Utility of the BioFire® FilmArray® Pneumonia Panel *plus* assay for syndromic testing of lower-respiratory tract infections in a low-middle-income setting

by

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Abbreviations

AMR: Antimicrobial resistance

AMS: Antimicrobial stewardship

BAL: Bronchoalveolar lavage

bla: Beta-lactamase encoding gene

CAP: Community-acquired pneumonia

CE: Conformité Européenne

CLSI: Clinical and Laboratory Standards Institute

CI: Confidence intervals

Colgent: Columbia blood agar with gentamicin

COVID-19: Coronavirus disease 2019

ESBL: Extended spectrum beta-lactamase

FA-PP: BioFire® FilmArray® Pneumonia Panel *plus*

GSH: Groote Schuur Hospital

HIV: Human Immunodeficiency Virus

ICU: Intensive care unit

IMP: Imipenem beta-lactamases

KPC: *Klebsiella pneumoniae* carbapenemase

LMICs: Low/middle-income countries

LRT: Lower respiratory tract

LRTI: Lower respiratory tract infection

MCC: MacConkey agar plate

MC&S: Microscopy, culture and susceptibility testing

MDR: Multidrug-resistant

MERS: Middle East Respiratory Syndrome Coronavirus

MREJ: SCCmec right-extremity junction

MRSA: Methicillin-resistant *Staphylococcus aureus*

n: Number

N/A: Not applicable

NDM: New Delhi metallo-beta-lactamase

NHLS: National Health Laboratory Services

NICD: National Institute for Communicable Diseases

NPA: Negative percent agreement

OXA-48-like: Oxacillinase beta-lactamases

PPA: Positive percent agreement

PCR: Polymerase Chain Reaction

RDTs: Rapid diagnostic tests

RSV: Respiratory Syncytial Virus

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

TA: Endotracheal aspirate

US FDA-cleared: United States Food and Drug Administration

VIM: Verona Integron-Encoded Metallo-Beta-Lactamase

WHO: World Health Organisation

Symbols

®: Registered trademark

°C: Degrees Celsius

g: Gram

h: Hour

L: Litre

m: Milli

μ: Micro

UTILITY OF THE BIOFIRE® FILMARRAY® PNEUMONIA PANEL *PLUS* ASSAY FOR SYNDROMIC TESTING OF LOWER-RESPIRATORY TRACT INFECTIONS IN A LOW-MIDDLE-INCOME SETTING

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Running title

Utility of BioFire for LRTI in LMIC

ABSTRACT

Background

Determining lower respiratory tract infection (LRTI) aetiology is complex. Culture-based methods are laborious with poor sensitivity. Molecular assays improve detection of potential pathogens, but incorrect interpretation of results may lead to inappropriate antimicrobial therapy.

Methods

The utility of the BioFire® FilmArray® Pneumonia Panel *plus* (FA-PP) to detect LRTI pathogens, and the potential impact on antimicrobial stewardship in a low-resource setting, were assessed. Routine LRT samples were included from adult patients with clinically suspected LRTI or with a concomitant blood culture at Groote Schuur Hospital and referring facilities. Culture and FA-PP results were compared, and pharmacy data analysed to determine appropriateness of antibiotic therapy.

Results

There was an 80% correlation between cultured LRTI pathogens and the FA-PP bin $\geq 10^7$ results. Compared with culture, the FA-PP detected substantially more pathogens (86.6% versus 17.9%) and produced a combined 100% positive percent agreement, and 88% negative percent agreement. The FA-PP detected bacterial/viral coinfections in 27% of samples. Correlation of FA-PP results with pharmacy data (n = 69) indicated a potential antibiotic change in 75% of cases, but this is difficult to accurately characterize without a 'gold standard' for treatment or complete clinical data.

Conclusions

The FA-PP increased the number of positive samples with typical bacteria, but the semi-quantitative reporting algorithm does not describe the correlation between the different bin values and colonization versus infection. This complicates result interpretation and may lead to inappropriate antimicrobial treatment. This study highlights the potential positive impact of rapid molecular assays for routine care in lower-income settings, but also underscores the interpretive challenges associated with these tests.

INTRODUCTION

Antibiotics are the cornerstone for pneumonia therapy and target the most common bacterial causes. It is often difficult to discern clinically and/or radiologically whether the cause of the pneumonia is viral, bacterial, or even non-infectious.[1] In the setting of community-acquired pneumonia (CAP) there is emerging evidence that viruses play a greater role than initially considered and implies that antibiotics may be unnecessarily prescribed in many instances.[1] Overprescribing of broad-spectrum antibiotics also occurs in the hospital setting where up to 50% of in-hospital patients may not have bacterial pneumonia.[2] This indiscriminate use of antibiotics may promote the development or selection of antimicrobial resistance (AMR), one of the leading WHO global health threats.[3]

CAP in adults can be caused by various bacteria, viruses, fungi and mixed infections may even occur. The South African guideline for managing CAP in adults report that *Streptococcus pneumoniae* is the most common bacterial cause, followed by *Haemophilus influenzae, Staphylococcus aureus* and *Klebsiella pneumoniae*.[4] Although the aetiology of CAP is highly variable between different areas, similar trends are observed globally.[3] Atypical bacteria such as *Legionella* and *Mycoplasma* species are less prevalent, but periodic increases in incidence and outbreaks are known to occur.[4] Some patients with CAP are more predisposed to infection by particular organisms for example patients with aspiration pneumonia are more likely to develop CAP due to anaerobic bacteria and patients with structural lung disease or underlying malignancies are more prone to infections with *Pseudomonas aeruginosa*.[4] Before the Coronavirus disease (COVID-19) pandemic, it was reported that Influenza virus was the most common cause of viral pneumonia in adults, followed by other respiratory viruses such as Respiratory Syncytial Virus, Rhinovirus and Adenovirus.[4] Due to the high prevalence of HIV in South Africa, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* are also important causes of pulmonary infections in our setting.[4]

The aetiology of hospital acquired pneumonia varies between different regions globally, but the most frequent pathogens isolated include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* species, and members of the *Enterobacterales* order.[3,5] The Gram-negative bacteria tend to be multi-drug resistant or even extensively drug resistant and are associated with higher mortality rates.[6]

Identifying the bacterial aetiology of lower respiratory tract infection (LRTI) in clinical respiratory samples using conventional culture methods is laborious and can take up to 5 days to finalize complex cultures and determine susceptibility profiles.[7] Implementing antimicrobial stewardship (AMS) principles in these settings is therefore challenging, and broad-spectrum antibiotics are generally continued until conventional microbiology results are available.[7,8]

Various factors contribute to the sensitivity and turnaround times of conventional cultures. These include failure to isolate the aetiological agent due to antimicrobial exposure prior to obtaining the sample, overgrowth of contaminating or normal microbiota, which may mask the pathogen, as well as failure of routine culture media to support the organism-specific growth requirements of atypical bacteria.[9,10]

Molecular methods have become an attractive alternative to conventional culture methods to detect bacterial pathogens. These tests can overcome the limitations of conventional microbiological methods by providing rapid and more sensitive results without the need for viable organisms. This may assist in making clinical decisions earlier, improve patient outcomes, and ultimately reduce the unnecessary use of antimicrobial agents.[8,11–13]

One such syndrome-specific system, which has been evaluated in numerous studies, is the BioFire® FilmArray® Pneumonia Panel *plus* (FA-PP; bioMérieux, Marcy l'Étoile, France). It is a rapid, cartridge-based, multiplex PCR assay detecting nucleic acids from various bacterial and viral respiratory pathogens causing pneumonia, as well as common AMR genes. This assay is approved for respiratory samples such as sputum, endotracheal aspirate (TA) and bronchoalveolar lavage (BAL) samples,[14] and is US FDA-cleared, CE-marked for *in vitro* diagnostic medical devices and Therapeutic Goods Administration-certified for diagnostic use.

This assay includes 15 typical pneumonia-causing bacteria (typical bacteria) that are reported semi-quantitatively, and 9 viruses and 3 atypical bacteria that are reported qualitatively. It also includes AMR genes for MDR organisms such as extended spectrum beta-lactamase (ESBL)- and carbapenemase-producing organisms and MRSA. The presence of AMR genes is reported qualitatively, but only when an associated bacterium is simultaneously detected by the panel. The manufacturer reported performance of the assay was as follows: The sensitivity and specificity for pathogens was 75-100% and 87.2-100% depending on the specimen type and target. The positive percent agreement (PPA) and negative percent agreement (NPA) for AMR genes was 80-100% and 87.5-100% depending on the target.[14]

Development of commercial multiplex panels for the diagnosis of LRTI is rapidly progressing, but limited data are available to guide informed clinical decision-making regarding the utility of rapid diagnostic tests (RDTs) in low/middle-income countries (LMICs). To address this gap, this single-centre, cross-sectional pilot study was performed, determining the diagnostic utility of the BioFire® FA-PP panel for detecting LRTIs to assess the potential AMS impact, pertaining to organism identification and/or mechanism of resistance.

METHODS

Design

A single-centre, cross-sectional, laboratory diagnostic study comparing the results of conventional microbiological investigations of LRTI specimens with that of the BioFire® FA-PP panel.

Setting

The study was conducted at the National Health Laboratory Services (NHLS), Microbiology Laboratory, C18, located at Groote Schuur Hospital (GSH), Cape Town, South Africa. GSH provides tertiary and quaternary care for a large population in the City of Cape Town Metropolitan, and both the hospital and the on-site NHLS laboratory serve as a referral centre for regional and district hospitals.

Ethics

Ethics approval for this work was granted by the University of Cape Town Human Research Ethics Committee (HREC reference number: 769/2020) and a waiver of the requirement for informed consent was obtained.

Study population

The study population included adult (≥18 years) inpatients and outpatients at GSH or any of the referring healthcare facilities. Samples were collected between 24 February and 3 April 2021 using a convenience sampling method. All LRTI (47 tracheal aspirates and 78 sputum) samples submitted to the NHLS laboratory by the treating clinician as part of routine patient care for routine microbiological investigations were considered for inclusion. Respiratory samples were included in the study if they included clinical suspicion of an LRTI as stated on the laboratory request form. Since the suspicion of LRTI is rarely indicated on laboratory request forms, this study included all respiratory tract samples without a recorded clinical indication, where a blood culture was submitted within 2 days of the respiratory tract sample, to serve as a proxy for sepsis attributable to severe respiratory infection. All selected samples had to be of sufficient volume to perform standard diagnostic tests and additional tests requested by the treating clinician, with a minimum of 500 µL residual sample for the FA-PP assay. Duplicate samples were only included if a previous sample from the same patient had been included >10 days prior.

Sample size

Using the MKmisc R package[15] to run the power diagnostic test script with the selected criteria of $\alpha = 0.05$, power = 0.8 and $\delta = 0.1$, an approximate sample size of 136 specimens was

required to determine the PPA and NPA assuming 95% sensitivity for the FA-PP assay based on published literature and an expected prevalence of detectable pathogens in 50% of selected samples.

Laboratory procedures

LRT samples that met the inclusion criteria were processed within 48 h of sample receipt at the laboratory and were refrigerated prior to processing. Routine microbiological investigations included Gram stain microscopy, culture and susceptibility testing (MC&S). The quality of sputum samples was assessed using the Bartlett scoring system, and samples with a score <1 were excluded from being processed for culture.[16] For semi-quantitative culture, chocolate blood agar, Colgent (Columbia blood agar with gentamicin) with the addition of a 10 µg/mL optochin disc, and a MacConkey agar plate (MCC) were inoculated and incubated in a carbon dioxide incubator, with the MCC agar plate aerobically, both at 35°C for 24 h. Respiratory pathogens were identified and graded by trained laboratory technologists and followed up according to standard operating procedures. The VITEK 2 instrument (bioMérieux, Marcy l'Étoile, France) was used to identify any bacterial growth deemed significant, and their susceptibility profiles determined and interpreted using the CLSI 2021 guidelines.[17]

The FA-PP assay was performed according to the manufacturer's instructions using 200 µl of sample. Approximately 500 µl of the residual sample was aliquoted once all routine laboratory testing was complete. The aliquot was refrigerated if the FA-PP assay was performed on the same day of sample receipt and routine laboratory investigations, otherwise the aliquot was stored at -80°C until the FA-PP assay could be performed. The amount of FA-PP runs that could be performed per day was limited by the instrument capacity. The remainder of the sample were also stored at -80°C for repeat testing if needed. The results of the FA-PP assay and routine laboratory testing were recorded by the investigator once all tests were finalized. FA-PP assay results were not communicated to the laboratory staff to prevent bias and the investigator only informed the treating clinicians of notifiable organisms detected.

Data collection and analysis

The results obtained from routine laboratory testing and the FA-PP assay, as well as basic patient demographics (age, gender, hospital, ward) available on the laboratory information system (TrakCare), and the electronic prescribing data obtained from the pharmacy, were captured in a Microsoft Excel spreadsheet. Descriptive statistical analyses were performed using Microsoft Excel and Stata software version 17.0 (StataCorp, College Station, TX, USA).

The FA-PP assay is able to detect common bacterial pathogens causing pneumonia and these were classified as either 'typical' or 'atypical'. The presence and absence of each typical

bacterial target as detected by the two methods were collated and the PPA and NPA were calculated for each bacterial target using GraphPad Prism version 9.3.1.

The FA-PP assay reports typical bacteria semi-quantitatively and this function is based on a binning algorithm where the relative number of PCR amplicons is measured and compared with an internal standard curve. Bins are reported as 10^4, 10^5, 10^6 or $\geq 10^7$ copies/mL, and each bin represents a density range of about 1 log unit, with upper and lower limits, for example: the 10^4 bin is equivalent to $10^{3.5}$ to $10^{4.5}$ copies/mL.[11,14]

The detection of AMR genes was compared with the phenotypic susceptibility test results obtained from the VITEK 2. Atypical bacteria in the FA-PP panel are not routinely tested for in the diagnostic laboratory and were therefore not compared with other methods.

Pharmacy records of included patients, where available, were reviewed by a clinical microbiologist to assess whether the prescribed antibiotic was appropriate for the FA-PP pathogens detected, based on current clinical microbiological practices. After completion of the FA-PP assay, and due to incomplete clinical data, the following assumptions were made for this analysis: (1) only typical bacteria detected in bin $\geq 10^7$ were considered significant; (2) all atypical bacteria detected on the FA-PP assay were regarded as significant; (3) resistance genes detected were only considered significant if a corresponding typical organism was also detected at bin $\geq 10^7$; (4) all antibacterial agents issued on the same day or within 2 days of sample collection were assessed for appropriateness; (5) if no antibiotics were issued on the same day or within 2 days of sample collection, antibacterial agents prescribed prior to sample collection were assessed, but only if sufficient doses were prescribed to overlap the sample collection day; (6) all antibacterial agents were prescribed for an LRTI. The bin $\geq 10^7$ value was considered significant as previous studies reported the overestimation of quantification using the FA-PP assay, when compared with culture.[10,18,19] Additionally more than 85% of bacteria considered as significant by culture were also reported with a bin $\geq 10^6$ or bin $\geq 10^7$ by Gastli *et al*.[20] and Yoo *et al*.[9], respectively. This approach was also based on the established practice in phenotypic microbiology of grading growth abundance and assigning significance to predominant growth or growth beyond a threshold. In other words, bacteria responsible for the respiratory tract infection will be present in higher bacterial loads compared to colonizing bacteria. The potential impact on patient treatment, using the results obtained using the FA-PP assay, were classified as ‘no change’, ‘escalation’, ‘de-escalation’ or ‘discontinuation’.

RESULTS

A total of 125 LRT samples (47 TAs and 78 sputum samples) were collected during the 5 week study period. The samples were collected from 123 patients with suspected LRTI, admitted or treated as an outpatient at GSH and from 11 surrounding referral hospitals. Most samples were collected from ICU patients, followed by general ward patients (Table 1).

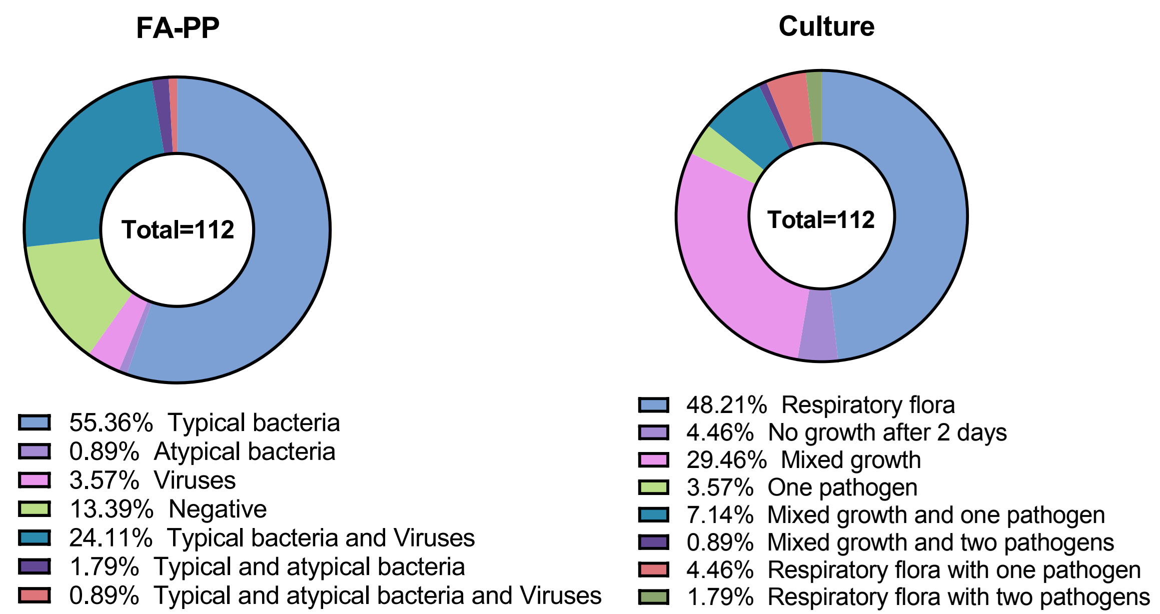
TABLE 1. Sample and patient characteristics

	Number (n=125)	Percentage
Sample type		
Sputum	78	62.4
Tracheal aspirate	47	37.6
Ward type		
ICU	47	37.4
General ward	43	34.4
Emergency centre	22	17.6
Outpatient clinic	3	2.4
Not indicated	10	8.0
Gender		
Male	80	64.0
Female	45	36.0
Age – years (Range)		
Combined median	47 (18-90)	
Male median	47 (18-90)	
Female median	45 (18-81)	

Of the 125 samples collected, 13 were rejected for further routine testing based on the Bartlett test results. The FA-PP assay detected pathogens in 86.6% (97/112) of the samples that were tested with both methods, and this included typical bacteria in 55.4% (62/112), atypical bacteria in 0.9% (1/112), viruses in 3.6% (4/112) and coinfections in 26.8% (30/112) of the samples. Multiple typical bacterial targets (ranging from 2 to 8) per sample were detected in 51.8% (58/112) of samples, whereas only a single typical bacterial target per sample was

detected in 30.4% (34/112) of samples. Routine culture only detected pathogens in 17.9% (20/112) of the samples tested (Figure 1).

