Molecular diagnostics improve the yield of diagnosis of community-acquired pneumonia and multidrug-resistant pathogens in hospitalised patients with HIV in a low-income setting

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Background. Community-acquired pneumonia (CAP) remains an important cause of morbidity and mortality in people with HIV (PWH), and antimicrobial resistance (AMR) leads to poor treatment outcomes. Better tests are required to overcome the low sensitivity of sputum Gram stain and culture for pneumonia diagnosis. Molecular diagnostic tests rapidly detect respiratory pathogens and markers of AMR, but few studies have examined their role in PWH.
Objectives. To investigate the additional yield of the Biofire FilmArray Pneumonia Panel *plus* (FilmArrayPN-PCR), an automated nested multiplex polymerase chain reaction system, over culture for diagnosis of CAP, and determine clinical predictors of AMR in PWH.
Methods. We enrolled adult PWH hospitalised with cough <2 months in a prospective cohort in Kampala, Uganda. Participants provided expectorated sputum samples for testing by FilmArrayPN-PCR and culture. We performed drug susceptibility testing of cultured sputum isolates and detection of genetic markers of AMR on sputum by FilmArrayPN-PCR.
Results. The 107 participants enrolled had a median (interquartile range) age of 40 (31 - 46) years, 50.5% (n=54/107) were female, and 74.8% (n=80/107) had recent antibiotic use. The median duration of cough was 3 (1 - 4) weeks. FilmArrayPN-PCR increased the detection of respiratory pathogens by 64.5% (95% confidence interval (CI) 54.8 - 73.1; p<0.001) and detected AMR in 25.2% (n=27/107). Baseline room air oxygen saturation <92% (adjusted odds ratio (aOR) 9.20; 95% CI 2.52 - 33.57; p=0.001) and prior antibiotic use (aOR 4.14; 95% CI 1.04 - 16.51; p=0.04) were independent predictors of AMR.
Conclusion. FilmArrayPN-PCR increased the diagnostic yield of pathogens, and a low baseline oxygen saturation (<92%) and prior antibiotic use were associated with an increased risk of AMR in hospitalised PWH with CAP.

Keywords. FilmArray, pneumonia, hospitalised, HIV, antimicrobial resistance.

Afr J Thoracic Crit Care Med 2025;31(2):e2415. https://doi.org/10.7196/AJTCCM.2025.v31i2.2415

Study synopsis
What the study adds. The Biofire FilmArray Pneumonia Panel *plus* detected 64.5% more respiratory pathogens compared with culture, and detected antimicrobial resistance (AMR) genes in 25.2% of patients with HIV hospitalised with community-acquired pneumonia (CAP). Baseline room air oxygen saturation <92% and prior antibiotic use were associated with nine times and four times increased odds of AMR, respectively.
Implications of the findi gs. Multiplex polymerase chain reaction (PCR) assays increase the speed of detection and diagnostic yield of respiratory pathogens and may be useful for diagnosis of AMR in hospitalised patients with HIV and CAP. The clinical implications of these findings should be evaluated further in prospective studies and cost-effectiveness studies to define the role of multiplex PCR tests in the patient care pathway.

Community-acquired pneumonia (CAP) is a common cause of morbidity and mortality in people living with HIV (PWH), especially those with advanced immune suppression.[1] The reduction in the frequency of pneumonia in PWH on antiretroviral therapy (ART) does not decline to levels in HIV-seronegative individuals.[2] As a result, PWH remain susceptible to pneumonia from a wide range of pathogens, which may need specific treatment.[3] This predisposition of PWH to a broad spectrum of lung infections underscores the need for a specific aetiological diagnosis.

Aetiological diagnosis of CAP remains a challenge because sputum Gram stain is nonspecific, and conventional culture-based techniques that can provide a specific diagnosis have low sensitivity and are not universally available.[4] The long turnaround time for sputum culture makes it impractical for initial antibiotic selection.[5] The sensitivity of culture-based tests is further compromised by prior antibiotic use,[6] yet early initiation of antibiotics is vital to prevent clinical deterioration of patients with severe CAP.[7] Furthermore, the infrastructure and capacity to perform sputum culture and drug susceptibility testing are limited in low-income settings. In Uganda, a low-income country with a high HIV burden, the last comprehensive hospital-based study on causes of CAP in PWH was published in 2001.[8] The epidemiology of CAP may have changed significantly, and updated studies are urgently needed. In addition, the increased emergence of antimicrobial resistance (AMR), which is estimated to cause 700 000 deaths globally per year, is a major public health challenge contributing to increased healthcare costs.[9] In many low-income settings where the vast majority of PWH reside, there are limited data on the epidemiology of CAP and AMR in PWH.

