistance genes found, this is the advised R_x***	***Third*** ***If resistance genes found, this is your advised R_x***

		Resistance Marker				
		None	CTX-M	KPC or OXA-48	IMP, NDM or VIM	*mecA* or *mecC*
	No organisms found	Antibiotics should be stopped unless there is clear evidence for probable or proven bacterial infection severe enough to warrant them				
	Any virus	Co-amoxiclav +antiviral if appropriate Cefuroxime +antiviral if appropriate Levofloxacin + antiviral if appropriate				
	Any virus + 1 or more bacteria	Treat as indicated for bacterial infection and add antiviral treatment where appropriate				

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Patient has no penicillin allergy

FilmArray
LRTI Panel - IUO

BIOFIRE
www.BioFireDx.com

Run Information

Sample ID:	IS014	**Run Date:**	10 May 2018 11:33 AM
Pouch:	LRTI Panel v2.0	**Controls:**	Passed
Run Status:	Completed	**Protocol:**	SPUTUM v3.3
Serial No.:	10466857	**Operator:**	INHALE INHALE (INHALE)
Lot No.:	602517	**Instrument:**	2FA01854

Detection Summary

Bacteria

		Bin (copies/mL)				
			10^4	10^5	10^6	≥10^7
Detected:	✓ ≥10^7	*Klebsiella pneumoniae* group				
	✓ 10^6	*Serratia marcescens*				

Antimicrobial Resistance Genes

Detected: ✓ CTX-M
✓ KPC

⚠ **Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility to the resistance drug class. Culture is required for species identification and susceptibility testing of isolates.

Atypical Bacteria

Detected: None

Viruses

Detected: ✓ Parainfluenza Virus

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Answer

- No penicillin allergy (in red on top of screen), therefore answer will be in a green line
- TWO organisms detected – *Klebsiella pneumoniae group* and *Serratia marcescens*
- *A.baumannii* is not present, therefore ignore the first three rows
- CTX-M and KPC resistance genes detected
- Parainfluenza virus detected

Go to page 6 of algorithm

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Prescribing Algorithm

First: What combination of bacteria have been found?						Second:	Third: if resistance genes found			
A. baumannii	*Enterobacteriales: E. aerogenes, E. cloacae, E. coli, K. pneumoniae ,K. oxytoca, Proteus sp., S. marcescens*	*P. aeruginosa*	*H. influenzae/M. catarrhalis*	*S. aureus*	*S. agalactiae, S. pneumoniae* or *S. pyogenes*	Therapy if no resistance genes	*mecA/C* found	CTX-M found	*C. pneumoniae, L. pneumophila* OR *M. pneumoniae*	Carbapenemase found
If NO *Acinetobacter*: but ≥1 *Pseudomonas*/Enterobacteriales found start here										
-	+ (at least one)		±	±	±	Piperacillin/ Tazobactam	Add Glycopeptide[10] OR Linezolid	Escalate to Meropenem	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
-	+ (at least one)		±	±	±	Meropenem	Add Glycopeptide[10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
-	Add Levofloxacin or Ciprofloxacin[9]	Add Levofloxacin or Ciprofloxacin[9]	Add Levofloxacin or Ciprofloxacin[9]	Add Glycopeptide[10] OR Linezolid	Add Glycopeptide[10] OR Linezolid	Colistin Combination as indicated	Add Glycopeptide[10] OR Linezolid	Discuss with Micro-biology	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
If NO *Acinetobacter* NO *Pseudomonas* & NO Enterobacteriaceae start here										
None of these			Any 2 or more of these			Co-amoxiclav	Add Glycopeptide[10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	
None of these			Any 2 or more of these			Levofloxacin	Add Glycopeptide[10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	
None of these			Any 2 or more of these			Levofloxacin	Add Glycopeptide[10] OR Linezolid		Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	

Footnotes

8. Add colistin in in areas of high carbapenem-resistance among *A. baumanii*.
9. Consider adding tigecycline of fosfomycin if fluoroquinolone resistance locally prevalent
10. Vancomycin or teicoplanin
11. Clarithromycin or azithromycin

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Answer

Answer is circled in red – Discuss with microbiology, this is because a carbapenemase had been found.

- CTX-M is an ESBL therefore Meropenem could be used if there was no carbapenemase, however a KPC was also found. KPC is a carbepenamase therefore the result needs to be discussed with microbiology

- Parainfluenza detected: We do not have a antiviral prescribing algorithm so manage this as you would usually

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93 **Appendix 8: Classification of Broad and Narrow Spectrum Antibiotics**

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Antibiotic	**Spectrum**
Individual Agents	
Amikacin	Broad
Amoxicillin	Narrow
Amoxicillin/clavulanate	Narrow
Azithromycin	Narrow
Cefotaxime	Broad
Ceftazidime	Broad
Ceftazidime/Avibactam	Broad
Ceftolozane/Tazobactam	Broad
Ceftriaxone	Broad
Cefuroxime	Narrow
Ciprofloxacin	Broad
Clarithromycin	Narrow
Clindamycin	Narrow
Colistin	Narrow
Co-Trimoxazole	Broad
Doxycycline	Narrow
Ertapenem	Broad
Erythromycin	Narrow
Flucloxacillin	Narrow
Fosfomycin	Broad
Gentamicin	Broad
Levofloxacin	Broad

Linezolid	Narrow
Meropenem	Broad
Moxifloxacin	Broad
Penicillin	Narrow
Piperacillin/Tazobactam	Broad
Teicoplanin	Narrow
Temocillin	Narrow
Tigecycline	Broad
Trimethoprim	Narrow
Vancomycin	Narrow
Examples of Combination Agents*	
Amoxicillin/ Flucloxacillin	Narrow
Amoxicillin/ Temocillin	Narrow
Amoxicillin/clavulanate / Clarithromycin	Narrow
Cefuroxime/ Flucloxacillin	Narrow
Clindamycin/ Teicoplanin	Narrow
Amoxicillin/clavulanate/ Temocillin	Broad

*If antibiotic combinations included broad-spectrum agents, then the combination was defined as broad-spectrum. Other combinations are listed above.