FIGURE 1: Proportions of pathogens detected using the FA-PP and routine laboratory testing.



Organisms not included in the FA-PP assay panel were detected in three samples using routine culture, including *Aeromonas hydrophila, Corynebacterium striatum* and *Morganella morganii*. The most commonly detected pathogen using both methods was *Haemophilus influenzae*, with 38.4% of samples using the FA-PP assay, and in 4.5% samples with routine testing. The second most common pathogen detected by the FA-PP was *Staphylococcus aureus*, while the second most common pathogens detected by culture were both *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (Table 2).

TABLE 2. Typical and atypical bacteria detected, with PPAs and NPAs

Typical bacteria	**Culture (n)**	**FA-PP (n)**	**PPA (95% CI)**	**NPA (95% CI)**
Haemophilus influenzae	5	43	100 (57-100)	64 (55-73)
Staphylococcus aureus	1	30	100 (5-100)	74 (65-81)
Streptococcus pneumoniae	4	28	100 (51-100)	78 (69-85)
Klebsiella pneumoniae	1	27	100 (5-100)	77 (68-83)
Enterobacter cloacae complex	2	18	100 (18-100)	85 (78-91)
Acinetobacter calcoaceticus-baumannii complex	2	16	100 (18-100)	87 (80-92)
Pseudomonas aeruginosa	4	11	100 (51-100)	94 (87-97)
Escherichia coli	0	10	-	91 (84-95)
Moraxella catarrhalis	0	9	-	92 (85-96)
Proteus species	0	6	-	95 (89-98)
Klebsiella oxytoca	0	4	-	96 (91-99)
Serratia marcescens	1	4	100 (5-100)	97 (92-99)
Klebsiella aerogenes	0	3	-	97 (92-99)
Streptococcus agalactiae	0	3	-	97 (92-99)
Streptococcus pyogenes	0	2	-	98 (94-100)
Total	**20**	**214**	**100 (84-100)**	**88 (87-90)**

Atypical bacteria	**Culture (n)**	**FA-PP (n)**
Legionella pneumophila	4[a]	4
Chlamydia pneumoniae	N/A	0
Mycoplasma pneumoniae	N/A	0
Total		**4**
Viral pathogens	**PCR (n)**	**FA-PP (n)**
Human Rhinovirus/Enterovirus	N/A	19
Parainfluenza virus	N/A	5
Coronavirus	N/A	4
Respiratory Syncytial Virus (RSV)	N/A	4
Adenovirus	N/A	2
Human Metapneumovirus	N/A	0
Influenza A	N/A	0
Influenza B	N/A	0
Middle East Respiratory Syndrome Coronavirus (MERS)	N/A	0
Total		**34**
Resistance genes	**Phenotypic (n)**	**FA-PP (n)**
CTX-M	3[b,c]	16

mecA/C and MREJ	0	6
NDM	2[c,d]	5
OXA-48 like	0	3
IMP	0	0
KPC	0	0
VIM	0	0
Total	**5**	**30**

N/A, not applicable

a 2 out of the 4 *L. pneumophila* detections confirmed with positive *L. pneumophila* serogroup 1 urinary antigen test, the rest confirmed with a PCR test for atypical pneumonia by the NICD as non-serogroup 1.

b Carbapenem susceptible *P. aeruginosa* (wild-type) was detected by routine laboratory testing, but FA-PP detected CTX-M, NDM, and OXA-48 like genes in the presence of *Acinetobacter* complex, *K. pneumoniae* and *P. aeruginosa*. Assumption that *P. aeruginosa* only associated with CTX-M.

c Carbapenem resistant *Acinetobacter baumannii* detected by routine laboratory testing, but FA-PP detected CTX-M and NDM genes in the presence of *Acinetobacter* complex and *K. pneumoniae*. Assumption that *A. baumannii* associated with both genes.

d Carbapenem resistant *Acinetobacter baumannii* detected by routine laboratory testing, but FA-PP detected the NDM gene in the presence of *Acinetobacter* complex and *P. aeruginosa*. Assumption made that *A. baumannii* associated with the NDM gene.

The most commonly detected virus was human rhinovirus/enterovirus. No tests for respiratory viruses, except for SARS-CoV-2, were requested as part of routine care and thus no comparison between methods was possible. The SARS-CoV-2 is not included in the FA-PP assay. *Legionella pneumophila* was detected in four samples; for three of these patients a *Legionella* urinary antigen test (serogroup 1) was not requested as part of routine testing. A confirmatory *Legionella* urinary antigen test (serogroup 1) was performed by the investigator following discussion of the FA-PP results with the treating clinicians. Two of these samples tested negative for *L. pneumophila* serogroup 1 and were later confirmed by the reference laboratory (National Institute for Communicable Diseases, NICD) as *L. pneumophila* non-serogroup 1.

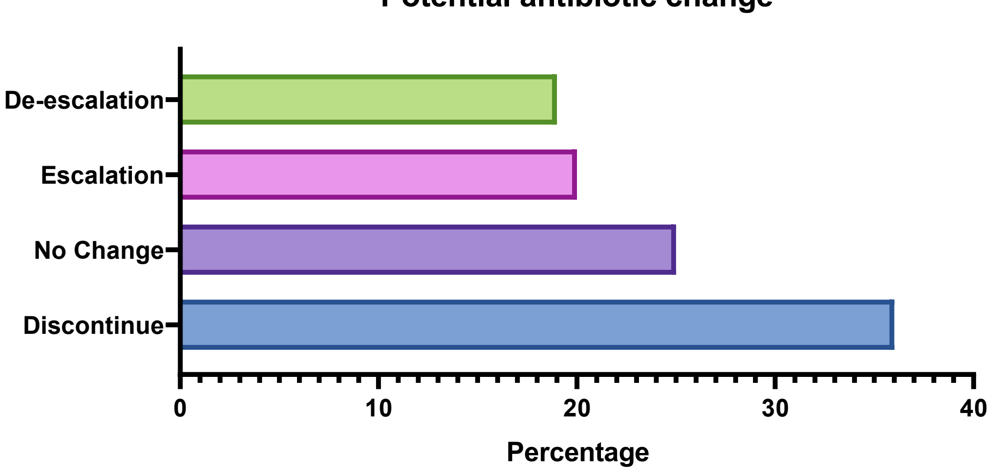
A total of 30 resistance genes were detected using the FA-PP assay. Of these, 22 samples had one gene detected, 1 sample had two genes, and 2 samples had three genes. The $bla_{\text{CTX-M}}$ gene was detected most commonly (14.3%), followed by *mecA/C* (5.4%), bla_{NDM} (4.5%) and $bla_{\text{OXA-48-like}}$ (2.7%).

The FA-PP detected pathogens in 11/13 sputum samples that were rejected for routine culture due to a low Bartlett score. This produced a total of 17 typical bacteria (ranging from 1 to 3 per sample) and 8 viral pathogens. Two samples also contained either the $bla_{CTX\text{-}M}$ or $bla_{OXA\text{-}48}$ resistance gene.

The qualitative assessment of the FA-PP assay to detect typical bacterial targets demonstrated a combined 100% PPA and 88% NPA compared with routine culture (Table 2). In 18/20 samples where bacteria were detected with both methods, the FA-PP reported a bin $\geq 10^7$ result. The concordance for semi-quantitation of the two methods was not determined due to the low number of pathogens detected by the routine laboratory testing.

Pharmacy data were available for 69/125 samples (67 participants) and in 75% (n = 52) of these cases a potential antibiotic change was possible based on the FA-PP assay results (Figure 2).

FIGURE 2: Potential antibiotic change based on FA-PP findings.
De-escalation, narrower-spectrum agents(or fewer agents) indicated; escalation, broader-spectrum agents (or additional agents) indicated; no change, agents appropriate; discontinue, no targets detected, only viral pathogens detected or bacteria detected with a bin value of less than 10^7.



DISCUSSION

Compared with conventional microbiology, novel and rapid panel-based diagnostic strategies offer clear advantages of a shorter turnaround time, increased sensitivity and the detection of fastidious microorganisms, including AMR determinants, and may thus offer substantial improvements in patient care. Numerous studies report the performance and benefits of respiratory syndromic panels such as the FA-PP assay; however, most were performed in

high-income countries using BAL specimens and samples collected from patients admitted to ICUs.[10,18,19,21] Additionally, the impact on patient-level outcomes have yet to be determined.[22]

We aimed to assess the utility of the FA-PP assay using all routine LRT samples submitted to a South African laboratory for routine microbiological investigations. The samples included were collected at various healthcare settings in order to assess patients with a range of disease severities. More than half of the samples collected were sputum samples. The routine laboratory testing had a poor yield, where ≥1 pathogen/s were detected in less than a quarter of samples. More pathogens were detected in TA samples, but 'mixed growth' or 'respiratory flora' were reported in a large number of both TA and sputum samples, which is common for these sample types.

The poor diagnostic yield of sputum samples from patients with suspected CAP is well described and for this reason the collection is discouraged in many guidelines, including the South African guideline for managing CAP[4] and ATS/IDSA[23], which indicate that MC&S has a limited impact on patient management and outcome and should only be requested in cases of severe disease and where there is a high risk for an infection with nosocomial pathogens.[23] In contrast, the IDSA guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia promote the collection of non-invasive samples over invasive samples due to the lack of evidence that invasive sampling improves clinical outcome.[24] Unfortunately, these non-invasive sample types are prone to contamination with upper respiratory tract commensals or colonizing microorganisms, especially in patients with chronic tracheostomies, where the tracheostomy tube is colonized.[11,13] A BAL may theoretically provide a 'superior' quality result due to the site-directed collection[13] limiting contamination, which may imply organisms cultured from these samples will most likely better reflect the true pathogen causing the LRTI, which further simplifies interpretation of the laboratory report. BAL is, however, not a realistic option for all patients due to limited resources in lower-income settings, as well as the invasiveness and complicated nature of specimen collection.

The use of the FA-PP assay increased the number of samples where typical bacteria were detected by 68.7%. This dramatic increase may appear as an increase in sensitivity over culture; however, we detected a concerning amount of additional typical bacteria, not detected with culture, using the FA-PP (194/214). A similarly increased positivity rate (63.3%) was also reported by Buchan *et al*.[10] However, that study was much larger and assessed 259 BAL samples, but only reported 73 additional bacterial targets. This finding is likely attributed to the non-invasive sample types in our study, which probably contained more contaminating commensals.

The FA-PP assay produced a PPA of 100% for 8/15 of the typical bacteria detected by culture, which was comparable with other studies evaluating the FA-PP. Buchan *et al*.,[10] Lee *et al*.,[19] Ginocchio *et al*.,[18] Mitton *et al*.,[25] Weber *et al*.[21] and Yoo *et al.*[9] all reported a PPA (or sensitivities) for bacterial pathogens of ≥90%. The combined NPA was 88% for the typical bacteria, with all targets having an NPA ≥64%, which was lower than those reported in the above studies, except for Yoo *et al*.,[9] who reported a specificity of 76.5%. Due to the lack of a 'gold standard' diagnostic test for LRTI these detections cannot, however, be described as false positives.[19]

Unfortunately, the interpretation of these additional detections using the FA-PP assay is complicated by a number of factors. PCR-based assays are more sensitive than routine culture and can detect genetic material of both viable and non-viable organisms. This is advantageous where antibiotics were administered prior to sample collection leading to some organisms being undetectable by culture. Since there were no records of antibiotics prior to sample collection or time of antibiotic administration for the participants in this study, no correlations could be made.

In cases where bacteria are cultured, the laboratory's protocol will guide reporting. Bacterial isolates that are grown in insufficient quantities, mixed with more than two potential pathogens, or where the growth is overwhelmed by normal respiratory microbiota may not be regarded as significant, and therefore not reported. Bacteria such as *H. influenzae* are fastidious to culture and may easily be overgrown by normal microbiota or lose viability[18] and may as a result be missed by culture. *H. influenzae* was the most common pathogen detected by both methods, but the FA-PP detected *H. influenzae* in 38 additional samples. The FA-PP also detected 29 additional samples with *S. aureus*. A similar finding was also described by Buchan *et al*.[10] and Ginocchio *et al*.[18]

The quality of the sample is also vital to ensure that significant typical bacterial pathogens are not missed by routine laboratory testing. We included 13 sputum samples that were rejected for routine culture in order to assess the value of these samples for detecting potential pathogens. Typical bacteria were detected by the FA-PP assay in nine of these samples and this most likely reflects contamination or colonization in the absence of inflammation. This illustrates the importance of quality assessment prior to culture or molecular tests for typical bacteria, because neither of these methods are able to separate colonizers from invasive pathogens.[13] However, quality scoring systems for sputum samples do not apply in LRTIs caused by atypical bacteria and viruses.[26] In fact, viral pathogens were detected in eight of these samples that were rejected due to poor quality. This demonstrates that future studies need to assess the value of a quality scoring system for the FA-PP to enable optimal detection of all clinically relevant pathogens.

The semi-quantitative reporting of bacterial results using the FA-PP assay, which is intended to simplify this discrimination, lacks the ability to definitively interpret organisms that may be colonizers.[11] Nearly all of the typical bacteria reported by the routine testing correlated with an FA-PP bin of $\geq 10^7$, but the majority of all the detected typical bacteria were also reported with a bin of $\geq 10^7$. In addition to the large number of typical bacteria that were detected per sample, a combination of bacterial and viral pathogens was also detected in 27% of samples. This further complicated the interpretation of results, since coinfections with bacterial and viral pathogens are possible, but the detection of a viral pathogen can also be due to asymptomatic carriage or a recent viral infection that led to a subsequent secondary bacterial infection.[27]

The rapid detection of AMR genes can lead to earlier escalation or de-escalation of therapy in patients, as well as earlier infection prevention and control interventions. Although the FA-PP assay detected resistance genes in some of the samples, the pathogen it was associated with was not always detected by culture and the results could therefore not be compared.

The assessment of potential impacts on antibiotic therapy using the FA-PP results was based on typical bacteria detected with a bin $\geq 10^7$ only, associated resistance genes, atypical bacteria and viruses, by applying local microbiology practices. In clinical practice, multiple factors must be considered before an antibiotic change is made. In this retrospective assessment with limited clinical information, we identified 52/69 cases where a potential antibiotic change was possible including de-escalation (19%), escalation (20%) and discontinuation of antibiotics (36%). This may have significant impacts on patient outcomes, AMS and infection prevention and control interventions.

As described by Hanson *et al*.,[13] the enhanced detection of a multiplex nucleic acid amplification test decreases the likelihood that important pathogens are missed, but also complicates result interpretation and ultimately patient management. The use of the FA-PP assay on routine samples and the reporting of results without the input of a clinical microbiologist or infectious diseases specialist may lead to overtreatment due to the large number of additional bacterial detections with unclear clinical significance. Advocating the use of these diagnostic assays without firmly establishing criteria for which patients would benefit most, how to meaningfully interpret the results, and how to treat accordingly, could in fact be counterproductive with regard to diagnostic 'best practice' and AMS.

This study had several limitations, most importantly the lack of a 'gold standard test', which complicated the comparison of methods. The small sample size produced wide CIs for comparisons and no definite conclusions could be made. The comparison was also limited by the poor yield of the bacterial culture as well as the lack of testing for viral pathogens and atypical bacteria as part of clinical practice. Complete records of clinical data would have

improved the assessment of potential antibiotic changes; also, information on antimicrobial exposure prior to sample collection would have simplified interpretation of the results. We were unable to categorise the cases as community-or hospital-acquired pneumonia due to the lack of clinical data, but this information would have improved the assessment of the FA-PP utility. We were also unable to compare the cost differential between the methods, and therefore definitive recommendations as to the value-added benefit of introducing such an assay in our setting cannot be made.

Future work should focus on determining semi-quantitative values where relative pathogen abundance may assist in determining the significance of a specific pathogen. Similarly, the timing of the specimen in the course of disease may be a confounder and requires elucidation.

CONCLUSIONS

Despite the limitations in our study, the FA-PP assay substantially increased the number of positive samples with typical bacteria. A potential antibiotic change was possible in 75% of cases. Our study highlights the potential impact of introducing rapid molecular assays in routine care in settings such as ours but underscores the interpretive challenges associated with novel rapid tests.

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TRANSPARENCY DECLARATIONS

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

M.V.D.W., C.M. and N.S. designed the study. M.V.D.W. performed the laboratory tests, analysed and interpreted the data. M.V.D.W., C.M., N.S. and A.B. wrote the manuscript.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, M.V.D.W, upon reasonable request.

DISCLAIMER

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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SUPPORTING INFORMATION

Appendix A: Additional tables

This table contains the routine MC&S and FA-PP results for each sample included in the study.