Molecular tests, such as the polymerase chain reaction (PCR), show promise in detecting pathogens causing CAP, as the PCR is less affected than culture by prior antibiotic use. In addition, the PCR identifies specific mutations associated with AMR. Multiplex PCR panels designed for pneumonia can diagnose several bacterial and viral pathogens simultaneously, leading to rapid diagnosis of pathogens causing pneumonia[10] that can be used to triage hospitalised patients, and can also identify AMR to optimise antibiotic selection. We therefore investigated the additional yield of the Biofire FilmArray Pneumonia Panel *plus* (FilmArrayPN-PCR) (bioMérieux, France) over culture-based methods for detection of lower respiratory tract pathogens and AMR in PWH, and determined the clinical predictors of AMR in hospitalised patients with CAP.

Methods

Study design and participants

We conducted a prospective cohort study at two referral hospitals in Kampala, Uganda (Kiruddu Hospital and China-Uganda Friendship Hospital), from 7 May 2019 to 22 February 2021. We performed laboratory testing at the Infectious Disease Institute Translational Laboratory and the Makerere University Medical Microbiology Laboratory.

Adult patients who were hospitalised with clinician-diagnosed CAP, had provided written informed consent, were PWH, and had cough <2 months were enrolled. Patients were excluded if they were unable to consent, had been hospitalised during the previous 4 weeks, had no chest radiograph to confirm radiographic pneumonia, could not produce sputum, or had a confirmed diagnosis of tuberculosis.

We administered a standard questionnaire to participants to obtain demographic information and a clinical history regarding their respiratory symptoms, risk factors for pneumonia, history of prior antibiotic use, and presence of comorbidities. A physical examination was conducted, and vital signs were measured and recorded. Oxygen saturation on room air was measured using pulse oximetry. Empirical antibiotic treatment was provided by the clinicians based on existing clinical care guidelines provided by the Ministry of Health, and switching of antibiotics was at the discretion of the attending clinicians.

Sample collection and testing

Two expectorated sputum samples were collected. One sample was sent to the laboratory for microscopy, culture and drug susceptibility testing as the standard of care, and the other sample was tested using FilmArrayPN-PCR. FilmArrayPN-PCR is a multiplex PCR assay approved by the US Food and Drug Administration. The assay identifies 18 commonly occurring bacterial pathogens, 9 respiratory viruses, and 7 antimicrobial resistance genes for extended beta-lactamases, carbapenemases, and *Staphylococcus* resistance (Supplementary Table 1, available online at http://coding.samedical.org/file/2340). Sputum quality was assessed using the Bartlett score prior to inoculation on culture media.[11] A sputum sample with <10 epithelial cells per low-power field and >25 pus cells per low-power field was considered good quality. Sputum culture was done on chocolate, blood and MacConkey agar. Isolates grown from sputum were suspended in Mueller Hinton broth and incubated in the automated BD Phoenix AST system (Becton Dickinson, USA) for rapid identification and antimicrobial susceptibility testing.

Study outcomes

We defined a potential pathogen likely to cause CAP as any positive result from sputum culture or FilmArrayPN-PCR. We defined AMR as present based on the results of sputum culture drug susceptibility testing or presence of molecular markers of resistance on FilmArrayPN-PCR.

Data management and statistical analysis

Double data entry was performed in Microsoft Access 2016 (Microsoft, USA), and statistical analysis was performed using Stata version 9.0 (StataCorp, USA). We calculated the proportions of patients with a potential pathogen likely to cause CAP and AMR by standard of care and FilmArrayPN-PCR and compared the concordance and incremental yield of FilmArrayPN-PCR relative to sputum culture and sensitivity. Participant demographic and clinical characteristics were summarised as means and medians for continuous variables, and categorical data were summarised as frequencies. Group comparisons for categorical variables were made with Fisher's exact test and for continuous variables using the rank-sum test. To investigate the association of clinical and demographic factors with drug resistance, we used bivariate and multivariate binary logistic regression analysis. A multivariate model was generated using variables with a p-value ≤0.20. Back stepwise regression procedures were used to develop the final multivariate model. A p-value <0.05 was considered significant.

Ethical considerations

All participants provided written informed consent to participate in the study. Ethics approval was obtained from the Makerere University School of Medicine Research and Ethics Committee

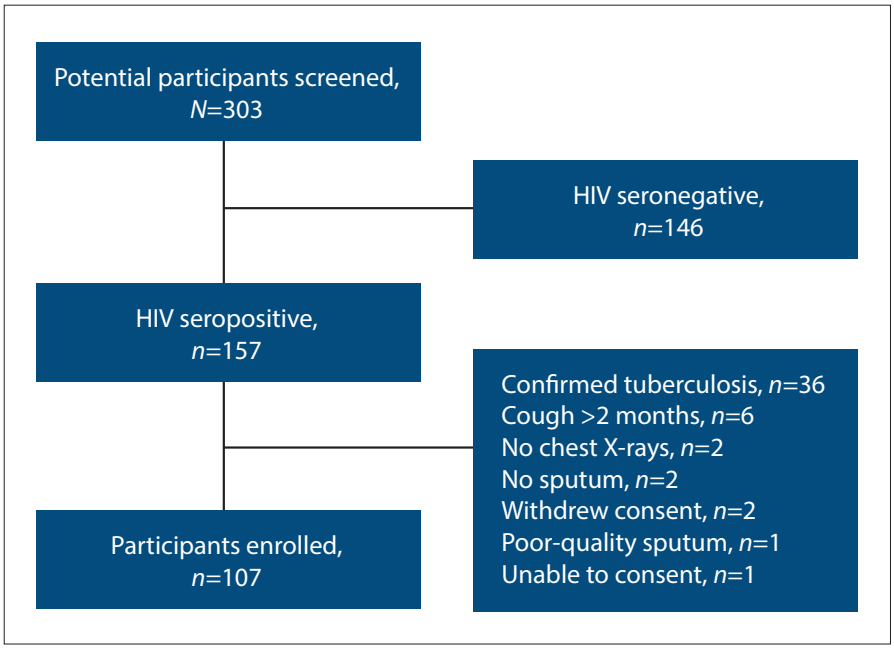

Fig. 1. Study profile.

(ref. no. 2006-017). The study was also approved by the Uganda National Council of Science and Technology (ref. no. 2006-19).

Results

Study participants

Of 303 adults with presumptive CAP who were screened, 107 were enrolled (Fig. 1). The baseline characteristics of the enrolled participants are shown in Table 1. Their median (interquartile range) age was 40 (31 - 46) years, and 54 (50.5%) were female. The median CD4 lymphocyte count was 167 (85 - 331) cells/μL. All the participants had a cough, with a median duration of 3 (1 - 4) weeks, and the cough was productive of sputum in 100 (93.5%). The median baseline oxygen saturation was 96% (92 - 98%) on room air. At baseline, 21 (19.6%) of the 107 study participants had oxygen saturation <92%, 31 (29.0%) had oxygen saturation 92 - 95%, and 55 (51.4%) had oxygen saturation >95%. Overall, 80 (74.8%) had recent use of antibiotics prior to hospitalisation (Table 1). The median duration of antibiotic use before hospitalisation was 5 (3 - 7) days. Overall, 68 participants (71.6%) reported ART use. On admission, 30 participants (28.0%) were on co-trimoxazole prophylaxis for *Pneumocystis jirovecii* pneumonia. Reported comorbidities included asthma (*n*=5; 4.7%), chronic obstructive lung disease (*n*=3; 2.8%), liver disease (*n*=2; 1.9%) and diabetes mellitus (*n*=1; 0.9%).

Microbiological diagnoses

In 94 participants (87.9%), FilmArrayPN-PCR (and sputum culture in 25 cases) revealed a possible aetiological agent for pneumonia (Table 2). Participants who had a shorter duration of cough were more likely to have a positive sputum culture than those with a longer duration of cough, although this did not apply to the FilmArrayPN diagnostic approach (Table 1), and participants with a positive culture were more likely to have low oxygen saturation compared with those with a negative culture (Table 1).

Standard of care (sputum culture)

Twenty-five patients (23.4%) had bacteria identified on sputum culture (Table 2). *Klebsiella pneumoniae* was the most frequent pathogen found in patients with a culture-based diagnosis (*n*=15/25; 60.0%). This was followed in frequency by *Streptococcus pneumoniae* (*n*=3/25; 12.0%) and *Acinetobacter baumannii* (*n*=3/25; 12.0%). All but 3 of these participants had received antibiotics (*A. baumannii n*=1, *S. pneumoniae n*=1 and *Streptococcus pyogenes n*=1) prior to hospitalisation.