Sample	Sample type	Microscopy					General culture results																				
		Neutrophils (grading)	Epithelial cells (grading)	Mucus (grading)	Bartlett score	Organisms observed	Culture result and/or amount of pathogens	Non FA-PP bacteria	FA-PP ACIBA	BIN ACIBA	Culture ACIBA	Grade ACIBA	FA-PP ENCLO	BIN ENCLO	Culture ENCLO	Grade ENCLO	FA-PP ECOLI	BIN ECOLI	Culture ECOLI	Grade ECOLI	FA-PP HAEIN	BIN HAEIN	Culture HAEIN	Grade HAEIN	FA-PP KLEAE	BIN KLEAE	Culture KLEAE
001	Sputum	3	2	1	2	Mix_oro	Mixgr+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
002	Sputum	1	1	1	1	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
003	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004	Sputum	1	1	1	1	Mix_oro	Mixgr	0	0	0	0	0	1	10^4	0	0	0	0	0	0	1	10^7	0	0	0	0	0
005	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
006	Sputum	3	1	1	3	Mix_oro	Mixgr	0	1	10^5	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
007	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
008	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009	Sputum	2	1	1	2	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	1	10^5	0	0	1	10^7	0	0	0	0	0
010	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
011	Sputum	3	3	1	1	Mix_oro +GNB	Mixgr	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0
012	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
013	Sputum	3	1	1	3	Mix_oro	Mixgr	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0
015	Sputum	3	3	1	1	Mix_oro	Mixgr	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0
016	Sputum	2	1	1	2	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
017	Sputum	3	3	0	0	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
018	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr+2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	1	10^7	0
019	Sputum	N/A	N/A	N/A	N/A	N/A	Mixgr+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
020	TA	2	N/A	N/A	N/A	Mix_oro	Mixgr+1	0	1	10^7	0	0	1	10^5	0	0	1	10^6	0	0	1	10^7	0	0	0	0	0
021	TA	3	N/A	N/A	N/A	Mix_oro+GNB	Mixgr+1	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
022	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr+1	0	1	10^7	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	1	10^7	0	0	0	0	0	0	1	10^7	0	0	0	0	0
024	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	1	10^5	0	0	1	10^4	0	0	0	0	0	0	1	10^6	0	0	0	0	0
025	TA	3	N/A	N/A	N/A	GPCB	Mixgr+1	MOGMM	0	0	0	0	1	10^7	0	0	1	10^7	0	0	1	10^7	0	0	1	10^7	0
026	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	1	10^7	0	0	1	10^7	0	0	1	10^5	0	0	0	0	0
027	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
028	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0
029	TA	3	N/A	N/A	N/A	Mix_oro+GNB	Mixgr	0	1	10^7	0	0	1	10^5	0	0	1	10^7	0	0	0	0	0	0	0	0	0
030	TA	1	N/A	N/A	N/A	Mix_oro	Mixgr+1	0	0	0	0	0	1	10^7	1	2	0	0	0	0	0	0	0	0	0	0	0
031	Sputum	3	3	1	1	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032	Sputum	2	2	1	1	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
033	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
034	Sputum	3	3	1	1	Mix_oro	Mixgr	0	0	0	0	0	1	10^4	0	0	1	10^4	0	0	1	10^4	0	0	0	0	0
035	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036	Sputum	2	1	1	2	Mix_oro	Mixgr	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
038	Sputum	3	1	1	3	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0
040	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041	TA	3	N/A	N/A	N/A	NBO	Mixgr+1	CORST	1	10^4	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0
042	TA	3	N/A	N/A	N/A	Mix_oro	Mixgr	0	0	0	0	0	1	10^4	0	0	1	10^5	0	0	0	0	0	0	0	0	0
043	Sputum	3	1	1	3	Mix_oro+GNB	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0
044	TA	3	N/A	N/A	N/A	GNC	1	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045	TA	3	N/A	N/A	N/A	NBO	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
046	TA	3	N/A	N/A	N/A	NBO	1	0	1	10^5	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0
047	TA	2	N/A	N/A	N/A	NBO	NG2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
048	TA	3	N/A	N/A	N/A	NBO	NG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
049	TA	3	N/A	N/A	N/A	NBO	NG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
050	TA	3	N/A	N/A	N/A	Mix_oro	NG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051	TA	1	N/A	N/A	N/A	NBO	NG2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052	Sputum	3	1	1	3	Mix_oro	Resp+2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0
053	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
054	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
055	Sputum	3	1	1	3	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
056	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0
057	Sputum	3	2	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
058	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
059	Sputum	3	3	1	1	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
060	Sputum	3	1	1	3	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
061	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
062	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0
063	Sputum	3	1	1	3	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
064	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
065	Sputum	1	1	1	1	Mix_oro	Resp	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
066	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
067	Sputum	3	2	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
068	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
069	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	1	10^4	0	0	0	0	0	0	1	10^6	0	0	0	0	0
070	Sputum	3	2	0	1	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
071	TA	3	N/A	N/A	N/A	Mix_oro+GNCB	Resp+1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0
072	Sputum	N/A	N/A	N/A	N/A	N/A	Resp+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
073	TA	3	N/A	N/A	N/A	Mix_oro+GNCB	Resp+2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0
074	TA	3	N/A	N/A	N/A	Mix_oro	Resp+1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
075	TA	1	N/A	N/A	N/A	Mix_oro	Resp	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
076	TA	1	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
077	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
078	TA	2	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
079	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0
080	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
081	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
082	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
083	Sputum	3	2	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
084	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
085	Sputum	2	1	1	2	Mix_oro	Resp+1	0	0	0	0	0	1	10^5	1	2	0	0	0	0	0	0	0	0	0	0	0
086	Sputum	3	2	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
087	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
088	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
089	Sputum	3	1	1	3	Mix_oro	Resp	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
090	Sputum	3	3	1	1	Mix_oro	Resp	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0
091	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
092	Sputum	3	1	1	3	Mix_oro	Resp	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
093	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
094	Sputum	2	1	1	2	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
095	Sputum	3	1	1	3	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
096	Sputum	1	1	1	1	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
097	Sputum	1	1	1	1	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
098	TA	3	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
099	TA	2	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	TA	2	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	TA	0	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	TA	3	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	TA	1	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
105	TA	2	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	TA	3	N/A	N/A	N/A	Mix_oro+GNB	Resp+1	AERHG	0	0	0	0	0	0	0	0	1	10^5	0	0	1	10^5	0	0	0	0	0
107	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	TA	2	N/A	N/A	N/A	NBO	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	TA	3	N/A	N/A	N/A	Mix_oro	Resp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	Sputum	1	2	1	0	Mix_oro	Saliva (Poor quality)	0	1	10^4	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^7	N/A	N/A	0	0	N/A
114	Sputum	1	3	0	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^6	N/A	N/A	0	0	N/A
115	Sputum	2	2	0	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	1	10^5	N/A	N/A	0	0	N/A	N/A	1	10^7	N/A	N/A	0	0	N/A
116	Sputum	1	2	1	0	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
117	Sputum	2	3	1	0	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
118	Sputum	1	3	0	-2	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
119	Sputum	1	2	0	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
120	Sputum	1	3	0	-2	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
121	Sputum	1	2	0	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^4	N/A	N/A	0	0	N/A
122	Sputum	2	3	1	0	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
123	Sputum	1	3	1	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
124	Sputum	1	3	1	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
125	Sputum	1	3	1	-1	Mix_oro	Saliva (Poor quality)	0	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A

Sample	Bacterial and viral targets																														
	Typical bacteria (FA-PP and culture results)																														
	Grade KLEAE	FA-PP KLEOX	BIN KLEOX	Culture KLEOX	Grade KLEOX	FA-PP KLEPN	BIN KLEPN	Culture KLEPN	Grade KLEPN	FA-PP MORCA	BIN MORCA	Culture MORCA	Grade MORCA	FA-PP PROSP	BIN PROSP	Culture PROSP	Grade PROSP	FA-PP PSEAE	BIN PSEAE	Culture PSEAE	Grade PSEAE	FA-PP SERMA	BIN SERMA	Culture SERMA	Grade SERMA	FA-PP STAAU	BIN STAAU	Culture STAAU	Grade STAAU	FA-PP STRAG	BIN STRAG	Culture STRAG
001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0	0	1	10^6	0	0	0	0	0
002	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
003	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0
004	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0
006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0
009	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0
010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0
011	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	1	10^5	0	0	0	0	0
012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0
013	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014	0	0	0	0	0	1	10^4	0	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017	0	0	0	0	0	1	10^7	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^6	0
018	0	0	0	0	0	1	10^4	0	0	1	10^7	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0
020	0	0	0	0	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	1	10^6	0	0	1	10^7	0	0	0	0	0
021	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	1	10^7	1	2	0	0	0	0	0	0	0	0	0	0	0
022	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023	0	1	10^7	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024	0	0	0	0	0	1	10^6	0	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
025	0	1	10^4	0	0	1	10^6	0	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
026	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	1	10^7	0	0	0	0	0
028	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029	0	0	0	0	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
030	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
033	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
034	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
035	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039	0	1	10^5	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
040	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
043	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
044	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0
045	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	2	0	0	0	0	0	0	0	0	0	0	0
046	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	1	2	0	0	0	0	0	0	0
047	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
048	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
049	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0
050	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
054	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
055	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
057	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
058	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
059	0	0	0	0	0	0	0	0	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
060	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
061	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
063	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
066	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
068	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
069	0	0	0	0	0	1	10^5	0	0	1	10^5	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
071	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
072	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	1	3	0	0	0
073	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
074	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0
075	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
076	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
077	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
079	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^7	0	0	0	0	0
080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
081	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
082	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
083	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
084	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
085	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
086	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
087	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
088	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
089	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
090	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
091	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
092	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
093	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
094	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
095	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
096	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
097	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
098	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
099	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^5	0	0	0	0	0
106	0	1	10^5	0	0	0	0	0	0	1	10^5	0	0	1	10^6	0	0	1	10^5	0	0	0	0	0	0	1	10^4	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10^4	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
114	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^7	N/A	N/A	0	0	N/A
115	N/A	0	0	N/A	N/A	1	10^5	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
116	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
117	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
118	N/A	0	0	N/A	N/A	1	10^6	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
119	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^5	N/A	N/A	1	10^4	N/A	N/A	0	0	N/A
120	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
121	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^4	N/A	N/A	0	0	N/A
122	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
123	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^4	N/A	N/A	0	0	N/A
124	N/A	0	0	N/A	N/A	0	0	N/A	N/A	1	10^6	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A
125	N/A	1	10^4	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	N/A

Sample										Atypical bacteria			Viruses												
	Grade STRAG	FA-PP STRPN	BIN STRPN	Culture STRPN	Grade STRPN	FA-PP STRPY	BIN STRPY	Culture STRPY	Grade STRPY	FA-PP CHLPN	FA-PP LEGPN	FA-PP MYCPN	FA-PP Adenovirus	FA-PP Coronavirus	FA-PP Human Metapneumovirus	FA-PP Human Rhinovirus/ Enterovirus	FA-PP Influenza A	FA-PP Influenza B	FA-PP MERS	FA-PP Parainfluenza virus	FA-PP RSV	FA-PP CTX-M	Phenotypic ESBL	FA-PP IMP	Phenotypic IMP
001	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
003	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
004	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
007	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
009	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
011	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
016	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
017	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
018	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
019	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
023	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
024	0	1	10^7	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
025	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
033	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
034	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
035	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
036	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
038	0	1	10^6	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
040	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
042	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
043	0	1	10^4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
044	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
045	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
046	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
047	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
048	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
049	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
050	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
054	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
055	0	0	0	0	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
056	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
057	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
058	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
059	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
060	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
061	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
063	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
064	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
066	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
068	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
069	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
070	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
071	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
072	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
073	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
074	0	1	10^7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
075	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
076	0	1	10^7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
077	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
079	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
081	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
082	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
083	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
084	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
085	0	0	0	0	0	1	10^5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
086	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
087	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
088	0	1	10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
089	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
090	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
091	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
092	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
093	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
094	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
095	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
096	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
097	0	1	10^5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
098	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
099	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
114	N/A	1	10^6	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
115	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
116	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
119	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
120	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
121	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
123	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
124	N/A	1	10^4	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
125	N/A	0	0	N/A	N/A	0	0	N/A	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Sample	Resistance genes									
	FA-PP KPC	Phenotypic KPC	FA-PP mecA/C and MREJ	Phenotypic MRSA	FA-PP NDM	Phenotypic NDM	FA-PP OXA-48 like	Phenotypic OXA-48	FA-PP VIM	Phenotypic VIM
001	0	0	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0	0	0
003	0	0	0	0	0	0	0	0	0	0
004	0	0	0	0	0	0	0	0	0	0
005	0	0	0	0	0	0	0	0	0	0
006	0	0	0	0	0	0	0	0	0	0
007	0	0	0	0	0	0	0	0	0	0
008	0	0	0	0	0	0	0	0	0	0
009	0	0	0	0	0	0	1	0	0	0
010	0	0	0	0	0	0	0	0	0	0
011	0	0	1	0	0	0	1	0	0	0
012	0	0	0	0	0	0	0	0	0	0
013	0	0	0	0	1	0	0	0	0	0
014	0	0	0	0	0	0	0	0	0	0
015	0	0	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0	0	0
017	0	0	0	0	0	0	0	0	0	0
018	0	0	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0	0	0
020	0	0	1	0	0	0	0	0	0	0
021	0	0	0	0	1	0	1	0	0	0
022	0	0	0	0	1	1	0	0	0	0
023	0	0	0	0	0	0	0	0	0	0
024	0	0	0	0	0	0	0	0	0	0
025	0	0	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0	0	0
030	0	0	1	0	0	0	0	0	0	0
031	0	0	0	0	0	0	0	0	0	0
032	0	0	0	0	0	0	0	0	0	0
033	0	0	1	0	0	0	0	0	0	0
034	0	0	1	0	0	0	0	0	0	0
035	0	0	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0	0	0
040	0	0	0	0	0	0	0	0	0	0
041	0	0	0	0	0	0	0	0	0	0
042	0	0	0	0	0	0	0	0	0	0
043	0	0	0	0	0	0	0	0	0	0
044	0	0	0	0	1	1	0	0	0	0
045	0	0	0	0	0	0	0	0	0	0
046	0	0	0	0	1	0	0	0	0	0
047	0	0	0	0	0	0	0	0	0	0
048	0	0	0	0	0	0	0	0	0	0
049	0	0	0	0	0	0	0	0	0	0
050	0	0	0	0	0	0	0	0	0	0
051	0	0	0	0	0	0	0	0	0	0
052	0	0	0	0	0	0	0	0	0	0
053	0	0	0	0	0	0	0	0	0	0
054	0	0	0	0	0	0	0	0	0	0
055	0	0	0	0	0	0	0	0	0	0
056	0	0	0	0	0	0	0	0	0	0
057	0	0	0	0	0	0	0	0	0	0
058	0	0	0	0	0	0	0	0	0	0
059	0	0	0	0	0	0	0	0	0	0
060	0	0	0	0	0	0	0	0	0	0
061	0	0	0	0	0	0	0	0	0	0
062	0	0	1	0	0	0	0	0	0	0
063	0	0	0	0	0	0	0	0	0	0
064	0	0	0	0	0	0	0	0	0	0
065	0	0	0	0	0	0	0	0	0	0
066	0	0	0	0	0	0	0	0	0	0
067	0	0	0	0	0	0	0	0	0	0
068	0	0	0	0	0	0	0	0	0	0
069	0	0	0	0	0	0	0	0	0	0
070	0	0	0	0	0	0	0	0	0	0
071	0	0	0	0	0	0	0	0	0	0
072	0	0	0	0	0	0	0	0	0	0
073	0	0	0	0	0	0	0	0	0	0
074	0	0	0	0	0	0	0	0	0	0
075	0	0	0	0	0	0	0	0	0	0
076	0	0	0	0	0	0	0	0	0	0
077	0	0	0	0	0	0	0	0	0	0
078	0	0	0	0	0	0	0	0	0	0
079	0	0	0	0	0	0	0	0	0	0
080	0	0	0	0	0	0	0	0	0	0
081	0	0	0	0	0	0	0	0	0	0
082	0	0	0	0	0	0	0	0	0	0
083	0	0	0	0	0	0	0	0	0	0
084	0	0	0	0	0	0	0	0	0	0
085	0	0	0	0	0	0	0	0	0	0
086	0	0	0	0	0	0	0	0	0	0
087	0	0	0	0	0	0	0	0	0	0
088	0	0	0	0	0	0	0	0	0	0
089	0	0	0	0	0	0	0	0	0	0
090	0	0	0	0	0	0	0	0	0	0
091	0	0	0	0	0	0	0	0	0	0
092	0	0	0	0	0	0	0	0	0	0
093	0	0	0	0	0	0	0	0	0	0
094	0	0	0	0	0	0	0	0	0	0
095	0	0	0	0	0	0	0	0	0	0
096	0	0	0	0	0	0	0	0	0	0
097	0	0	0	0	0	0	0	0	0	0
098	0	0	0	0	0	0	0	0	0	0
099	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	1	0	0	0
120	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0	0	0	0
124	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0

Abbreviations	
ACIBA	*Acinetobacter calcoaceticus-baumannii* complex
AERHG	*Aeromonas hydrophila group*
CHLPN	*Chlamydia pneumoniae*
CORST	*Corynebacterium striatum*
ECOLI	*Escherichia coli*
ENCLO	*Enterobacter cloacae* complex
ESBL	Extended spectrum beta-lactamase
FA-PP	BioFire® FilmArray® Pneumonia Panel *plus*
GNB	Gram negative bacilli
GNC	Gram negative cocci
GNCB	Gram negative cocco-bacilli
GPCB	Gram positive cocco-bacilli
HAEIN	*Haemophilus influenzae*
IMP	Imipenem beta-lactamases
KLEAE	*Klebsiella aerogenes*
KLEOX	*Klebsiella oxytoca*
KLEPN	*Klebsiella pneumoniae*
KPC	*Klebsiella pneumoniae* carbapenemase
LEGPN	*Legionella pneumophila*
MERS	Middle East Respiratory Syndrome Coronavirus
Mix_oro	Mixed oropharyngeal flora
Mixgr	Mixed growth
MOGMM	*Morganella morganii*
MORCA	*Moraxella catarrhalis*
MREJ	SCCmec right-extremity junction
MRSA	Methicillin-resistant *Staphylococcus aureus*
MYCPN	*Mycoplasma pneumoniae*
N/A	Not applicable
NBO	No bacteria observed
NDM	New Delhi metallo-beta-lactamase
NG2	No growth after 2 days
OXA-48	Oxacillinase beta-lactamases
PROSP	*Proteus species*
PSEAE	*Pseudomonas aeruginosa*
Resp	Respiratory flora
RSV	Respiratory Syncytial Virus
SERMA	*Serratia marcescens*
STAAU	*Staphylococcus aureus*
STRAG	*Streptococcus agalactiae*
STRPN	*Streptococcus pneumoniae*
STRPY	*Streptococcus pyogenes*
TA	Tracheal aspirate
VIM	Verona Integron-Encoded Metallo-Beta-Lactamase
Culture and FA-PP results	
0	Absent
1	Present
Microscopy grading	
1	Scanty
2	Moderate
3	Numerous
Culture grading	
1	Light growth
2	Moderate growth
3	Heavy growth

Appendix B: Ethics and hospital approval letter

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences

Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

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23 November 2020

HREC REF: 769/2020

Dr C Moodley
Division of Medical Microbiology
NHLS, Room 5.26, Level 5, Entrance 2
Falmouth Building
Email: c.moodley@uct.ac.za
Student: vwsmen001@myuct.ac.za

Dear Dr Moodley

PROJECT TITLE: THE UTILITY OF A MULTIPLEX PCR TO RAPIDLY DIAGNOSE VENTILATOR-ASSOCIATED PNEUMONIA (VAP) TO FACILITATE ANTIMICROBIAL STEWARDSHIP, IN A LMIC SETTING (MMED CANDIDATE: DR M VD WESTHUYZEN)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study, subject to finding out from SAHPRA if the FA-IP is a device.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 November 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Mene van der Westhuyzen will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

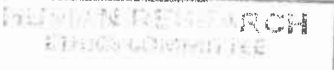
Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.



02 MAR 2021

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
☑ Approved	☑ Type of review: Expedited	☐ Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature HREC Chairperson / Designee	Date	5/3/21

Note: All major amendments must include a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown.