FilmArrayPN-PCR

FilmArrayPN-PCR identified a possible bacterial aetiological diagnosis in 89 study participants (83.2%) and viral aetiology in 53 (49.5%) (Table 2). The most frequent bacterium identified on FilmArrayPN-PCR was *Haemophilus influenzae* (*n*=50/89; 46.7%). The semi-quantitative determination of DNA for *H. influenzae* was $>10^4$ genomic copies/mL in 46/50 (46.7%). Other bacterial pathogens identified by FilmArrayPN-PCR included *K. pneumoniae* (*n*=29/107; 27.1%), *S. pneumoniae* (*n*=27/107; 25.2%) and *Staphylococcus aureus* (*n*=24/107; 22.4%).

The majority of viral infections identified were due to human rhinovirus/enterovirus (*n*=31/107; 29.0%) and non-SARS-CoV-2 coronaviruses (*n*=14/107; 13.1%) (Table 2). Forty-eight participants (44.9%) had mixed infections with both bacterial and viral pathogens.

Comparison of FilmArrayPN-PCR with sputum culture

FilmArrayPN-PCR increased the detection of potential pathogens associated with CAP by 64.5% (95% confidence interval (CI) 54.8 - 73.1) (Table 3). All the participants with a positive sputum culture also had bacteria identified by FilmArrayPN-PCR; however, only 80.0% (*n*=20/25) of the results were concordant. In the discordant 5 participants, sputum culture identified *S. pyogenes* in 2, *A. baumannii* in another 2, and *Pseudomonas aeruginosa* in 1. FilmArrayPN-PCR identified *H. influenzae* in 3 participants (1 also had *P. aeruginosa*), 1 had both *S. pneumoniae* and *Mycoplasma pneumoniae*, and 1 had *S. aureus*. Overall, FilmArrayPN-PCR identified more than one pathogen in 14 participants (56.0%) with a positive sputum culture. In 13 participants (12.1%), no pathogens were detected by either diagnostic test.

Antimicrobial resistance

Of all the study participants, 34 (31.8%) had evidence of AMR on sputum culture or on FilmArrayPN-PCR (Supplementary Table 2, available online at http://coding.samedical.org/file/2341). Twenty of these participants (58.8%) had AMR detected on sputum culture and susceptibility testing, while 27 (79.3%) had resistance gene mutations detected on FilmArrayPN-PCR. Thirteen participants (38.2%) had resistance detected by both methods. Of the 13 who had AMR detected on both tests, 12 were diagnosed with *K. pneumoniae* and 1 with *A. baumannii*. All 12 *K. pneumoniae* organisms identified had CTX-M (cefotaxime-Munich beta-lactamase) resistance mutations and 2 additionally had carbapenemase (NDM (New Delhi metallo-beta-lactamase) and OXA-48-like)-associated

Table 1. Baseline characteristics of study participants with community-acquired pneumonia compared by diagnostic approach

Characteristic	All (*N*=107), *n* (%)‡	SoC*		*p*-value	FilmArrayPN-PCR†		*p*-value
		Negative (*n*=82; 76.6%), *n* (%)‡	Positive (*n*=25; 23.4%), *n* (%)‡		Negative (*n*=13; 12.1%), *n* (%)‡	Positive (*n*=94; 87.9%), *n* (%)‡	
Sociodemographic features							
Age (years), median (IQR)	40 (31.0 - 46.0)	40.2 (31.0 - 46.0)	37.4 (31.1 - 41.2)	0.31	40.2 (32.1 - 46.3)	39.7 (31.0 - 45.2)	0.50
Female	54 (50.5)	42 (51.2)	12 (48.0)	0.82	7 (53.9)	47 (50.0)	1.00
Smoking	22 (20.6)	19 (23.2)	3 (12.0)	0.27	3 (23.1)	19 (20.2)	0.73
Alcohol use	76 (71.0)	58 (70.7)	18 (72.0)	1.00	8 (61.5)	68 (72.3)	0.52
Biomass fuel use	74 (69.2)	68 (82.9)	6 (24.0)	<0.001	11 (84.6)	63 (67.0)	0.34
Clinical symptoms							
Duration of cough (weeks), median (IQR)	3.0 (1.0 - 4.0)	3 (2 - 4)	2 (1 - 4)	0.05	2 (2 - 4)	3 (1 - 5)	0.51
Sputum production	100 (93.5)	75 (91.5)	25 (100.0)	0.20	10 (76.9)	90 (95.7)	0.04
Fever	83 (77.6)	63 (76.8)	20 (80.0)	1.00	13 (100)	70 (74.5)	0.04
Chest pain	74 (69.2)	54 (65.9)	20 (80.0)	0.22	9 (69.2)	65 (69.2)	1.00
Difficulty breathing	62 (57.9)	44 (53.7)	18 (72.0)	0.11	8 (61.5)	54 (57.5)	1.00
Wheeze	21 (19.6)	19 (23.2)	2 (8.0)	0.15	3 (23.1)	18 (19.2)	0.72
Weight loss	90 (84.1)	68 (82.9)	22 (88.0)	0.76	12 (92.3)	78 (83.0)	0.69
Physical examination							
Temperature (°C), median (IQR)	36.8 (36.4 - 37.5)	36.8 (36.3 - 37.2)	37.1 (36.5 - 37.8)	0.29	36.9 (36.4 - 37.1)	36.8 (36.4 - 37.5)	0.71
Heart rate (bpm), median (IQR)	92 (82 - 104)	93 (79 - 105)	89 (86 - 96)	0.68	99 (92 - 106)	89 (82 - 102)	0.09
Respiratory rate (/min), median (IQR)	24 (20 - 26)	23.5 (20 - 26)	24 (21 - 28)	0.36	24 (20 - 28)	23.5 (20 - 26)	0.52
Oxygen saturation (%), median (IQR)	96 (92 - 98)	96 (94 - 98)	92 (90 - 96)	0.004	97 (94 - 98)	95 (92 - 98)	0.41
Laboratory result							
CD4 count (cells/μL), median (IQR)	167 (85 - 331)	196 (75 - 340)	125 (94 - 280)	0.45	172 (125 - 484)	167 (75 - 331)	0.61
Treatment history							
Receiving ART	68 (71.6)	52 (73.2)	16 (66.7)	0.60	6 (50.0)	62 (74.7)	0.09
Pneumocystis prophylaxis	30 (28.0)	25 (34.3)	5 (25.0)	1.00	4 (30.8)	26 (34.5)	1.00
Prior antibiotic use	80 (74.8)	58 (70.7)	22 (27.5)	0.12	9 (69.2)	71 (75.5)	0.73

SoC = standard of care; FilmArrayPN-PCR = Biofire FilmArray Pneumonia Panel plus; IQR = interquartile range; ART = antiretroviral therapy.
*SoC (positive or negative) refers to potential pathogens causing community-acquired pneumonia detected (positive) or not (negative) on sputum culture.
†FilmArrayPN-PCR (positive or negative) refers to potential respiratory pathogens detected (positive) or not (negative) on the FilmArrayPN-PCR.
‡Except where otherwise indicated.

resistance mutations. The *A. baumannii* organism diagnosed with resistance had an OXA-48-like mutation. All *K. pneumoniae* organisms identified had multidrug resistance (MDR) for up to seven antibiotics. All the *K. pneumoniae* MDR organisms had phenotypic resistance to cefotaxime, and 11 had resistance to cefuroxime. Imipenem was the only antibiotic to which these organisms were universally susceptible.

Fourteen participants had genotypic resistance mutations but did not have phenotypic resistance demonstrated on sputum culture. The resistance mutations identified were CTX-M in 13 of these participants and mecAC/MREJ in 1 participant. Three participants with CTX-M had additional mutations due to NDM (*n*=2) and mecAC/MREJ (*n*=1). Seven participants had phenotypic resistance, but had no genotypic resistance mutations detected. Three of these participants had *S. pneumoniae*, 2 *K. pneumoniae*, 1 *A. baumannii* and 1 *P. aeruginosa*.