Comments from the HREC to the Principal Investigator:
Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	26/02/2021	
HREC REF Number	HREC REF 769/2020	
Protocol title	The utility of a multiplex PCR to rapidly diagnose Ventilator-Associated Pneumonia to facilitate antimicrobial stewardship, in a LMIC setting	
Protocol number (if applicable)	N/A	
Principal Investigator	Dr Clinton Moodley	
Department / Office Internal Mail Address	Division of Medical Microbiology, National Health Laboratory Service, Groote Schuur Hospital and University of Cape Town, South Africa	
1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	☒ Major	☐ Minor
1.2 Does this protocol receive US Federal funding?	☐ Yes	☒ No

1.3 If the amendment is a major amendment <u>and</u> receives US Federal Funding, does the amendment require full committee approval? **Note:** Any protocol amendments for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please email an electronic copy to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval. This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.
Protocol version 6, 26/02/2021

3. Protocol status (tick ✓)

☐	Open to enrolment
☒	No participants have been enrolled
☐ ☐ ☐ ☐	Closed to enrolment (tick ✓) Research-related activities are ongoing Research-related activities are complete, long-term follow-up only Research-related activities are complete, data analysis only

4. Proposed changes will affect: (tick ✓ all the categories that apply)

	Protocol
☒	Study objectives, design (including investigator's brochure, clinical activities, study length)
☐	Study instruments, questionnaires, interview schedules
☐	Sample size
☒	Recruitment methods
☒	Eligibility criteria (inclusion and exclusion criteria)
☐	Drug/device (composition, amount, schedule, route of administration, combination with other drugs/devices, safety information)
☒	Data collection/ analysis
☐	Principal Investigator. (Please attach revised conflict of interest and PI declaration statements. Refer: sections 7 and 8.4 in the New Protocol Application Form FHS013)
☐	Consent form and information sheet
☐	Recruitment materials (e.g. advertisements)
☐	Administrative (e.g. change in sponsor's name, change in contact information)
☐	Other. Please specify:

4.1 In your opinion, will there be any **increase** in risk, discomfort or inconvenience to participants?	☐ Yes	☒ No
If yes, please provide a detailed justification/explanation:		

4.2 What follow-up action do you propose for participants who are already enrolled in the study?	
☐	Inform current participants as soon as possible
☐	Re-consent current participants with revised consent/assent forms (append)
☐	No action required
☒	Other. Please describe: No participants have been enrolled

5. Detailed description of the change(s)

Please attach, for each amendment, a summary of all changes which clearly indicates:

i. Old wording (e.g. ~~strikethrough~~ text, CHANGED FROM and CHANGED TO)
ii. New wording (e.g. *italicized*, **bold**, tracked)
iii. Detailed rationale/ justification/ explanation for each change

6. Ethics Review Levy – cost including vat

Cost for Major Amendments - R3 691.20
(Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from charges)

For invoicing purposes, please provide:	
Sponsor's name	
Contact person	
Address	
Telephone number	
Email Address	

7. Signature

My signature certifies that I will maintain the anonymity and/ or confidentiality of information collected in this research. If at any time I want to share or re-use the information for purposes other than those disclosed in the original approval, I will seek further approval from the HREC.

Signature of PI	Signed by candidate	Date	26/02/2021

HUMAN RESEARCH ETHICS COMMITTEE
10 NOV 2021
HEALTH SCIENCES FACULTY UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	30-11-2022
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	[signature]	Date Signed	11/11/2021

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	09/11/2021		
HREC REF Number	769/2020	Current Ethics Approval was granted until	30/11/21
Protocol title	The utility of a multiplex PCR to rapidly detect lower respiratory infection pathogens to facilitate antimicrobial stewardship, in a LMIC setting.		
Protocol number (if applicable)	Version 6(26/02/2021)		
Are there any sub-studies linked to this study?	☐ Yes	☒ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Clinton Moodley		
Department / Office Internal Mail Address	Division of Medical Microbiology, National Health Laboratory Service, Groote Schuur Hospital and University of Cape Town, South Africa		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.1 Does this protocol receive US Federal funding?	☐ Yes	☒ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	Description	New fee (Vat Incl.)	tick ✓
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	✓
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. **Invoice billing – Directly to Sponsor**

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person	
Telephone number	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
☒	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☒	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	125
Number of participants enrolled, since last HREC Progress report (continuing review)	N/A
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	N/A

6. Cumulative summary of participants

Total number of participants who provided consent	N/A
Number of participants determined to be ineligible (i.e. after screening)	N/A
Number of participants currently active on the study	N/A

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Number of participants completed study (without events leading to withdrawal)	N/A
Number of participants withdrawn at participants' request (i.e. changed their mind)	N/A
Number of participants withdrawn by PI due to toxicity or adverse events	N/A
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	N/A
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	0
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
In this observational and laboratory diagnostic comparative study, 125 respiratory samples were collected from patients with suspected pneumonia. These samples were collected as part of routine care,and included sputum and tracheal aspirate samples that were send to the Microbiology department for microscopy, culture and susceptibility testing. We also performed a multiplex panel, the BioFire FilmArray Pneumonia Panel plus, on all the samples and are in the process of comparing the results and analysing the data collected.

8. Protocol violations and exceptions (tick ✓ all that apply)

☒	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐	No Prior amendments have been made since the original approval
☒	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
No adverse events occurred.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☒ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☒ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☒ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☒ Not applicable
		DSMB report attached	☐ Yes	☐ No	☒ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☒ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☒	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.
This is an observational and laboratory diagnostic comparative study of patients with suspected pneumonia. The samples that were used, were collected as part of routine care and there was no increased risk or safety concerns for the patients.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes		☒ No	
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☒ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	Signed by candidate	Date	9/11/2021

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**				
☑ Approved	Annual progress report	Approved until/next renewal date	30 11 23	
☐ Not approved	See attached comments			
Signature Chairperson of the HREC/ Designee	[signature]	Date Signed	21/1/2023	

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC
Many apologies for the delay. We note the appropriate submission [signature]

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	24/11/2022		
HREC REF Number	769/2020	Current Ethics Approval was granted until	30/11/22
Protocol title	The utility of a multiplex PCR to rapidly detect lower respiratory infection pathogens to facilitate antimicrobial stewardship, in a LMIC setting.		
Protocol number (if applicable)	Version 6(26/02/2021)		
Are there any sub-studies linked to this study?	☐ Yes	☒ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Clinton Moodley		
Department / Office Internal Mail Address	Division of Medical Microbiology, National Health Laboratory Service, Groote Schuur Hospital and University of Cape Town, South Africa		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

HUMAN RESEARCH ETHICS COMMITTEE
19 JAN 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

1.1 Does this protocol receive US Federal funding?	☐ Yes	☒ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	✓
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person	
Telephone number	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
☒	Closed to enrolment (tick ✓)
☒	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	125
Number of participants enrolled, since last HREC Progress report (continuing review)	N/A
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	N/A

6. Cumulative summary of participants

Total number of participants who provided consent	N/A
Number of participants determined to be ineligible (i.e. after screening)	N/A
Number of participants currently active on the study	N/A

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Number of participants completed study (without events leading to withdrawal)	N/A
Number of participants withdrawn at participants' request (i.e. changed their mind)	N/A
Number of participants withdrawn by PI due to toxicity or adverse events	N/A
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	N/A
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	0
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
In this study we determined the utility of the BioFire FilmArray Pneumonia Panel plus by comparing the results of this multiplex PCR with routine MC&S. Respiratory samples (n=125) that were send to the NHLS microbiology laboratory for routine testing were used. The laboratory work and data-analysis were completed. The manuscript was submitted for publication, and we are currently addressing reviewer comments.

8. Protocol violations and exceptions (tick ✓ all that apply)

☒	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐	No Prior amendments have been made since the original approval
☒	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
No adverse events occurred.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☒ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☒ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☒ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☒ Not applicable
		DSMB report attached	☐ Yes	☐ No	☒ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☒ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☒	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.
This is an observational and laboratory diagnostic comparative study of patients with suspected pneumonia. The samples that were used, were collected as part of routine care and there was no increased risk or safety concerns for the patients.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes		☒ No	
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and Information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☒ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	Signed by candidate	Date	24/11/2022

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
e-mail: *Bernadette.Eick@westerncape.gov.za*

Dr Clinton Moodley
DIVISION OF MEDICAL MICROBIOLOGY

E-mail: c.moodley@uct.ac.za / vwsmen001@myuct.ac.za

Dear Dr Moodley,

RESEARCH PROJECT: The Utility of A Multiplex PCR to Rapidly Diagnose Ventilator-Associated Pneumonia (VAP) To Facilitate Antimicrobial Stewardship, In A LMIC Setting (MMed. Dr M. van der Westhuyzen)

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **30 November 2021, subject to the approval of Professor Ivan Joubert.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) Confidentiality must always be maintained.
d) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
e) **No patient folders may be removed from the premises or be inaccessible.**
f) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Please contact Michelle Riley (Patient Fees) at ext. 2276 to ascertain if there will be charges for conducting the Research and to obtain a quote or to discuss charges
m) Kindly submit a copy of the publication or report to this office on completion of the research.
n) At no time should any posters encouraging patients to partake in research, be displayed within a clinical area.
o) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.

I would like to wish you every success with the project.

Yours sincerely

Signed by candidate

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 19 January 2021

C.C. Mr. L. Naidoo / Dr H. Aziz / Professor I. Joubert

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Appendix C: JAC-Antimicrobial Resistance author guidelines

Instructions for Authors

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Background and Scope of the Journal

Background

JAC-Antimicrobial Resistance was founded in 2019 by the British Society for Antimicrobial Chemotherapy (BSAC) as part of its mission to be a leading influencer of responsible antimicrobial

use globally and a provider of peer-reviewed research publications and education resources on antimicrobials. Any profits from the Journal will be used by the BSAC to further these objectives. It is an online only open access Journal with archived quarterly issues.

JAC-Antimicrobial Resistance strives to be a unique open access Journal that encompasses traditional research and clinical practice, and education with an emphasis on stewardship. For clarity in this document we will refer to these two intertwined strands of *JAC-AMR* as *JAC-AMR* (Education) and *JAC-AMR* (Research).

JAC-AMR (Education)

Background

The education component of *JAC-AMR* is an online repository for educational resources intended to support learning and increase knowledge about antimicrobial stewardship practice, antimicrobial resistance, behaviour change and more, for a worldwide audience. What is unique is that *JAC-AMR* will provide commentary and peer review on the educational resources. This will take the form of a structured review of the content of the resource. These reviews will be candid and serve to guide users of the resources as to the type of resource, intended audience, expected time to complete the resource and so on, as well as an assessment of any gaps or weaknesses in the resource. Unlike a research review of a submitted manuscript, the Editor will be encouraged to give a sometimes subjective view of its overall value; the emphasis here is not always on high-level scientific critique but pragmatic and practical assessment of the value of the resource, both in the healthcare setting it is intended for, as well as an assessment of its broader value.

As the library of resources grows, we intend that there will be an opportunity for users of the platform to rate the content on a range of metrics to include relevance, ease of use, achievement of learning outcomes and so on.

Aims

JAC-AMR will gather a wide range of educational resources intended for a wide-ranging local, national and international audience in a variety of healthcare and academic settings, with differing levels of prior knowledge and experience of issues relating to antimicrobial resistance and antimicrobial stewardship in humans. However, where stewardship in veterinary practice has been developed in conjunction with human practice we would be keen to consider these resources.

There will be a focus on resources to support learners in low- and middle-income countries (LMICs), and resources which are available in more than one language will be featured where possible. In addition to this geographic and educational diversity, the platform will also guide users to access resources most suited to their needs, via a searchable database. The range of resources will be broad in outlook, ranging from educational courses hosted by academic institutions, websites which provide news and comment, to resources from professional societies and national institutions plus clearly identified sponsored 'Supplement' educational and content from the pharmaceutical, diagnostic and vaccines industry.

Scope

JAC-AMR welcomes suggestions of educational resources for review by the Editors from a wide variety of sectors, particularly those intended for LMICs and users whose first language is not English.

Content should be educational and from across the healthcare communities (hospitals, primary care, outpatients, long-term care facilities, rural clinics and so on) but is not limited to traditional models of learning – innovative learning solutions are welcomed, whether this is interactive online learning (Massive Open Online Courses), webinars or video lectures, blogs, vlogs , podcasts, instructive interviews or case histories, webcasts, educational games/applications, patient stories and other approaches.

Sponsored 'Supplement' commercially produced and focused content will also be considered, provided it has an educational component. This will be clearly marked with the 'Supplement'

article type. Supplement content with no clear educational focus will be hosted at the discretion of the editorial board, and will always be clearly signposted as such. We wish to encourage commercial organisations to use *JAC-AMR* as a high-quality peer reviewed platform to host their educational resources.

Resources that are free to access are encouraged and welcomed and will be our primary focus. However, all educational content will be considered, including that which requires a fee for user assessment and certification.

Content can be submitted for review and inclusion by professional societies, individuals with interest/expertise in AMR/AMS, national bodies, national/governmental institutions, academic institutions, healthcare institutions, commercial organizations, not for profit organizations, charities, NGOs and others with relevant educational resources to submit.

The submission of educational resources that address the topics listed below are welcomed and encouraged:

- medical, pharmaceutical, nursing or allied health professional, undergraduate healthcare (medical, nursing, dentists, pharmacy) students, public health or quality improvement resources relating to antimicrobial resistance, antimicrobial use and stewardship
- mode of action of antimicrobial agents, mechanisms of resistance, methods of determining susceptibility
- basic microbiology and immunology
- other aspects of antimicrobial resistance, stewardship and use that have an educational focus; these may include veterinary clinical practice where resources have been developed with human stewardship practice
- the appropriate use of antimicrobial agents, to include the pharmacokinetics/pharmacodynamics, administration and management of antimicrobials, including route, duration and outpatient parenteral antimicrobial therapy (OPAT) and complex outpatient oral and parenteral antimicrobial therapy (COPAT) settings
- the role of non-antimicrobial approaches, novel diagnostics or new diagnostic approaches and diagnostic stewardship in

infection management, the role of vaccines in preventing disease

- pharmaceutical aspects of antimicrobial use – stability, compatibility, bioavailability; pharmacological aspects of antimicrobial use – adverse effects/toxicity, drug interactions, dosage adjustment in organ failure
- recognition and management of drug allergies and intolerances; allergy testing and de-labelling of non-allergic patients
- patient, carer and public education campaigns
- news, campaign and comment driven websites dealing with issues relating to antimicrobial stewardship and antimicrobial resistance with a global focus
- data warehouses, surveillance/informatics providing information and resources relating to antimicrobial consumption and quality, resistance and impact
- behaviour change/implementation science approaches to antimicrobial stewardship

There is no specific preference for the format of the educational resources, and all approaches to providing innovative methods of reaching, engaging with and educating audiences in the field of antimicrobial use, antimicrobial resistance and related subjects will be considered. All the posted educational resource reviews will have a unique document object identifier (doi) and with this metrics of use will be available. *JAC-AMR* cannot take any responsibility for keeping the original educational content URLs up to date.

Authors who are unsure about whether their intended submission meets the aims and scope of the *JAC-AMR* are welcome to contact the Editorial Office (jacamr.EditorialOffice@oup.com). At all times the Editorial team will have a final say in whether a resource is hosted and the content of the review. Any objections can be referred to the Editor in chief.

JAC-AMR (Research)

Aims

JAC-AMR (research journal) publishes clinically oriented opinions, reviews and original articles that advance the science and knowledge of antimicrobial resistance, stewardship and use relating to antibacterial, antifungal, antiviral and antiprotozoal agents. The Journal publishes primarily in human medicine, but articles in veterinary medicine will be considered, provided they fall within the scope of a regional or global 'one health' approach to antimicrobials.

Scope

JAC-AMR particularly welcomes high-quality, original articles on behaviour change research, clinical trials, education research, epidemiology, health services and observational research, quality improvement science, and systematic reviews/meta-analyses that have a clear, contemporaneous, clinical message in one or more of the following areas:

- the practice of evidence-based education, medicine, public health or quality improvement relating to antimicrobial resistance, stewardship and use
- other aspects of antimicrobial resistance, stewardship and use that fulfil the above criteria
- the pharmacokinetics/pharmacodynamics, administration and management of antimicrobials, including in the outpatient parenteral antimicrobial therapy (OPAT) and complex outpatient oral and parenteral antimicrobial therapy (COPAT) settings
- other aspects of OPAT and COPAT (as defined above) that fulfil the above criteria
- the clinical use of non-antimicrobial approaches in infection management
- the clinical use of novel diagnostics or new diagnostic approaches in infection management
- diagnostic stewardship relating to clinical infection management

- clinical aspects of antimicrobial adverse effects, allergies/intolerances or drug–drug interactions
- the shared patient–prescriber decision-making approach to clinical infection management

Articles should have ideally have regional significance or applicability (where ‘regional’ is defined as work that has clear relevance beyond the centre or country in which it was undertaken); however, outstanding single-centre studies with broader generalizability may be considered for peer review at the discretion of the Editor. The Journal will not consider case reports or case series of fewer than 10 cases.

Viewpoint and review articles:

- will usually be solicited by one of the Editors
- unsolicited viewpoint or review articles will be considered only after prior discussion with one of the Editors and will be subject to an article processing charge

Authors who are unsure about whether their intended submission meets the aims and scope of the *JAC-AMR* are welcome to contact the Editorial Office (jacamr.EditorialOffice@oup.com)

Acceptance rate and processing times

Not applicable as yet

Appeals

Authors wishing to lodge an appeal against a decision can do so by contacting the Senior Editor responsible for the decision directly and by copying in the Editorial Office.

Editorial Office Contact Information

The contact details for the *JAC-AMR* Editorial Office are as follows:

E-mail: jacamr.EditorialOffice@oup.com

Processing of Papers

Where to submit

All material to be considered for publication should be submitted in electronic form via the Journal's online submission system at https://mc.manuscriptcentral.com/jac-amr

Given that you can produce a file of your paper through a word processing package of some description, you only need the three following items to access and use the system: access to the website via a web browser, Adobe Acrobat Reader (which can be downloaded free of charge from http://www.adobe.com/) and an e-mail account.

Authors must comply with the stipulations in the Instructions to Authors.

Article types and format

All documents should be double spaced, and the margins should not be excessively wide. A clear, legible single font (which is readily available internationally) and point size should be employed throughout. For symbols, please use the 'insert symbol' function and ONLY select characters from the 'normal text' subset. *All submitted articles should be line numbered (using continuous line numbers). To do this in Word, use File, Page Setup, Layout, Line Numbers and select continuous line numbering. Please DO NOT insert page numbers (as the pdf proof created by the online submission system will automatically be page numbered).*

All articles should include a title page comprising: article title; author names and their affiliations (each affiliation address must be given separately and in full); telephone, fax and e-mail contact details for the corresponding author; and a short running title. In addition, all articles must include a Funding section (if reporting original research) and a Transparency declarations section.

Article titles. All articles reporting the results of original research must have a descriptive title. For example ‘Effect of streptomycin in tuberculosis’ is acceptable; ‘Streptomycin cures

tuberculosis' is not acceptable. Viewpoint articles are permitted to have declarative titles. Please note that claims of priority are not permitted in article titles as such claims are impossible to verify; only history will reveal the first example. For instance 'First NDM-1 *Escherichia coli* isolated in Andorra' would not be permitted. Authors are permitted to indicate in the article that, to the best of their knowledge, a finding is the first of its kind.