Factors associated with antimicrobial resistance

Using multiple logistic regression analysis, we examined the association of demographic and baseline clinical characteristics with AMR (Table 4). Age and gender were confounders of the association between prior antibiotic use and AMR. Factors independently associated with increased odds of AMR included baseline oxygen

Table 2. Aetiology of bacterial and viral causes of community-acquired pneumonia identified on sputum culture and FilmArrayPN-PCR

Pathogens identified	Sputum culture	FilmArrayPN-PCR*
Bacteria identified	*n*=25 patients	*n*=89 patients
Haemophilus influenzae	1	50
Klebsiella pneumoniae	15	29
Streptococcus pneumoniae	3	27
Staphylococcus aureus	0	24
Enterobacter cloacae	0	14
Moraxella catarrhalis	0	13
Acinetobacter baumannii	3	12
Escherichia coli	0	6
Streptococcus agalactiae	0	5
Streptococcus pyogenes	2	4
Pseudomonas aeruginosa	1	2
Klebsiella oxytoca	0	2
Proteus spp.	0	2
Atypical bacteria		*n*=2 patients
Mycoplasma pneumoniae	Not done	1
Chlamydia pneumoniae	Not done	1
Legionella pneumoniae	Not done	0
Viruses identified		*n*=53 patients
Rhinovirus	Not done	31
Coronavirus	Not done	14
Respiratory syncytial virus	Not done	6
Influenza A virus	Not done	3
Parainfluenza virus	Not done	3
Adenovirus	Not done	3
Human metapneumovirus	Not done	2
Influenza B virus	Not done	0
Middle East respiratory syndrome coronavirus	Not done	0

FilmArrayPN-PCR = Biofire FilmArray Pneumonia Panel plus.
*Some patients had more than one pathogen identified.

Table 3. Comparison of SoC v. FilmArrayPN-PCR results for the detection of pathogens causing community-acquired pneumonia

Test	*n* (%) (95% CI)
SoC	
Negative	82 (76.6) (67.5 - 83.8)
Positive	25 (23.4) (16.2 - 32.5)
FilmArrayPN-PCR	
Negative	13 (12.1) (7.1 - 20.0)
Positive	94 (87.9) (80.0 - 92.9)
Levels of agreement/disagreement	
FilmArrayPN-PCR positive and SoC positive	25 (23.4) (16.2 - 32.5)
FilmArrayPN-PCR positive and SoC negative	69 (64.5) (54.8 - 73.1)
FilmArrayPN-PCR negative and SoC positive	0
FilmArrayPN-PCR negative and SoC negative	13 (12.1) (7.1 - 20.0)
Overall level of agreement	
All are positive or all are negative	38 (35.5) (26.9 - 45.2)
Not all agree	69 (64.5) (54.8 - 73.1)

SoC = standard of care; FilmArrayPN-PCR = Biofire FilmArray Pneumonia Panel plus; CI = confidence interval.

saturation <92% (adjusted odds ratio (aOR) 9.20; 95% CI 2.52 - 33.57; *p*=0.001), baseline oxygen saturation 92 - 95% (aOR 3.16; 95% CI 0.99 - 10.10; *p*=0.05) and prior antibiotic use (aOR 4.14; 95% CI 1.04 - 16.51; *p*=0.04).

Discussion

Our study has several findings with important implications for the clinical care of PWH and presumptive CAP. Using FilmArrayPN-PCR led to a 64.5% increase in the detection of potential respiratory pathogens compared with traditional sputum culture-based methods. Accurate diagnoses of pathogens causing pneumonia and accompanying antibiotic resistance are critical in people with HIV and advanced immune suppression, who are at increased risk of poor outcomes.[3] The increased yield of FilmArrayPN-PCR and faster turnaround for identification of respiratory pathogens can lead to instant treatment decision-making. An additional advantage of the multiplex PCR assays is the ability to detect pathogens despite prior antibiotic use.[12]

Murphy *et al.*[13] conducted a multicentre evaluation of FilmArrayPN-PCR and found a sensitivity of >95% and a specificity of >91% compared with culture. Similar increased yields of multiplex PCR assays have also been reported in other studies, especially in HIV-negative populations.[14] A few studies have addressed the usefulness of multiplex PCR assays in PWH. In one of these studies, Maartens *et al.*[15] in Cape Town, South Africa, performed multiplex PCR of sputum in PWH with World Health Organization danger signs and cough, and found a high prevalence of tuberculosis (52%), CAP (32%) and *P. jirovecii* pneumonia (9.2%). Probable bacterial infection (>10^5 copies/mL) was detected in 47% of participants.

The epidemiology of pathogens causing CAP varies according to geographical region. In the present study, *H. influenzae* was the bacterial pathogen most frequently detected by FilmArrayPN-PCR, while multidrug-resistant *K. pneumoniae* was most frequent on sputum culture. The most common pathogens in CAP globally are *S. pneumoniae* and *H. influenzae*.[3] However, in the context of HIV-related immunosuppression, there are higher rates of invasive disease due to *H. influenzae*, which may be associated with high morbidity and

Table 4. Logistic regression analysis for factors associated with antimicrobial resistance in HIV-positive patients with community-acquired pneumonia

Characteristic	OR (95% CI)	*p*-value	aOR (95% CI)	*p*-value
Age (years)				
≤29	1.00		1.00	
30 - 39	1.08 (0.36 - 3.29)	0.89	1.26 (0.32 - 4.99)	0.75
40 - 49	0.65 (0.21 - 2.01)	0.45	0.64 (0.16 - 2.57)	0.53
≥50	0.48 (0.10 - 2.24)	0.35	0.58 (0.09 - 3.68)	0.57
Female	1.16 (0.51 - 2.61)	0.73	1.24 (0.46 - 3.36)	0.68
Smoking	0.76 (0.27 - 2.16)	0.61		
Subjective fever	1.53 (0.55 - 4.28)	0.42		
Any weight loss	4.14 (0.89-19.25)	0.07		
Haemoptysis	3.85 (1.52 - 9.77)	<0.001		
Chest pain	2.74 (1.01 - 7.46)	0.05		
Difficulty in breathing	1.51 (0.65 - 3.51)	0.33		
Sputum production	2.96 (0.34 - 25.6)	0.33		
Wheeze	0.08 (0.01 - 0.63)	0.02	0.12 (0.01 - 1.00)	0.05
Oxygen saturation (%)				
96 - 100	1.00		1.00	
92 - 95	3.16 (1.11 - 9.05)	0.03	3.16 (0.99 - 10.10)	0.05
<92	14.38 (4.29 - 48.12)	<0×001	9.20 (2.52 - 33.57)	0.001
Temperature (°C)				
<36.4	1.00			
36.4 - 37.5	1.45 (0.50 - 4.26)	0.50		
>37.5	2.62 (0.78 - 8.75)	0.12		
Heart rate >100 bpm	0.43 (0.16 - 1.18)	0.10		
Respiratory rate >20/min	2.03 (0.83 - 4.98)	0.12		
Receiving ART	1.21 (0.46 - 3.19)	0.69		
Recent antibiotic use	5.06 (1.40 - 18.24)	0.01	4.14 (1.04 - 16.51)	0.04
Pneumocystis prophylaxis	1.35 (0.56 - 3.29)	0.51		
CD4 count (cells/µL)				
<200	1.00			
200 - 350	0.83 (0.25 - 2.75)	0.76		
>350	0.40 (0.09 - 1.77)	0.23		

OR = odds ratio; aOR = adjusted odds ratio; ART = antiretroviral therapy.

mortality.[16] A fundamental question is how many of these potential pathogens were actual pathogens. It is usually difficult to differentiate between colonisation and infection, since some of these pathogens are also commensals in the oropharynx. The majority of the patients with a diagnosis of *H. influenzae* had $>10^4$ genomic copies, indicating a relative abundance of this organism in sputum. The significance of this semi-quantitative measure when compared with colony-forming units on standard culture is still not well established. However, in a study by Park *et al.*,[17] the colonisation density of the upper respiratory tract was associated with a confirmed microbiological diagnosis of *H. influenzae* infection. It is not surprising that on culture, drug-resistant *K. pneumoniae* was the dominant species identified, while fastidious organisms such as *S. pneumoniae* and *H. influenzae* were less frequent because 74.8% of the participants had received prior antibiotics.

On FilmArrayPN-PCR, rhinovirus infections were the commonest identified, followed by coronaviruses (non-SARS-CoV-2). The significance of these viral infections is not well understood, but rhinovirus/enterovirus and endemic coronavirus infections commonly present as self-limited diseases in immune-competent individuals. However, they may be associated with severe infections, including CAP and intensive care unit infections, in immunosuppressed hosts, the elderly, and patients with significant underlying conditions.[18] They have also been reported as mixed infections with bacteria. In the EPIC multicentre pneumonia study, rhinovirus infections were also the commonest cause of pneumonia.[19] Multiplex PCR assays therefore enable identification of viruses in the respiratory samples, to provide insight into alternative diagnoses or co-pathogens.