Original articles and Brief reports must have a structured synopsis. The headings for the structured synopsis are as follows: Background (optional), Objectives, Patients and methods (or Methods), Results, and Conclusions.

Original articles. There is a limit of 3500 words in the main text of the article (everything from the Introduction to the end of the Discussion). Papers must be written as concisely as possible. Original articles are divided into the following sections: Synopsis (250 words maximum), Introduction, Materials (or Patients) and methods, Results, Discussion, Acknowledgements, Funding, Transparency declarations and References. Repetition of content between sections must be avoided. A combined Results and Discussion section is acceptable.

Brief reports. These should have the same format as Original articles, but should have no more than two figures/tables, should have a maximum of 20 references and should not exceed 1500 words of main text.

Correspondence. Letters on topics of concern or interest in the field of antimicrobial chemotherapy, particularly arising from papers or letters already published in the Journal. These should be addressed to the Editor-in-Chief and must not exceed 800 words, one figure or table and 10 references.

Systematic review articles. There is no length limit for this format. A systematic review, as defined by the Cochrane Handbook, is 'A review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review. Statistical methods (meta-analysis) may or may not be used to analyse

and summarize the results of the included studies.’ They should include a structured synopsis (with appropriate headings; these may differ from the headings used for Original articles etc.).

Review articles. There is no length limit for this format. These generally aim to give an overview of a field suitable for a wide audience, and they should include a synopsis (250 words maximum). Most reviews are invited. We are pleased to consider unsolicited reviews, but authors are encouraged to consult the Editor-in-Chief in advance of writing to avoid duplicating commissioned material.

Viewpoint. These articles are usually in the region of 800–1000 words and may contain the expression of opinion as well as fact. They should address a topical subject, perhaps taking a particular viewpoint and throwing new light on a current debate. A leading article should include a short synopsis (150 words maximum) that should convey the topics and ideas the article covers. Those wishing to contribute a Leading article are encouraged to contact the Editor-in-Chief to discuss their ideas before writing to prevent clashes with any articles already in the pipeline.

For debate. These articles should air contentious issues or discuss controversies so as to stimulate discussion in the Journal on any given topic on antimicrobial chemotherapy. Articles should be as clear and concise as possible, consist of 800–2500 words and must be accompanied by an unstructured synopsis of up to 150 words.

Please note that on publication all Original articles and Brief reports, as well as Antimicrobial practice papers, will be published under the heading of Original research so that articles on similar topics can be grouped together when assigned to an issue. In addition, each piece of Correspondence will be published as either a Research letter or a Letter to the Editor.

Educational resources for review. Readers are encouraged to make suggestions of Educational resources that they feel are suitable and deserving of review in JAC-AMR. Suggestions, in the form of an e-mail containing a hyperlink to the resource and a brief description of its content, and why you feel it is worthy of

review in JAC-AMR, should be sent to the Editorial Office at jacamr.EditorialOffice@oup.com.

Peer review

JAC-AMR follows single-blind peer review.

After preliminary examination of the submission by Editorial Office staff to check that all the necessary elements are present, the manuscript is passed to the Editor-in-Chief. The Editor-in-Chief then assigns the manuscript to an appropriate Senior Editor. The Senior Editor is then responsible for selecting an Editor to handle the manuscript. Manuscripts can be rejected immediately by the Editor-in-Chief, a Senior Editor or an Editor without further peer review. The assigned Editor is responsible for selecting referees and obtaining referee reports.

The usual number of referees is two, however, the Editors reserve the right to make a decision on a manuscript on the basis of one referee report, and the Editors reserve the right to seek the opinion of two or more referees if they judge this to be necessary or desirable. Viewpoints and Correspondence are not routinely sent for external refereeing, but the Editor-in-Chief, Senior Editors and Editors reserve the right to seek the opinion of one or more external referees if they judge this to be necessary or desirable. Senior Editors, Editors and referees are asked to consider whether they have any conflicts of interest when they are assigned a manuscript, and if necessary to decline to handle it. See the section ‘Conflicts of interest’ for more information on this subject.

If an Editor decides upon rejection of a manuscript, it is passed back to the handling Senior Editor for approval of this decision. All rejection correspondence therefore originates from a Senior Editor. Authors should regard rejection as final and only resubmit if they have been invited to do so. Manuscripts may be rejected for a number of reasons, including: (i) they may be of only peripheral interest and perhaps more suitable for submission to a different journal; (ii) they may be, in the opinion of the reviewers, scientifically flawed; (iii) they may be unclear or overly long; or (iv) they may not make a significant contribution to the literature.

Requests that a revised version of a manuscript be submitted for consideration are sent direct to the corresponding author from the Editor responsible. Any revised version should be submitted within 6 weeks of the revision request or the Journal reserves the right to consider the manuscript as a new submission that may be subject to further refereeing.

The Editor-in-Chief, Senior Editors and Editors reserve the right to request more rounds of revision and resubmission/refereeing, or reject a manuscript outright, if they judge that any revised version does not adequately address the concerns raised by the referees and the Editor. Once the Editor is satisfied that a revised version has adequately dealt with any points raised they may accept the manuscript.

Authors can appeal against a decision by contacting the handling Senior Editor, but unless there has been a gross misunderstanding of the submitted article by the Editor and referees, rejection appeals are not likely to be successful. Authors should appreciate that if they resubmit an article that has been rejected without substantially modifying it in line with the suggestions of the Editor and referees, it is almost certain to be rejected again.

After acceptance the manuscript is sent for copy-editing and typesetting prior to production of proofs for author correction. The Journal maintains the right to edit any manuscript to the extent necessary to achieve clarity and precision of expression and to conform with English usage and the Journal's conventions. Please note that if authors ignore requests to conform with Journal style at the revision stage, these changes may be enforced during copy-editing and proof production.

Articles submitted by Editors of the Journal

JAC-AMR does not bar Editors (including Senior Editors and the Editor-in-Chief) from submitting manuscripts to the Journal. Articles submitted by Editors are handled in the same fashion as other articles subject to the following considerations: these manuscripts are never assigned to the submitting Editor, or an Editor from the same institution; the submitting Editor is unable to access details of their manuscript through the online

submission system; and, like other authors, the submitting Editor will not know the identity of the handling Editor (in cases of rejection) or referees.

Supplement articles

Supplement manuscripts are subject to peer review and may be rejected. Unless specialist external expertise is required, this peer review is conducted among the team of Editors that is dealing with the Supplement.

Guidelines

Guidelines that have undergone proper public consultation will normally only be subjected to peer review by members of the Editorial Board.

Proofs

An e-mail containing a link to the proof is sent to the corresponding author. The proof should be read carefully, paying particular attention to any tables, figures and references, and corrections (and answers to any queries) should be submitted to the *JAC-AMR* Editorial Office as soon as possible. Authors should pay particular attention that they check any dosage directions, owing to the seriousness of any error entering the printed record. Extensive changes at the proof stage are not permitted. Authors may be charged for correction of their non-typographical errors. The Journal reserves the right not to comply with changes marked on the Author's proof if these are contrary to the style set down in the Instructions to Authors.
In the event of important developments in a field that affect the paper arising after the final revision, a 'Note added in proof' may be permitted. Please note that Supplementary data files are largely unedited and are not proofed out.

Once all the corrections have been made by the typesetters, the article is then posted on *JAC-AMR* Advance Access

Late corrections, Advance Access and Errata

Authors should check articles carefully before submission and resubmission to ensure errors are kept to an absolute minimum. Authors must treat the proof as the LAST CHANCE they will have to make corrections to their article. Corrections that are requested once an article has appeared in Advance Access will entail a higher level of scrutiny. The Journal takes a very dim view of corrections requested at this stage that should have been dealt with earlier, and reserves the right to refuse to make further changes.

After publication online, the only avenue available to correct an article is the publication of a linked Erratum. The purpose of an Erratum is to correct items that affect the scientific validity of a piece of research. The Journal will refuse to publish an Erratum if the correction requested does not affect the scientific validity of the article (hence requests to correct author names or address details, funding information, or collaborator names or locations, for example, will be refused). This is why it is of the utmost importance that authors pay the necessary attention to ensuring articles are correct at every stage and treat the proof as the last available opportunity for corrections.

Journal Policies

Material offered for publication must be original, unpublished and not under simultaneous consideration by another journal. Any previous publication of the material (including abstracts in conference proceedings or posters, or in a clinical trials results database) must be declared in the covering letter, as well as in the Acknowledgements section of the paper. For these purposes the posting of essentially raw data on a website without significant analysis, is not considered to represent prior publication. In addition, authors must include in the covering letter details of ANY previous submission of the work to *JAC-AMR* that has been rejected. The manuscript number of the earlier submission must be provided, as well as a point-by-point response to the comments made in the decision e-mail for the previous submission.

Authors should not fragment their research into least publishable units. Authors must be aware that *JAC-AMR* may decline to publish articles if this approach becomes evident.

Authors are fully responsible for the accuracy of all data in their articles.

JAC-AMR reserves the right to use plagiarism detection software on any submitted material.

Authors are responsible for adhering to relevant legislation in their country regarding research in humans or animals and the reporting of data from routine patient care.

JAC-AMR is a member of the Committee on Publication Ethics (COPE), and strives to adhere to its code of conduct and guidelines. For further information see http://www.publicationethics.org. Authors are also expected to behave ethically and unacceptable practices include: (i) plagiarism; (ii) fabrication or falsification of data; (iii) omission of legitimate authors, Funding information or financial conflicts of interest; (iv) inclusion of authors who have not made a significant contribution to the design and execution of the work described; and (v) redundant/duplicate publication.

In-press papers or papers under editorial consideration

In-press and submitted papers that are important for the review of a paper MUST be uploaded when the paper is submitted and referred to in the covering letter that accompanies the submission. Authors should be aware of the issues of redundant/duplicate publication. For further information, please see the following Editorial:

Reeves DS, Wise R, Drummond CWE. Duplicate publication: a cautionary tale. *J Antimicrob Chemother* 2004; **53** : 411-2.

Sequence data

When reporting sequences they must be submitted to one of the three major databases and an accession number must be provided at latest in the first revised version.

If a sequence has been submitted but an accession number has not yet been provided or the sequence is not yet available to the public then authors must submit the annotated sequence data as Supplementary data for scrutiny by the Editor and referees. Articles will not be permitted to enter the review process without the sequence data.

Supplementary data

Please note that it is also possible to submit files containing Supplementary data. The Supplementary data (for example large tables of MICs, or a questionnaire) can be lodged with the version of the paper published online as an extra resource for readers. Supplementary data is largely unedited and is not proofed out so authors should ensure that they provide high-quality, accurate files. In addition, authors must ensure that they cite the Supplementary data within the article. Please contact the Editorial Office if you require further details.

Authorship

The authorship of the paper should be confined to those who have made a significant contribution to the design and execution of the work described. In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of article may indicate the contribution made by each author if they wish.

JAC recommends that authors review the ICMJE criteria for authorship before submission (http://www.icmje.org/#author).

Author signed submission forms

Please do not supply signed submission forms when an article is submitted.

If your article is accepted, the Editorial Office will generate an article-specific signed submission form template and pass this to the Corresponding author for signature.

Please note that copied and pasted ‘graphics’ of signatures are NOT permitted owing to the possibility of fraud. Digital signatures, properly verified by the issuing organization (such as Adobe for instance) are permitted.

Articles cannot be published until the signed form has been received.

Changes in authorship

The author list of any submission should be decided upon and fixed BEFORE submission. Other than in exceptional circumstances the Journal does not allow addition or removal of author names after submission. A satisfactory explanation for any proposed changes in authorship will be required. We will also require consent from any person whose name has been removed indicating that they agree to the removal of their name from the author list. Owing to the complexity of these rules we strongly advise authors to fix the author list before submission and not to attempt to make changes later.

'Umbrella' groups and authorship

Many large collaborative studies are organized under a group name that represents all of the participants. *JAC-AMR* will not accept a group name as an 'author' of an article. All articles must have at least one named individual as author. Authors of large collaborative studies should list the author(s) of the article and follow this with 'on behalf of the [GROUP NAME]'. The names of all of the participants should then be listed in the Acknowledgements section. If this is done under a heading ‘Members of [GROUP NAME]’ those persons listed will be accorded ‘Collaborator’ status on the PubMed record for the published article.

Professional medical writers and editorial assistance

Professional medical writers and other forms of writing assistance have an important role to play in the clear communication of scientific results. However, unless this role is openly explained and acknowledged unfounded suspicions about this role will continue. *JAC-AMR* encourages the open and precise description of any such assistance received by authors in

relation to any article. It is possible that writers may qualify for authorship of a manuscript; we recommend that authors review the ICMJE criteria for authorship before submission (http://www.icmje.org/#author).

The precise role of the writer or service in the origin or preparation of the manuscript must be declared in the Transparency declarations section; we recommend that the name of the writer (and their agency where applicable) or the service is provided. If this support was funded, the source must be declared in the Funding section.

Responsibilities of the corresponding author

For each paper submitted to *JAC-AMR* there must be a single corresponding author. As the representative of the authors, the corresponding author must ensure that all authors are given access to submitted and revised versions of papers. The corresponding author is responsible for the collation of the authors' signatures on submission forms and also the collation and communication of proof corrections to the Journal. The corresponding author should be the signatory of the publication licence form. As the authors' nominated representative, the corresponding author will be held primarily accountable for any failure to comply with the Instructions to Authors or generally accepted standards of good practice. This does not absolve other authors of responsibility, however.

The corresponding author will act as the primary contact for correspondence regarding the paper, and as such authors should take care not to appoint a corresponding author likely to be absent for extended periods (such as a sabbatical) during the consideration of the paper as this is likely to cause unacceptable delays.

Please note that papers submitted via ScholarOne Manuscripts must be submitted through the account of the corresponding author listed on the paper, not through the account of one of the other authors or the account of a third party who is not on the author list. This is to ensure that there can be no argument regarding the identification of the corresponding author. In addition, the authors listed during the submission process on

the ScholarOne Manuscripts website must fully match the author list of the actual submitted article.

Ethics

All articles in *JAC-AMR* describing research in humans or animals must include an 'Ethics' heading as the first section in the Patients and methods or Methods section. Authors must include in this section all relevant statements regarding approvals, licences, informed consent and so on, as applicable.

Research involving humans

Authors must indicate in the Ethics section whether the research was conducted in accordance with the Declaration of Helsinki and national and institutional standards. If approval was obtained from an Ethics Committee the authors must clearly name the ethics committee responsible if more than one institution is involved. The approval/reference number must be listed in the Ethics section of the article. Written informed consent must be obtained from study participants and the existence of this consent must be stated in the article. Authors must supply the relevant approval numbers from Ethics committees or other bodies.

Patient privacy. Patients have a right to privacy. Any information that might result in identification of individuals must be omitted, especially if it is not directly clinically relevant. Patient age, sex, admission dates and co-morbidities should be removed as far as possible. If it is possible that a patient could be identified, the authors must obtain written informed consent from the individual(s) concerned and state that this has been obtained in the article. Publication consent forms should be retained by the authors and not supplied to the Journal. If the patient is deceased the next of kin should be contacted. If consent cannot be obtained the authors must explain the circumstances briefly in the article, as well as in detail in the covering letter. In rare circumstances where relevant clinical details mean that the patient can be identified, the patient/next of kin must be shown the manuscript before submission and made aware as part of the informed consent process that the article may appear on the internet.

Case reports. Authors must avoid the temptation to recite the entire clinical history of the patient at the start of a case report and should retain only those elements that are pertinent. Reciting the entire clinical history greatly increases the chances that the patient could be identified. Dates of treatment must be removed or converted to timespans for the same reason.

Research involving animals

Authors must state their compliance with relevant institutional and national standards for animal care and experimentation, together with the details of any authorities that licensed the experiments.

JAC-AMR supports the use of the ARRIVE Guidelines and articles reporting research in animals must include a completed ARRIVE checklist, which must be uploaded with the article so it is available for the scrutiny of the Editor and referees.

Funding

ALL papers submitted to *JAC-AMR* reporting original research MUST include a 'Funding' section. This section should appear after the 'Acknowledgements' section.

Details of all funding sources for the work in question must be given.

Authors must list any internal funding. If no specific funding has been received then this should be clearly stated; equally if data have been generated as part of the routine work of an organization, this too should be stated. Ongoing financial support for any of the authors should also be included under the Funding heading.

If a professional medical writer or similar service was involved in the origin or preparation of a manuscript and this support was funded, the source must be declared in the Funding section.

Sources of funding may of course still be thanked in the Acknowledgements section, but should not be listed again in the Transparency declarations (see below), unless there is an

important reason for doing so. For example if the funder played any decision-making role in the research this must be stated.

The following rules should be followed:

The sentence should begin: 'This work was supported by …'

- The full official funding agency name should be given, i.e. 'the National Cancer Institute at the National Institutes of Health' or simply 'National Institutes of Health' not 'NCI' (one of the 27 subinstitutions) or 'NCI at NIH' (full RIN-approved list of UK funding agencies is at http://www.rin.ac.uk/files/List-of-major-UK-research-funders.pdf)
- Grant numbers should be complete and accurate and provided in brackets as follows: '(grant number ABX CDXXXXXX)'
- Multiple grant numbers should be separated by a comma as follows: '(grant numbers ABX CDXXXXXX, EFX GHXXXXXX)'
- Agencies should be separated by a semi-colon (plus 'and' before the last funding agency)
- Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number 'to (author initials)'.

An example is given here: 'This work was supported by the National Institutes of Health (P50 CA098252 and CA118790 to R. B. S. R.) and the Alcohol & Education Research Council (HFY GR667789).

Crossref Funding Data Registry

In order to meet your funding requirements authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about the CHORUS initiative please click here.

Conflicts of interest

Conflicts of interest have the potential to affect authors, referees and Editors (including Senior Editors and the Editor-

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Editors. The Editor-in-Chief, Senior Editors and Editors register their interests (including personal and business interests) with the BSAC. The BSAC Register of Interests is held at BSAC Headquarters, is updated periodically and is available for inspection. When an article is assigned to a Senior Editor or an Editor they are reminded to consider whether there are any potential conflicts of interest, and if so, to discuss them with the handling Senior Editor or the Editor-in-Chief, who will come to a decision as to whether it is appropriate for them to act on the article, or whether it should be reassigned.

Transparency declarations

In the interests of openness, ALL papers submitted to *JAC-AMR* MUST include a 'Transparency declarations' section (which should appear at the end of the paper, before the 'References' section). We suggest authors concentrate on transparency declarations (i.e. conflicts of interest) of a financial nature, although relevant non-financial disclosures can also be made. Authors should consider making a declaration if they answer 'Yes' to any of the following questions:

1. Have you in the period of research leading up to this publication accepted any of the following from an organization (including government departments or granting bodies) that may in any way be financially affected by the conclusions of your article (e.g. reimbursement for attending a symposium, a fee for speaking, a consultancy fee, funds for research other

than directly for this work, funds for a member of staff, any other substantial material benefit)?