Another significant finding in the present study was detection of AMR in one-third of the patients with CAP. FilmArrayPN-PCR detected resistance gene mutations to commonly used antibiotics in 79.3% of the study participants. Only 38.2% had resistance identified by both culture and FilmArrayPN-PCR. This discordance between phenotypic and genotypic resistance has also been observed in other studies. Lee *et al.*[20] investigated the role of FilmArrayPN-PCR for the detection of determinants of AMR compared with the minimum inhibitory concentration (MIC) method, and found similar discrepancies. The determinants of AMR are multifactorial. For the participants who had AMR detected by the MIC method but had no resistance mutations detected on FilmArrayPN-PCR, this failure to detect resistance mutations may be due to the limited selection of mutation targets in the FilmArrayPN-PCR, or because they had fewer pathogens than the detection limit of the assay. On the other hand, for those who had determinants of resistance identified on FilmArrayPN-PCR but had no phenotypic resistance, the determinants detected may not be ascribed to the pathogens cultured, since these mutations can be associated with a wide range of pathogens.[21] Multidrug-resistant pathogens were identified, especially *K. pneumoniae*, *A. baumannii* and *S. aureus*. This worrying trend of AMR, which has been observed globally, is of public health importance as it threatens to negate the progress in antimicrobial treatment for CAP.[22,23] The presence of such infections rapidly reduces treatment options for patients with pneumonia, hence the need for regular antimicrobial surveillance in hospitalised patients.

Hypoxia (oxygen saturation <92%) was associated with nine times increased odds of AMR. Hypoxia may reflect severe pneumonia as a result of non-response to treatment. Prior antibiotic use was also associated with increased odds of AMR. Based on these findings, patients with CAP and hypoxia should be targeted for sputum culture and drug susceptibility testing, and PCR testing to detect AMR. A study by Shindo *et al.*[24] found that prior antibiotic use and immune suppression were independent predictors of drug-resistant pathogens in patients with CAP. Similarly, Prina *et al.*[25] found that previous antibiotic use was associated with an increased risk of infection with *P. aeruginosa*, extended-spectrum beta-lactamase-positive *Enterobacteriaceae* and methicillin-resistant *S. aureus* (PES) organisms, increasing 30-day mortality.[25]

Study limitations

Limitations of this study include the lack of longitudinal follow-up of the participants to assess the clinical outcomes of those who had AMR identified but were on standard therapy. Secondly, 74.8% of participants in our study had taken antibiotics prior to hospitalisation, which may select for a population with suboptimal response to initial antibiotics in an ambulatory setting and therefore skew the aetiological profile of CAP, but reflects the reality of hospitalised CAP. Thirdly, the reference standard for the diagnosis of CAP is imperfect, since sputum culture, the traditional gold standard, has low sensitivity and blood cultures were not performed. On the other hand, multiplex PCR assays may have high sensitivity but can also have high false-positive rates in the real world. Finally, FilmArrayPN-PCR does not detect opportunistic infections such as *P. jirovecii*, *Mycobacterium tuberculosis* and *Cryptococcus*, which occur commonly in PWH, and only detects a selected number of antimicrobial resistance mutations that may not fully represent important infections and common resistance patterns in a high HIV burden setting.

Conclusion

The FilmArray pneumonia panel increased the yield for diagnosis of both potential bacterial and viral infections causing CAP in hospitalised PWH in Uganda. In addition, prior antibiotic use and hypoxia were associated with an increased risk of AMR. Further evaluation of the significance of these findings and the cost-effectiveness of the molecular tests should be done in prospective studies.

Data availability. The datasets generated and analysed during the present study are available from the corresponding author (WW) on reasonable request. Any restrictions or additional information regarding data access can be discussed with the corresponding author.

Declaration. None.

Acknowledgements. We acknowledge the contributions of Magezi Yusuf and Kate Nabakiibi with data collection, and Edson Mwebesa with statistical analysis. We thank the Infectious Disease Research Collaboration, Medical and Molecular Laboratories Ltd, China-Uganda Friendship Hospital and Kiruddu Hospital for enabling this research.

Author contributions. WW, AA, FS, AC, DRB and LH contributed to the design of the work. All authors contributed to data acquisition, analysis, or interpretation of data for the work. All authors contributed to drafting the work or reviewing it critically for important intellectual content, and all authors approved the final version to be published.

Funding. This study was supported by funding from Biofire Diagnostics LLC and bioMérieux SA, the European and Developing Countries Clinical

Trials Partnership (TMA2018SF-2465), and the National Institutes of Health (NIH R01 HL128156 and NIH R01 HL143998).

Confl cts of interest. None.

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Submitted 11 July 2024. Accepted 14 August 2024. Published 2 June 2025.