2. Do you directly own any stocks or shares in a company that might be financially affected by the conclusions of your article?

3. Has the funder of the research played any decision-making role in the design, execution, analysis or reporting of the research?

4. Have you received the assistance of a professional medical writer or similar service? [The precise role of the writer or service in the origin or preparation of the manuscript must be declared and we recommend that the name of the writer (and their agency where applicable) or the service is provided.]

5. Have you accepted any reimbursement for preparing your article?

Authors should either include appropriate declarations or state 'None to declare'. Importantly, the declarations should be kept as concise as possible, should avoid giving financial details (e.g. sums received, numbers of shares owned etc.), and should be restricted to declarations that are specific to the paper in question. Authors will of course need to consider whether or not the transparency declarations need to be amended when revisions are submitted.

The burden of responsibility rests with all authors, who must ensure that appropriate declarations are included. The corresponding author will be responsible for obtaining the relevant information from all of their co-authors. By signing a submission form each author is stating that they have made any necessary transparency declaration. All authors should carefully consider the embarrassment and potential damage to their reputation that could result should they fail to declare an interest that is revealed subsequently.

If only some authors need to make a declaration it must be made clear that the remaining authors have nothing to declare, for example:

'A. B. has received funds for speaking at symposia organized on behalf of Panacea Ltd and has also received funds for research

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All papers submitted to *JAC-AMR* must include a Transparency declarations section; papers that do not include such a section will not enter the review process; they will be returned to the corresponding author so that the appropriate section can be added. Following resubmission the paper will then be progressed to peer review.

In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of articles may indicate the contribution made by each author if they wish.

Other useful information

In some instances (often when the authors themselves have no interests to declare) it may be helpful to readers as background information to give brief details of organizations that do have an interest but do not appear elsewhere in the article, for example 'Fantastazole is owned by Wonder Pharmaceuticals'.

Misconduct

We will energetically pursue accusations of misconduct directed at authors, Editors or referees and have a number of sanctions at our disposal including the option to inform employers about accusations and ask them to mount their own internal investigations. Accusations should not be made lightly or in the absence of the likelihood of supporting evidence being obtainable. The Journal may take the view that accusations are malicious if supporting evidence cannot be found and may direct sanctions against accusers in such cases. Any accusation of misconduct should be addressed to the Editor-in-Chief (unless it involves the Editor-in-Chief, in which case it should be directed to the President of BSAC). *JAC-AMR* is a member of COPE and will follow its guidelines on the handling of investigations into research misconduct.

Clinical trials/Randomized controlled trials

Registration and data publication

Authors must register their trials in one of the databases dedicated to registration of trials. In addition, authors must state the database and provide the unique registration number – both in the abstract and in the main body of the paper.

JAC-AMR will consider for publication clinical trials for which there has been prior publication of trial data in results databases (such as http://www.clinicalstudyresults.org), however, authors MUST declare in the covering letter and the Acknowledgements section of the article that they have previously published data in a results database.

Contributions

The contribution of each author must be clearly stated in the Transparency declarations section, after the information on conflicts of interest.

Reporting standards

All involved in the publication of health intervention research have a duty to patients and society at large to ensure that this research is reported in a complete, accurate and transparent fashion. This includes authors, referees, Editors and Journals. *JAC-AMR* takes this responsibility seriously and endorses the work of organizations such as the EQUATOR network (http://www.equator-network.org/), an international initiative that seeks to improve the reliability and value of the medical research literature.

There is a wide range of reporting guidelines, each specific for different types of study. Some of those for study types that are frequent in *JAC-AMR* are mentioned specifically below. Authors should consult the EQUATOR network website (http://www.equator-network.org/) for links to the latest versions of guidelines, which are organized by the study type.

Randomized controlled trials

Authors should comply with the Consolidated Standards of Reporting Trials (CONSORT) statement (www.consort-statement.org) and use the resources within it (for example the checklist and flow diagram) to ensure they have addressed potential criticisms and provided all necessary information. Authors should include a CONSORT flow diagram in their article, and provide a copy of the completed checklist.

Systematic reviews and meta-analyses

For systematic reviews and meta-analyses of randomized controlled trials authors should comply with the PRISMA statement (which replaces the QUORUM statement), which consists of a checklist and flow diagram (http://www.prisma-statement.org/index.htm). Authors should include a PRISMA flow diagram in their article, and provide a copy of the completed checklist.

Outbreaks and intervention studies in nosocomial infection

Authors should comply with the ORION statement (www.idrn.org/orion.php), which is the CONSORT equivalent for infection control studies. Its purpose is to increase the quality of research and reporting in the area of nosocomial infection.

Economic evaluations

Authors of articles describing economic evaluations of antimicrobial interventions are encouraged to make use of the following resources, where applicable, in order to ensure that their work is both optimal and adequately described.

International Society of Pharmacoeconomics and Outcomes Research (ISPOR) Checklist for retrospective database studies, which can be accessed at: https://www.ispor.org/heor-resources/good-practices-for-outcomes-research/article/a-checklist-for-retrospective-database-studies

Quality of Health Economic Studies (QHES) Instrument. See Table 1 in: http://www.amcp.org/data/jmcp/Formulary Management-53-61.pdf

Observational epidemiology studies

Authors of articles reporting observational epidemiology studies should follow the STROBE guidelines (https://www.strobe-statement.org/index.php?id=strobe-home) and complete the relevant checklist for the type of study they have conducted. The completed checklist should be supplied as part of the article submission process.

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JAC-AMR supports the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a digital object identifier (DOI). Data citations should include the minimum information recommended by DataCite:

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Journal Style

General

In addition to reading the information provided here, authors should consult a recent issue of the Journal for the layout and conventions used.

The past tense should be used throughout for description of the results of the paper, the present tense should be used when referring to previously established and generally accepted results.

Where possible SI units should be used.

Please ensure that characters with a similar appearance are consistent throughout the document and not from different Unicode sub ranges as with the Greek Delta.

Language editing

Particularly if English is not your first language, before submitting your manuscript you may wish to have it edited for correct usage of English. This is not a mandatory step, but may help to ensure that the academic content of your paper is fully understood by journal Editors and reviewers. Language editing does not guarantee that your manuscript will be accepted for publication. If you would like information about such services, please click https://academic.oup.com/journals/pages/authors/language_services. There are other specialist language editing companies that offer similar services and you can also use any of these. Authors are liable for all costs associated with such services.

Spelling

British spelling should be used. Spelling should follow that of the Oxford Dictionary for Scientific Writers and Editors and where this gives no guidance the Concise Oxford Dictionary. Spelling of drug names should conform with that given in the latest edition of the British National Formulary (published by the British Medical Association and the Royal Pharmaceutical Society of Great Britain and available online at http://www.bnf.org/bnf), but please note that *JAC-AMR* will continue to use methicillin (not meticillin).

Abbreviations

Non-standard abbreviations should be defined at the first occurrence and introduced only where multiple use is made. See here for abbreviations that may be used without definition, as well as antimicrobial abbreviations (which may be used in Tables and Figures).

Dosage frequencies and routes of administration

Latin dosage frequency abbreviations are not permitted (qd, bd, bid, tds etc.), however, constructions q12h, q8h and so on are permitted as there is less likelihood of confusion. Routes of administration other than intramuscular (im) and intravenous (iv), which may be abbreviated after definition, should be given in full in English.

MICs

Please note that all MIC data in *JAC* must be expressed in terms of mg/L (not μg/mL).

Nomenclature

Authors are required to check and ensure that in all instances the most up to date nomenclature is being used.

Bacterial nomenclature

When genus and species are given together use a capital letter for the genus and a lowercase letter for the species and italicize both e.g. *Staphylococcus aureus*. After the initial use in the text of the full name of an organism the generic name should then be abbreviated to the initial letter, e.g. *E. coli*.

When the genus is used as a noun or adjective use lowercase roman unless the genus is specifically referred to e.g. 'staphylococci and streptococci' but 'organisms of the genera *Staphylococcus* and *Streptococcus*'.

The name of an order has an initial capital but is not italicized, e.g. Enterobacteriaceae. For genera in the plural, use lowercase roman, e.g. salmonellae.

When the species is used alone use lowercase e.g. viridans streptococci. For trivial names, use lowercase roman e.g. meningococcus.

Authors should use bacterial names present in the *Approved List of Bacterial Names*, Amended Edition (1989), Skermanm, V.B.D., McGowan, V. & Sneath, P.H.A., Eds, ASM Press, Washington, DC, USA (ISBN 1-55581-014-4), with subsequent alterations validly

published by announcement in Validation Lists of the *International Journal of Systematic and Environmental Microbiology* (formally the *International Journal of Systematic Bacteriology*).

Genetic and amino acid nomenclature

Bacterial genetics. Genotype designations are indicated with italic lowercase three-letter locus codes (e.g. *par*, *his*, *ara*). If several loci are involved in a related function the individual loci are designated by the addition of an uppercase italic letter to the locus code (*parC*, *ompF*).

Phenotype designations (for example the protein product of a bacterial gene) are given in roman type with an initial capital letter (OmpF, LacZ).

Erythromycin gene nomenclature should follow that described in: Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J & Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; 43: 2823–30.

Yeast genetics. Wild-type alleles are all uppercase and italicized (*LEU*2), mutant alleles are all lowercase and italicized (*leu*2) and gene products are capitalized on the first letter and are not italicized (Leu2).

General. Authors should ensure that they confine discussion of changes in amino acid sequence to the context of the protein (e.g. OmpF) and nucleotide changes to the context of the gene (e.g. *ompF*). Please also be aware of the difference between a mutant (a strain with one or more mutations) and a mutation (a change in the sequence of the genetic material).

Amino acids. The full residue names or three-letter abbreviations are preferred in the text (e.g. a methionine residue at position 184 should be symbolized Met-184). The single letter codes may be used in figures. Amino acid changes should be designated Met-184→Val or M184V.

When comparing nucleotide or amino acid sequences authors should exercise care in the use of the term homology. Homology

should only be used when a common evolutionary origin is being implied; it is incorrect to give a percentage homology between two sequences. The wing of a bird and the human arm are homologous structures (they are believed to have a common evolutionary origin), homology cannot be quantified. For sequence comparison authors should use the terms identity and similarity. Sometimes 'equivalent' or 'counterpart' is more appropriate than 'homologue'.

Beta-lactamase nomenclature

The allocation of new beta-lactamase names and numbers is handled centrally to avoid confusion in the literature.

It is essential that authors check new alleles against the existing list and submit new ones for the unambiguous allocation of new numbers or names.

An article discussing the background, the system and how to submit is available here: https://journals.asm.org/doi/10.1128/aac.00333-22.

Macrolide-lincosamide-streptogramin resistance determinant nomenclature

Nomenclature for macrolide-lincosamide-streptogramin resistance determinants should follow the structure suggested by: Roberts MC, Sutcliffe J, Courvalin P *et al.* Nomenclature for macrolide and macrolide-lincosamide-streptogramin B antibiotic resistance determinants. *Antimicrob Agents Chemother* 1999; **43** : 2823–30. A new gene must have ≤79% amino acid identity with all previously characterized MLS genes before receiving a new unique name. Adding subscripts or superscripts to established genes is not acceptable. See: http://faculty.washington.edu/marilynr/. Before submitting a sequence to GenBank or submitting a manuscript for publication, please contact Professor Marilyn Roberts (marilynr@uw.edu). Once a new name has been assigned you must indicate in your article that you have received approval by the nomenclature centre for the new gene name.

Tetracycline resistance determinant nomenclature

Nomenclature for tetracycline resistance determinants should follow that suggested by: Levy SB, McMurry LM, Barbosa TM *et al.* Nomenclature for new tetracycline resistance determinants. *Antimicrob Agents Chemother* 1999; **43**: 1523–4. A new gene must have ≤79% amino acid identity with all previously characterized *tet* genes before receiving a new unique name. Adding subscripts or superscripts to established genes is not acceptable. See: http://faculty.washington.edu/marilynr/. The Levy Group is responsible for coordinating the naming of new *tet* genes and before submitting a sequence to GenBank or submitting a manuscript for publication, please contact Professor Marilyn Roberts (marilynr@uw.edu). Once a new name has been assigned you must indicate in your article that you have received approval by the nomenclature centre for the new gene name.

qnr gene/allele nomenclature

Authors submitting articles reporting the identification of new *qnr* genes or alleles must provide evidence that they have contacted the relevant clearinghouse (https://www.ncbi.nlm.nih.gov/pathogens/submit-beta-lactamase/) to deposit the new sequence data and receive a unique designation. Authors should consult Jacoby G, Cattoir V, Hooper D *et al. qnr* gene nomenclature. *Antimicrob Agents Chemother* 2008; **52**: 2297–9.

mcr gene/allele nomenclature

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FICI data

Fractional inhibitory concentration index (FICI) experiments are performed in order to study drug interactions and they must be interpreted in the following way:

FICI<=0.5 = synergy

FICI>4.0 = antagonism

FICI>0.5-4 = no interaction

For further information please see the following Editorial:

Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother* 2003; **52**: 1.

Microarray data

Authors of articles containing microarray data must ensure that the full datasets are lodged with an appropriate publicly available online database (the data must not be supplied for publication as Supplementary data alongside the article). The data should be supplied with the submitted article if they are not already publicly available. The name of the database and the accession numbers should be provided in the article. Authors must ensure that their data are available for public scrutiny from the online publication date of their article at the latest.

Chemistry

General nomenclature. The IUPAC recommendations on chemical nomenclature should be followed [*IUPAC Compendium of Chemical Terminology* (1987, ISBN 0 632 01767 8, Blackwell Scientific Publications, Oxford]. All chemical names are run together except those of acids, acetals, esters, ethers, glycosides, ketones and salts, which are printed as separate words; hyphens are used to separate numbers, Greek letters and some configurational prefixes, e.g. *p*-nitrophenol. Italics are used for certain prefixes, e.g. *cis*-, *trans*- and *N*. Small capitals are used for dextro- and laevo- prefixes, e.g. L-glutamine.

Drugs. Spelling of drug names should conform with that given in the latest edition of the British National Formulary. Chemical or generic names of drugs should be used; trade names may be referred to once only upon first use of the generic or chemical name. The content of proprietary formulations should be given if relevant. Generic names should not be abbreviated in the text; abbreviations may be used in Tables if there is limited space. If compounds are referred to by code name or company number either the structure or a reference to a paper illustrating the structure must be given, any previous code names or designations should be given on first use.

Supplier locations are required for all smaller/local suppliers.

References

Authors are responsible for the accuracy of all references, which must be checked against the original material. Reference citations should be restricted to those that are essential for introducing the purpose and context of the paper, describing methods that are not given in detail, and for discussing the results and any relevant issues raised by them. Authors are responsible for ensuring that references are quoted accurately and not taken out of context. References must not be cited in the synopsis.

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References should be cited in the text using sequential numbers. Superscript numbers should be used and should be placed after any punctuation. When referring to several references, separate individual numerals by a comma or a hyphen for a range greater than two references. For instance: This was first discovered by

Jones,[1] and later confirmed by several other groups of investigators.[2,3,5-7]

Papers accepted for publication, but not yet published, may be included in the reference list; they should be listed as 'in press', with the name of the journal and the likely year of publication. Submitted work should be quoted as 'unpublished results'. Personal communications and unpublished results, which are permitted in the text only, must include the initials and surnames of all the workers involved; for the former citation, the person's affiliation must be stated, e.g. '(J. Bloggs, NIH, personal communication)', and documentary evidence (an e-mail will suffice) from the person quoted, showing their agreement to be so quoted, must be provided (the agreement must include the exact wording that appears in the paper).

All references should be listed numerically at the end of the text. Each reference should be preceded by a bold number (not superscript). Please see the following examples. Failure to conform to Journal style will result in the manuscript being returned to authors.

Examples

Journal reference (<= three authors)
Sanschagrin F, Levesque RC. A specific peptide inhibitor of the class B metallo-B-lactamase L-1 from *Stenotrophomonas maltophilia* identified using phage display. *J Antimicrob Chemother* 2005; **55**: 252–5.

Journal reference (> three authors)
Williams I, Gabriel G, Cohen H *et al.* Zidovudine-the first year of experience. *J Infect* 1989; **18** Suppl 1: 23–31.

Journal reference (online journal)
Bell A, Lewandowski K, Myers R *et al.* Genome sequence analysis of Ebola virus in clinical samples from three British healthcare workers, August 2014 to March 2015. *Euro Surveill* 2015; **20:** pii=21131.

Whole book
Long HC, Blatt MA, Higgins MC *et al. Medical Decision Making.* Boston: Butterworth-Heinemann, 1997.

Book chapter

Manners T, Jones R, Riley M. Relationship of overweight to hiatus hernia and reflux oesophagitis. In: Newman W, ed. *The Obesity Conundrum.* Amsterdam: Elsevier Science, 1997; 352–74.

CLSI methods

Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S15.* CLSI, Wayne, PA, USA, 2005.

Meeting abstract

Hou Y, Qiu Y, Vo NH *et al.* 23-O derivatives of OMT: highly active against *H. influenzae*. In: *Abstracts of the Forty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2003.* Abstract F-1187, p. 242. American Society for Microbiology, Washington, DC, USA.

Online material

References to online material should be given in the reference list. Please note that URLs for the suppliers of materials must not be given in either the text or the references. The Journal does not accept any responsibility for the content of web pages cited.

NB – it is no longer necessary to provide the ‘date last accessed’ for URLs.

Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents.* Department of Health and Human Services. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

Tables

These should be employed sparingly and should be generally comprehensible without reference to the text. Each table should be supplied on a separate sheet and numbered consecutively using Arabic numerals in the order they are referred to in the text. Each must have a brief descriptive heading. Column

headings must clearly explain the content of the column and indicate any units used. Footnotes should be kept to a minimum.

Tables must be created using the Table function in Word; they must not be inserted as images. Each data item should occupy a single cell and return characters should not be used within any Table. *JAC-AMR* reserves the right to move complicated Tables to online-only Supplementary data.

Figures

These must be employed sparingly to demonstrate important specific points. Figures should be numbered using Arabic numerals in the order in which they are referred to in the text. *In figure LEGENDS, symbols should be described in words (e.g. filled circles, open squares etc.).*

Wherever possible, figures should be two-dimensional. Authors should NOT supply 'three-dimensional' figures unless this is actually necessary to represent the data. The quality of reproduction in *JAC-AMR* is limited by the quality of the submitted material. All figures must be of high quality—they should be sharply focused, have good contrast and any lettering must be clear and legible. Colour illustrations can be reproduced if there is sufficient scientific merit in doing so.

The figures supplied with the accepted version will be considered final. Please ensure the figures are of sufficient clarity that you would be happy with them in the print version. Later correction or replacement of figures is not permitted unless significant errors are present. Correction of Figures at the proof stage will cause considerable delay to article publication.

Guidance for preparation of Figures

Figures should be sized to fit a single column of the Journal where possible (88 mm) or a double column if necessary (180 mm). The preferred font for lettering is Times; lettering should have an upper case height of 2 mm and a lower case height of 1 mm at publication size (corresponding to point size 8). Line thickness should be set at 0.5 points. Shading used on line drawings should be clear and distinctive; shades of grey and

heavy stippling do not reproduce well. Lines and symbols should be drawn boldly enough to withstand reduction. The preferred symbols are filled circles, open circles, filled squares, open squares, filled triangles and open triangles, and should be no smaller than 1 mm (height/diameter) at publication size. Part labels should be lower case letters within parentheses, e.g. (a), (b), (c) etc.

Authors must be ready to supply original gel pictures if requested to do so.

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Clinical characteristics, microbiology and outcomes of a cohort of patients treated with ceftolozane/tazobactam in acute care inpatient facilities, Houston, Texas, USA

Can’t keep it SECRET: system evaluation of carbapenem restriction against empirical therapy

The impact of COVID-19 on multidrug-resistant organisms causing healthcare-associated infections: a narrative review

Appendix D: JAC-Antimicrobial Resistance reviewer comments and author responses

Author responses

Reviewer: 1

1. Comment

I am confused as to why LRT samples were included from patients with clinically suspected LRTI or bacteremia. Does that mean that bacteremia without a pneumonia were included? Or is it implied that patients had at least a suspected pneumonia since the FA-PP was sent?

Response

The inclusion criteria are not explicitly defined in the manuscript and have been amended to clarify as follows (Line 135-142): 'Since the suspicion of LRTI is rarely indicated on laboratory request forms, this study included all respiratory tract samples without a recorded clinical indication, where a blood culture was submitted within 2 days of the respiratory tract sample, to serve as a proxy for sepsis attributable to severe respiratory infection.'

The rationale for this selection was that a blood culture taken within a short time period of respiratory sampling, may act as an indicator of suspected sepsis with the respiratory system suspected as a source.

2. Comment

The study's intent is to compare performance of FA-PP with routine culture – but is really just comparing findings (as opposed to performance) since there is no gold standard? That is fine, but just needs to be described accurately.

Response

Changes made in manuscript to clarify that the FA-PP and MC&S results were compared and not the performance of the assay to routine microbiological testing.

3. Comment

Similarly, the sample size was calculated to address the "research question" but unclear what the specific research question is, other than to compare performance, but that is somewhat vague.

Response

The sample size calculation was performed to ensure sufficient samples were tested to compare the utility of the FA-PP to routine culture to ensure positive and negative percent agreement (PPA and NPA) could be reliably determined. (Line 151-152) While not the only relevant measurands, they are important to the ultimate assessment of utility thus

necessitating a power analysis using the indicated parameters. The manuscript has been amended to reflect this.

4. Comment

Please define PPA and NPA better. I assume it means when 100% PPA that all the routine micro culture findings were also detected on FA-PP…but how does that translate into the performance of the FA-PP? It may be finding dead organisms or colonized organisms. Please translate and contextualize PPA and NPA, how they relate to sensitivity and specificity.

Response

PPA and NPA are standard terms when describing the performance of an assay relative to a non-gold standard reference test as was done in this case. In the discussion we highlight that even though the FA-PP in our study had a high PPA and NPA, a concerning number of additional bacteria were detected which might be dead or colonizing organisms. The main benefit of this assay is the likely improved analytical sensitivity reflected by the high PPA to culture, but unfortunately it can also lead to inappropriate management as noted in the discussion.

5. Comment

Regarding figure 2, in which >30% of cases could have resulted in discontinuation. Is that based on viral findings on FA-PP or negative findings on FA-PP? If a viral etiology uncovered, antibiotics could be discontinued. But if negative – does that mean antibiotics could be discontinued in a patient with a clinical pneumonia and was sick? I would think not.

Response

Criteria for discontinuation were: no targets detected, only viruses were detected or an insignificant amount of bacteria detected (defined as bin value of less than 10^7). While we agree that discontinuing antibiotics is a complex decision based on multiple factors, we did not asses clinical findings or alternative etiologies such as Mycobacterium tuberculosis, SARS-CoV2 or pneumocystis. An assessment of “antibiotics discontinuation” should thus be read as “Biofire FA-PP results supports the discontinuation of antibiotics taking into account clinical factors and the limitations of the assay”. The criteria used were added to the manuscript (see figure 2) for greater clarity to convey this implication. Clinical correlation of assay use and its real-world impact on antimicrobial stewardship is outside the intended scope of this paper which is aimed at laboratorians evaluating the potential role for FA-PP in their laboratory.

6. Comment

Is BioFire Film Array validated in sputum? Might want to clarify and reference that in background.

Response

The assay is validated for all used sample types as per the package insert (sputum-like specimens (induced or expectorated sputum, or endotracheal aspirates) or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL). This has been added to the introduction. (Line 83-84)

7. Comment

Can you clarify why only bin with 10^7 were chosen as significant? (as opposed to a lower number)

Response

The FA-PP bin values represent the relative genomic copies of bacterial nucleic acid per millilitre of specimen. In this analysis we made the assumption that the bacteria responsible for the respiratory tract infection will be present in high bacterial loads, as well as evidence in previous studies that the FA-PP overestimates quantification compared to culture. We added this clarification to the manuscript and references to relevant literature. (Line 220-225)

8. Comment

Spell out AMS abbreviation first time used in abstract

Response

This has been added to the manuscript.

Reviewer: 2

1. Comment

Methods: There needs to be a more detail presentation regarding how many of each LRT specimen types were included (sputum/TA/bronch wash/BAL).

Response

This was added to the methods section and is also specified in the result section. (Line 131 and 230/table 1)

2. Comment

Line 137: Was this criteria applied to all?

Response

This was applied to sputum only as per laboratory SOP. Clarification was added in the methods section (Line 158)

3. *Comment*

Line 175: The determination of impact is somewhat unclear. Was this determined by multiple pharmacists or just one? I considering results only >10^7 significant truly real life? Also, the potential for de-escalation should be stratified by appropriate or inappropriate based on culture results. In line 180 it says resistance genes were only considered significant if a viable organism was reported...though the instrument will not report these without a viable organism... Overall this analysis is confusing and adds little to the manuscript. I would recommend either greater detail or omission.

Response

The potential antibiotic change analysis was based on the FA-PP results, applying local microbiology practices, and reviewed by a clinical microbiologist. (Line 209-211) We agree that this analysis might not reflect real life, but this was a retrospective review of the available data with limited clinical information. When interpreting the FA-PP report in clinical practice multiple factors must be considered before an antibiotic change is made. (Line 393-395)

The FA-PP bin values represent the relative genomic copies of bacterial nucleic acid per millilitre of specimen. In this analysis we made the assumption that the bacteria responsible for the respiratory tract infection will be present in high bacterial loads, as well as evidence in previous studies that the FA-PP overestimates quantification compared to culture. We added this clarification to the manuscript. (Line 220-225) Thus, this approach try to limited the excessive use of antibiotics that might occur due the increased analytical sensitivity of this assay.

The criteria for the relevance of resistance genes detected was clarified to '3) resistance genes detected were only considered significant if a corresponding typical organism was detected at $\geq 10^7$ (line 215-216)

The authors disagree that this analysis adds little value and argue that this analysis is critical to the manuscript as a practical approach to using the FA-PP reported pathogens and antibiotic resistance genes to guide antimicrobial therapy choices, as the results are available rapidly, compared to conventional culture results which may take 48 – 72 hours. This section has been amended to clarify the analysis and results obtained.

4. *Comment*

Line 315: Does *this mean they failed the Bartlet score though were still included?*

Response

Yes, sputum samples that failed by Bartlett score were included as the Bartlett score has not been validated as a method of excluding samples intended for the detection of viruses and atypical pathogens such as Legionella. In fact, the GSH virology laboratory does not use any such microscopy based pre-screening method for sample exclusion. Viral pathogens were detected in these poor-quality samples and demonstrated that future studies need to assess the efficacy of a quality scoring system for the FA-PP. This has been added to the results. (Line 369-373)

Appendix E: Published manuscript

JAC Antimicrob Resist
https://doi.org/10.1093/jacamr/dlac139

JAC-Antimicrobial Resistance

Utility of the BioFire® FilmArray® Pneumonia Panel *plus* assay for syndromic testing of lower respiratory tract infections in a low/middle-income setting

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Background: Determining lower respiratory tract infection (LRTI) aetiology is complex. Culture-based methods are laborious with poor sensitivity. Molecular assays improve detection of potential pathogens, but incorrect interpretation of results may lead to inappropriate antimicrobial therapy.

Methods: The utility of the BioFire® FilmArray® Pneumonia Panel *plus* (FA-PP) to detect LRTI pathogens, and the potential impact on antimicrobial stewardship in a low-resource setting, were assessed. Routine LRT samples were included from adult patients with clinically suspected LRTI or with a concomitant blood culture at Groote Schuur Hospital and referring facilities. Culture and FA-PP results were compared, and pharmacy data analysed to determine appropriateness of antibiotic therapy.

Results: There was an 80% correlation between cultured LRTI pathogens and the FA-PP bin $\geq 10^7$ results. Compared with culture, the FA-PP detected substantially more pathogens (86.6% versus 17.9%) and produced a combined 100% positive percent agreement, and 88% negative percent agreement. The FA-PP detected bacterial/viral coinfections in 27% of samples. Correlation of FA-PP results with pharmacy data ($n = 69$) indicated a potential antibiotic change in 75% of cases, but this is difficult to accurately characterize without a 'gold standard' for treatment or complete clinical data.

Conclusions: The FA-PP increased the number of positive samples with typical bacteria, but the semi-quantitative reporting algorithm does not describe the correlation between the different bin values and colonization versus infection. This complicates result interpretation and may lead to inappropriate antimicrobial treatment. This study highlights the potential positive impact of rapid molecular assays for routine care in lower-income settings, but also underscores the interpretive challenges associated with these tests.

Introduction

Antibiotics are the cornerstone for pneumonia therapy and target the most common bacterial causes. It is often difficult to discern clinically and/or radiologically whether the cause of the pneumonia is viral, bacterial, or even non-infectious.[1] In the setting of community-acquired pneumonia (CAP) there is emerging evidence that viruses play a greater role than initially considered and implies that antibiotics may be unnecessarily prescribed in many instances.[1] Overprescribing of broad-spectrum antibiotics also occurs in the hospital setting where up to 50% of in-hospital patients may not have bacterial pneumonia.[2] This indiscriminate use of antibiotics may promote the development or selection of antimicrobial resistance (AMR), one of the leading WHO global health threats.[3]

Identifying the bacterial aetiology of lower respiratory tract infection (LRTI) in clinical respiratory samples using conventional culture methods is laborious and can take up to 5 days to finalize complex cultures and determine susceptibility profiles.[4] Implementing antimicrobial stewardship (AMS) principles in these settings is therefore challenging, and broad-spectrum antibiotics are generally continued until conventional microbiology results are available.[4,5] Various factors contribute to the sensitivity and turnaround times of conventional cultures. These include failure to isolate the aetiological agent due to antimicrobial exposure prior to obtaining the sample, overgrowth of contaminating or normal microbiota, which may mask the pathogen, as well as failure of routine culture media to support the organism-specific growth requirements of atypical bacteria.[6,7]

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Molecular methods have become an attractive alternative to conventional culture methods to detect bacterial pathogens. These tests can overcome the limitations of conventional microbiological methods by providing rapid and more sensitive results without the need for viable organisms. This may assist in making clinical decisions earlier, improve patient outcomes, and ultimately reduce the unnecessary use of antimicrobial agents.[5,8–10]

One such syndrome-specific system, which has been evaluated in numerous studies, is the BioFire® FilmArray® Pneumonia Panel *plus* (FA-PP; bioMérieux, Marcy l'Étoile, France). It is a rapid, cartridge-based, multiplex PCR assay detecting nucleic acids from various bacterial and viral respiratory pathogens causing pneumonia, as well as common AMR genes. This assay is approved for respiratory samples such as sputum, endotracheal aspirate (TA) and bronchoalveolar lavage (BAL) samples,[11] and is US FDA-cleared, CE-marked for *in vitro* diagnostic medical devices and Therapeutic Goods Administration-certified for diagnostic use.

This assay includes 15 typical pneumonia-causing bacteria (typical bacteria), 9 viruses, 3 atypical bacteria, as well as AMR genes for MDR organisms such as ESBL- and carbapenemase-producing organisms and MRSA. The presence of AMR genes is reported qualitatively, but only when an associated bacterium is simultaneously detected by the panel.

Development of commercial multiplex panels for the diagnosis of LRTI is rapidly progressing, but limited data are available to guide informed clinical decision-making regarding the utility of rapid diagnostic tests (RDTs) in low/middle-income countries (LMICs). To address this gap, this single-centre, cross-sectional pilot study was performed, determining the diagnostic utility of the BioFire® FA-PP panel for detecting LRTIs to assess the potential AMS impact, pertaining to organism identification and/or mechanism of resistance.

Methods

Design

A single-centre, cross-sectional, laboratory diagnostic study comparing the results of conventional microbiological investigations of LRTI specimens with that of the BioFire® FA-PP panel.

Setting

The study was conducted at the National Health Laboratory Services (NHLS), Microbiology Laboratory, C18, located at Groote Schuur Hospital (GSH), Cape Town, South Africa. GSH provides tertiary and quaternary care for a large population in the City of Cape Town Metropolitan, and both the hospital and the on-site NHLS laboratory serve as a referral centre for regional and district hospitals.

Ethics

Ethics approval for this work was granted by the University of Cape Town Human Research Ethics Committee (HREC reference number: 769/2020) and a waiver of the requirement for informed consent was obtained.

Study population

The study population included adult (≥18 years) inpatients and outpatients at GSH or any of the referring healthcare facilities. Samples were collected between 24 February and 3 April 2021 using a convenience sampling method. All LRTI (47 tracheal aspirates and 78 sputum) samples submitted to the NHLS laboratory by the treating clinician as part of routine patient care for routine microbiological investigations were considered for inclusion. Respiratory samples were included in the study if they included clinical suspicion of an LRTI as stated on the laboratory request form. Since the suspicion of LRTI is rarely indicated on laboratory request forms, this study included all respiratory tract samples without a recorded clinical indication, where a blood culture was submitted within 2 days of the respiratory tract sample, to serve as a proxy for sepsis attributable to severe respiratory infection. All selected samples had to be of sufficient volume to perform standard diagnostic tests and additional tests requested by the treating clinician, with a minimum of 500 μL residual sample for the FA-PP assay. Duplicate samples were only included if a previous sample from the same patient had been included >10 days prior.

Sample size

Using the MKmisc R package[12] to run the power diagnostic test script with the selected criteria of $\alpha=0.05$, power$=0.8$ and $\delta=0.1$, an approximate sample size of 136 specimens was required to determine the positive percent agreement (PPA) and negative percent agreement (NPA) assuming 95% sensitivity for the FA-PP assay based on published literature and an expected prevalence of detectable pathogens in 50% of selected samples.

Laboratory procedures

LRT samples that met the inclusion criteria were processed within 48 h of sample receipt at the laboratory. Routine microbiological investigations included Gram stain microscopy, culture and susceptibility testing (MC&S). The quality of sputum samples was assessed using the Bartlett scoring system, and samples with a score <1 were excluded from being processed for culture.[13] For semi-quantitative culture, chocolate blood agar, Colgent (Columbia blood agar with gentamicin) with the addition of a 10 μg/mL optochin disc, and a MacConkey agar plate (MCC) were inoculated and incubated in a carbon dioxide incubator, with the MCC agar plate aerobically, both at 35°C for 24 h. Respiratory pathogens were identified and graded by trained laboratory technologists and followed up according to standard operating procedures. The VITEK 2 instrument (bioMérieux, Marcy l'Étoile, France) was used to identify any bacterial growth deemed significant, and their susceptibility profiles determined and interpreted using the CLSI 2021 guidelines.[14]

The FA-PP assay was performed according to the manufacturer's instructions using 200 μL of the residual sample once all routine laboratory testing was complete. The results of the FA-PP assay and routine laboratory testing were recorded by the investigator once all tests were finalized. FA-PP assay results were not communicated to the laboratory staff to prevent bias and the investigator only informed the treating clinicians of notifiable organisms detected.

Data collection and analysis

The results obtained from routine laboratory testing and the FA-PP assay, as well as basic patient demographics (age, gender, hospital, ward) available on the laboratory information system (TrakCare), and the electronic prescribing data obtained from the pharmacy, were captured in a Microsoft Excel spreadsheet. Descriptive statistical analyses were performed using Microsoft Excel and Stata software version 17.0 (StataCorp, College Station, TX, USA).

The FA-PP assay is able to detect common bacterial pathogens causing pneumonia and these were classified as either 'typical' or 'atypical'. The presence and absence of each typical bacterial target as detected by the two methods were collated and the PPA and NPA were calculated for each bacterial target using GraphPad Prism version 9.3.1.

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The FA-PP assay reports typical bacteria semi-quantitatively and this function is based on a binning algorithm where the relative number of PCR amplicons is measured and compared with an internal standard curve. Bins are reported as 10^4, 10^5, 10^6 or $\geq 10^7$ copies/mL, and each bin represents a density range of about 1 log unit, with upper and lower limits, for example: the 10^4 bin is equivalent to $10^{3.5}$ to $10^{4.5}$ copies/mL.[8,11]

The detection of AMR genes was compared with the phenotypic susceptibility test results obtained from the VITEK 2. Atypical bacteria in the FA-PP panel are not routinely tested for in the diagnostic laboratory and were therefore not compared with other methods.

Pharmacy records of included patients, where available, were reviewed by a clinical microbiologist to assess whether the prescribed antibiotic was appropriate for the FA-PP pathogens detected, based on current clinical microbiological practices. After completion of the FA-PP assay, and due to incomplete clinical data, the following assumptions were made for this analysis: (1) only typical bacteria detected in bin $\geq 10^7$ were considered significant; (2) all atypical bacteria detected on the FA-PP assay were regarded as significant; (3) resistance genes detected were only considered significant if a corresponding typical organism was also detected at bin $\geq 10^7$; (4) all antibacterial agents issued on the same day or within 2 days of sample collection were assessed for appropriateness; (5) if no antibiotics were issued on the same day or within 2 days of sample collection, antibacterial agents prescribed prior to sample collection were assessed, but only if sufficient doses were prescribed to overlap the sample collection day; (6) all antibacterial agents were prescribed for an LRTI. The bin $\geq 10^7$ value was considered significant as previous studies reported the overestimation of quantification using the FA-PP assay, when compared with culture.[7,15,16] Additionally more than 85% of bacteria considered as significant by culture were also reported with a bin $\geq 10^6$ or bin $\geq 10^7$ by Gastli *et al.*[17] and Yoo *et al.*,[6] respectively. The potential impact on patient treatment, using the results obtained using the FA-PP assay, were classified as 'no change', 'escalation', 'de-escalation' or 'discontinuation'.

Results

A total of 125 LRT samples (47 TAs and 78 sputum samples) were collected during the 5 week study period. The samples were collected from 123 patients with suspected LRTI, admitted or treated as an outpatient at GSH and from 11 surrounding referral hospitals. Most samples were collected from ICU patients, followed by general ward patients (Table 1).

Of the 125 samples collected, 13 were rejected for further routine testing based on the Bartlett test results. The FA-PP assay detected pathogens in 86.6% (97/112) of the samples that were tested with both methods, and this included typical bacteria in 55.4% (62/112), atypical bacteria in 0.9% (1/112), viruses in 3.6% (4/112) and coinfections in 26.8% (30/112) of the samples. Routine culture only detected pathogens in 17.9% (20/112) of the samples tested (Figure 1).

Organisms not included in the FA-PP assay panel were detected in three samples using routine culture, including *Aeromonas hydrophila*, *Corynebacterium striatum* and *Morganella morganii*. The most commonly detected pathogen using both methods was *Haemophilus influenzae*, with 38.4% of samples using the FA-PP assay, and in 4.5% samples with routine testing. The second most common pathogen detected by the FA-PP was *Staphylococcus aureus*, while the second most common pathogens detected by culture were both *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (Table 2).

The most commonly detected virus was human rhinovirus/enterovirus. No tests for respiratory viruses, except for SARS-CoV-2, were requested as part of routine care and thus no comparison between methods was possible. *Legionella pneumophila* was detected in four samples; for three of these patients a *Legionella* urinary antigen test (serogroup 1) was not requested as part of routine testing. A confirmatory *Legionella* urinary antigen test (serogroup 1) was performed by the investigator following discussion of the FA-PP results with the treating clinicians. Two of these samples tested negative for *L. pneumophila* serogroup 1 and were later confirmed by the reference laboratory (National Institute for Communicable Diseases, NICD) as *L. pneumophila* non-serogroup 1.

A total of 30 resistance genes were detected using the FA-PP assay. Of these, 22 samples had one gene detected, 1 sample had two genes, and 2 samples had three genes. The $bla_{\text{CTX-M}}$ gene was detected most commonly (14.3%), followed by *mecA/C* (5.4%), bla_{NDM} (4.5%) and $bla_{\text{OXA-48-like}}$ (2.7%).

The FA-PP detected pathogens in 11/13 sputum samples that were rejected for routine culture due to a low Bartlett score. This produced a total of 17 typical bacteria (ranging from 1 to 3 per sample) and 8 viral pathogens. Two samples also contained either the $bla_{\text{CTX-M}}$ or $bla_{\text{OXA-48}}$ resistance gene.

The qualitative assessment of the FA-PP assay to detect typical bacterial targets demonstrated a combined 100% PPA and 88% NPA compared with routine culture (Table 2). In 18/20 samples where bacteria were detected with both methods, the FA-PP reported a bin $\geq 10^7$ result. The concordance for semi-quantitation of the two methods was not determined due to the low number of pathogens detected by the routine laboratory testing.

Pharmacy data were available for 69/125 samples (67 participants) and in 75% ($n = 52$) of these cases a potential antibiotic change was possible based on the FA-PP assay results (Figure 2).

Table 1. Sample and patient characteristics

	Number (n = 125)	Percentage
Sample type		
Sputum	78	62.4
TA	47	37.6
Ward type		
ICU	47	37.4
General ward	43	34.4
Emergency centre	22	17.6
Outpatient clinic	3	2.4
Not indicated	10	8.0
Gender		
Male	80	64.0
Female	45	36.0
Age, years (range)		
Combined median	47 (18–90)	
Male median	47 (18–90)	
Female median	45 (18–81)	

Discussion

Compared with conventional microbiology, novel and rapid panel-based diagnostic strategies offer clear advantages of a

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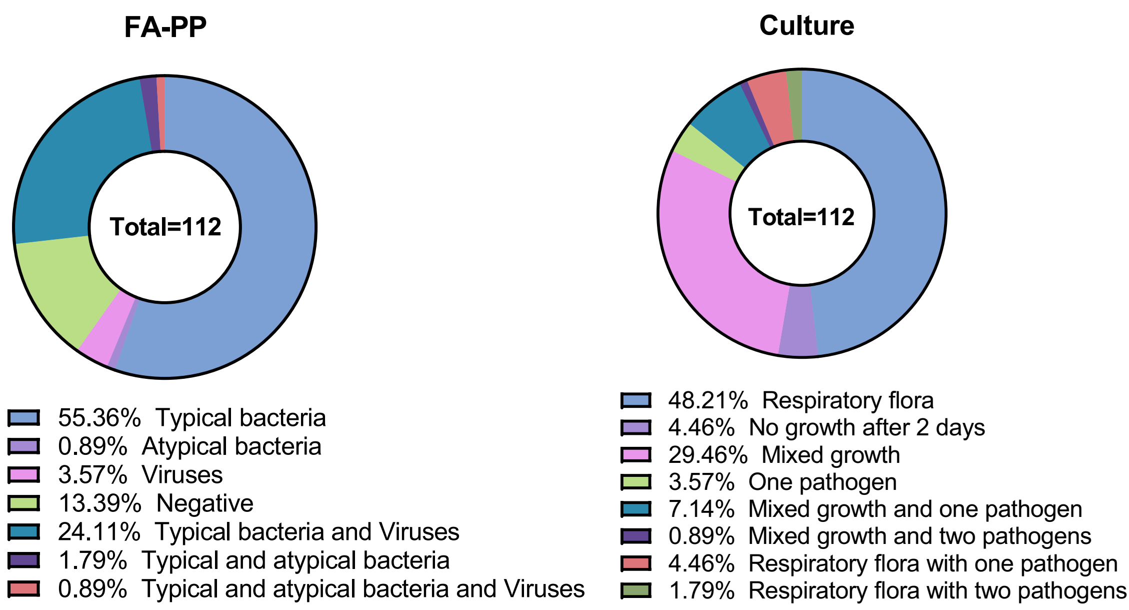

Figure 1. Proportions of pathogens detected using the FA-PP and routine laboratory testing.

shorter turnaround time, increased sensitivity and the detection of fastidious microorganisms, including AMR determinants, and may thus offer substantial improvements in patient care. Numerous studies report the performance and benefits of respiratory syndromic panels such as the FA-PP assay; however, most were performed in high-income countries using BAL specimens and samples collected from patients admitted to ICUs.[7,15,16,18] Additionally, the impact on patient-level outcomes have yet to be determined.[19]

We aimed to assess the utility of the FA-PP assay using all routine LRT samples submitted to a South African laboratory for routine microbiological investigations. The samples included were collected at various healthcare settings in order to assess patients with a range of disease severities. More than half of the samples collected were sputum samples. The routine laboratory testing had a poor yield, where ≥1 pathogen/s were detected in less than a quarter of samples. More pathogens were detected in TA samples, but 'mixed growth' or 'respiratory flora' were reported in a large number of both TA and sputum samples, which is common for these sample types.

The poor diagnostic yield of sputum samples from patients with suspected CAP is well described and for this reason the collection is discouraged in many guidelines, including the South African guideline for managing CAP[20] and ATS/IDSA,[21] which indicate that MC&S has a limited impact on patient management and outcome and should only be requested in cases of severe disease and where there is a high risk for an infection with nosocomial pathogens.[21] In contrast, the IDSA guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia promote the collection of non-invasive samples over invasive samples due to the lack of evidence that invasive sampling improves clinical outcome.[22] Unfortunately, these non-invasive sample types are prone to contamination with upper respiratory tract commensals or colonizing microorganisms, especially in patients with chronic tracheostomies, where the tracheostomy tube is colonized.[8,10] A BAL may theoretically provide a 'superior' quality result due to the site-directed collection[10] limiting contamination, which may imply organisms cultured from these samples will most likely better reflect the true pathogen causing the LRTI, which further simplifies interpretation of the laboratory report. BAL is, however, not a realistic option for all patients due to limited resources in lower-income settings, as well as the invasiveness and complicated nature of specimen collection.

The use of the FA-PP assay increased the number of samples where typical bacteria were detected by 68.7%. This dramatic increase may appear as an increase in sensitivity over culture; however, we detected a concerning amount of additional typical bacteria, not detected with culture, using the FA-PP (194/214). A similarly increased positivity rate (63.3%) was also reported by Buchan *et al.*[7] However, that study was much larger and assessed 259 BAL samples, but only reported 73 additional bacterial targets. This finding is likely attributed to the non-invasive sample types in our study, which probably contained more contaminating commensals.

The FA-PP assay produced a PPA of 100% for 8/15 of the typical bacteria detected by culture, which was comparable with other studies evaluating the FA-PP. Buchan *et al.*,[7] Lee *et al.*,[16] Ginocchio *et al.*,[15] Mitton *et al.*,[23] Weber *et al.*[18] and Yoo *et al.*[6] all reported a PPA (or sensitivities) for bacterial pathogens of ≥90%. The combined NPA was 88% for the typical bacteria, with all targets having an NPA ≥64%, which was lower than those reported in the above studies, except for Yoo *et al.*,[6] who reported a specificity of 76.5%. Due to the lack of a 'gold standard' diagnostic test for LRTI these detections cannot, however, be described as false positives.[16]

Unfortunately, the interpretation of these additional detections using the FA-PP assay is complicated by a number of factors.

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Table 2. Typical and atypical bacteria detected, with PPAs and NPAs

Typical bacteria	Culture (n)	FA-PP (n)	PPA (95% CI)	NPA (95% CI)
H. influenzae	5	43	100 (57–100)	64 (55–73)
S. aureus	1	30	100 (5–100)	74 (65–81)
S. pneumoniae	4	28	100 (51–100)	78 (69–85)
Klebsiella pneumoniae	1	27	100 (5–100)	77 (68–83)
Enterobacter cloacae complex	2	18	100 (18–100)	85 (78–91)
Acinetobacter calcoaceticus-baumannii complex	2	16	100 (18–100)	87 (80–92)
P. aeruginosa	4	11	100 (51–100)	94 (87–97)
Escherichia coli	0	10	—	91 (84–95)
Moraxella catarrhalis	0	9	—	92 (85–96)
Proteus species	0	6	—	95 (89–98)
Klebsiella oxytoca	0	4	—	96 (91–99)
Serratia marcescens	1	4	100 (5–100)	97 (92–99)
Klebsiella aerogenes	0	3	—	97 (92–99)
Streptococcus agalactiae	0	3	—	97 (92–99)
Streptococcus pyogenes	0	2	—	98 (94–100)
Total	20	214	100 (84–100)	88 (87–90)

Atypical bacteria	Culture (n)	FA-PP (n)
L. pneumophila	4[a]	4
Chlamydia pneumoniae	N/A	0
Mycoplasma pneumoniae	N/A	0
Total		4

Viral pathogens	PCR (n)	FA-PP (n)
Human rhinovirus/enterovirus	N/A	19
Parainfluenza virus	N/A	5
Coronavirus	N/A	4
Respiratory syncytial virus (RSV)	N/A	4
Adenovirus	N/A	2
Human metapneumovirus	N/A	0
Influenza A	N/A	0
Influenza B	N/A	0
Middle East respiratory syndrome coronavirus (MERS)	N/A	0
Total		34

Resistance genes	Phenotypic (n)	FA-PP (n)
CTX-M	3[b],c	16
mecA/C and MREJ	0	6
NDM	2[c],d	5
OXA-48-like	0	3
IMP	0	0
KPC	0	0
VIM	0	0
Total	5	30

N/A, not applicable.

[a]Two out of the four L. pneumophila detections confirmed with positive L. pneumophila serogroup 1 urinary antigen test, the rest confirmed with a PCR test for atypical pneumonia by the NICD as non-serogroup 1.

[b]Carbapenem-susceptible P. aeruginosa (WT) was detected by routine laboratory testing, but FA-PP detected CTX-M, NDM and OXA-48-like genes in the presence of Acinetobacter calcoaceticus-baumannii complex, K. pneumoniae and P. aeruginosa. Assumption made that P. aeruginosa only associated with CTX-M.

[c]Carbapenem-resistant A. baumannii detected by routine laboratory testing, but FA-PP detected CTX-M and NDM genes in the presence of Acinetobacter calcoaceticus-baumannii complex and K. pneumoniae. Assumption that A. baumannii associated with both genes.

[d]Carbapenem-resistant A. baumannii detected by routine laboratory testing, but FA-PP detected the NDM gene in the presence of Acinetobacter calcoaceticus-baumannii complex and P. aeruginosa. Assumption made that A. baumannii associated with the NDM gene.

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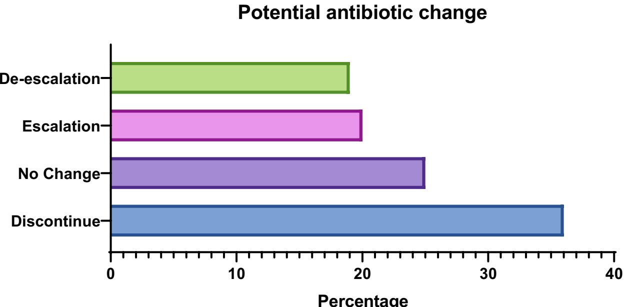

Figure 2. Potential antibiotic change based on FA-PP findings. De-escalation, narrower-spectrum agents (or fewer agents) indicated; escalation, broader-spectrum agents (or additional agents) indicated; no change, agents appropriate; discontinue, no targets detected, only viral pathogens detected or bacteria detected with a bin value of less than 10^7.

PCR-based assays are more sensitive than routine culture and can detect genetic material of both viable and non-viable organisms. This is advantageous where antibiotics were administered prior to sample collection leading to some organisms being undetectable by culture. Since there were no records of antibiotics prior to sample collection or time of antibiotic administration for the participants in this study, no correlations could be made.

In cases where bacteria are cultured, the laboratory's protocol will guide reporting. Bacterial isolates that are grown in insufficient quantities, mixed with more than two potential pathogens, or where the growth is overwhelmed by normal respiratory microbiota may not be regarded as significant, and therefore not reported. Bacteria such as *H. influenzae* are fastidious to culture and may easily be overgrown by normal microbiota or lose viability[15] and may as a result be missed by culture. *H. influenzae* was the most common pathogen detected by both methods, but the FA-PP detected *H. influenzae* in 38 additional samples. The FA-PP also detected 29 additional samples with *S. aureus*. A similar finding was also described by Buchan *et al.*[7] and Ginocchio *et al.*[15]

The quality of the sample is also vital to ensure that significant typical bacterial pathogens are not missed by routine laboratory testing. We included 13 sputum samples that were rejected for routine culture in order to assess the value of these samples for detecting potential pathogens. Typical bacteria were detected by the FA-PP assay in nine of these samples and this most likely reflects contamination or colonization in the absence of inflammation. This illustrates the importance of quality assessment prior to culture or molecular tests for typical bacteria, because neither of these methods are able to separate colonizers from invasive pathogens.[10] However, quality scoring systems for sputum samples do not apply in LRTIs caused by atypical bacteria and viruses.[24] In fact, viral pathogens were detected in eight of these samples that were rejected due to poor quality. This demonstrates that future studies need to assess the value of a quality scoring system for the FA-PP to enable optimal detection of all clinically relevant pathogens.

The semi-quantitative reporting of bacterial results using the FA-PP assay, which is intended to simplify this discrimination, lacks the ability to definitively interpret organisms that may be colonizers.[8] Nearly all of the typical bacteria reported by the routine testing correlated with an FA-PP bin of $\geq 10^7$, but the majority of all the detected typical bacteria were also reported with a bin of $\geq 10^7$. In addition to the large number of typical bacteria that were detected per sample, a combination of bacterial and viral pathogens was also detected in 27% of samples. This further complicated the interpretation of results, since coinfections with bacterial and viral pathogens are possible, but the detection of a viral pathogen can also be due to asymptomatic carriage or a recent viral infection that led to a subsequent secondary bacterial infection.[25]

The rapid detection of AMR genes can lead to earlier escalation or de-escalation of therapy in patients, as well as earlier infection prevention and control interventions. Although the FA-PP assay detected resistance genes in some of the samples, the pathogen it was associated with was not always detected by culture and the results could therefore not be compared.

The assessment of potential impacts on antibiotic therapy using the FA-PP results was based on typical bacteria detected with a bin $\geq 10^7$ only, associated resistance genes, atypical bacteria and viruses, by applying local microbiology practices. In clinical practice, multiple factors must be considered before an antibiotic change is made. In this retrospective assessment with limited clinical information, we identified 52/69 cases where a potential antibiotic change was possible including de-escalation (19%), escalation (20%) and discontinuation of antibiotics (36%). This may have significant impacts on patient outcomes, AMS and infection prevention and control interventions.

As described by Hanson *et al.*,[10] the enhanced detection of a multiplex nucleic acid amplification test decreases the likelihood that important pathogens are missed, but also complicates result interpretation and ultimately patient management. The use of the FA-PP assay on routine samples and the reporting of results without the input of a clinical microbiologist or infectious diseases specialist may lead to overtreatment due to the large number of additional bacterial detections with unclear clinical significance. Advocating the use of these diagnostic assays without firmly establishing criteria for which patients would benefit most, how to meaningfully interpret the results, and how to treat accordingly, could in fact be counterproductive with regard to diagnostic 'best practice' and AMS.

This study had several limitations, most importantly the lack of a 'gold standard test', which complicated the comparison of methods. The small sample size produced wide CIs for comparisons and no definite conclusions could be made. Complete records of clinical data would have improved the assessment of potential antibiotic changes; also, information on antimicrobial exposure prior to sample collection would have simplified interpretation of the results. We were also unable to compare the cost differential between the methods, and therefore definitive recommendations as to the value-added benefit of introducing such an assay in our setting cannot be made.

Future work should focus on determining semi-quantitative values where relative pathogen abundance may assist in determining the significance of a specific pathogen. Similarly, the timing of the specimen in the course of disease may be a confounder and requires elucidation.

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Conclusions

Despite the limitations in our study, the FA-PP assay substantially increased the number of positive samples with typical bacteria. A potential antibiotic change was possible in 75% of cases. Our study highlights the potential impact of introducing rapid molecular assays in routine care in settings such as ours but underscores the interpretive challenges associated with novel rapid tests.

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Transparency declarations

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author contributions

M.V.D.W., C.M. and N.S. designed the study. M.V.D.W. performed the laboratory tests, analysed and interpreted the data. M.V.D.W., C.M., N.S. and A.B. wrote the manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author, M.V.D.W, upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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