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Research Article

Diagnostic Performance of a Multiplex PCR in Identifying Pneumonia Micro-Organisms in Sputum of HIV Positive Patients in Uganda

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ABSTRACT

Background: Pneumonia remains a frequent cause of morbidity and mortality among HIV infected people in sub-Saharan Africa. A disease with such a burden requires an appropriate diagnostic modality to allow a logical approach to guide treatment. Conventional diagnostic methods although available are not fully utilized due to associated diagnostic limitations, and as such present challenges in the successful management of pneumonia. It is therefore necessary to evaluate a multiplex Polymerase Chain Reaction (PCR) assay that is rapid, sensitive and specific in detection of pneumonia microorganisms hence improving time to antimicrobial therapy.

Objective: We aimed to compare the performance of the Bio Fire® Film Array® pneumonia multiplex PCR panel against a composite reference standard.

Methods: Between November 2019 to February 2020, we conducted a diagnostic cross-sectional study among HIV positive clients accessing care at Mulago national referral hospital in Kampala. All consenting patients meeting the inclusion criteria were educated on quality sputum collection and two sputum samples obtained from them for analysis. Bartlett's grading was done on sputum samples prior to bacterial culture. Multiplex PCR tests were done on the second sputum samples. Data was analysed using STATA V14. A composite reference was used as the Gold standard.

Results: Compared to the composite reference standard, the sensitivity was 90.3%, specificity of 44.6%, positive predictive value of 36.8%, negative predictive value of 91.3%, Area under the ROC Curve of 0.680, the Kappa statistic was 0.23 and a 57% agreement between the two tests.

Conclusions: A high sensitivity and low specificity demonstrated by Multiplex PCR makes it a good screening test but not a confirmatory test for detecting pneumonia microorganisms in sputum.

Keywords: Pneumonia; HIV; Multiplex PCR; Conventional diagnostic methods; Diagnostic performance; Diagnostic Techniques; Film Array; Translational research; Uganda

INTRODUCTION

Pneumonia remains a global health problem with a high rate of morbidity and mortality [1]. The incidence is increased about 35-fold among Human Immunodeficiency Virus (HIV) infected persons despite the significant advances made in HIV treatment [2].

In Africa, although population-level pneumonia incidence data is sparse, major burden of disease as highlighted by hospital registry data shows that pneumonia is among the most common reasons for HIV adult hospitalization accounting for 6%-15% of in-hospital mortality amongst HIV positive adults [3]. A community surveillance study in East Africa (rural Kenya), estimated the incidence of pneumonia to be between 5 cases per 1000 person years in HIV-negative and 67 cases per 1000 person years in HIV positive individuals [4]. In Uganda about 12% of inpatient mortality is attributed to pneumonia (UNAS, CDDEP 2015).

A disease with such significant cause of mortality and morbidity would require an appropriate diagnostic method to identify the causative microorganisms and hence allow a logical approach

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to treatment [5]. This is however not the case, as pneumonia is often misdiagnosed and inappropriately treated due to diagnostic limitations presented by available conventional diagnostic methods (APUA, 2011) [6]. These limitations include decreased sensitivity (30%-40%) especially in patients with prior antibiotic treatment [7], labour intensive and slow turnaround time in availing results [8-10]. The limitations present challenges in the successful management of pneumonia and as such, demand the evaluation of a user-friendly and more sensitive, rapid diagnostic test for pneumonia diagnosis.

The BioFire® FilmArray® pneumonia multiplex Polymerase Chain Reaction (PCR) panel is an FDA approved molecular diagnostic test capable of identifying 33 targets in sputum (18 bacteria, 8 viruses and 7 antimicrobial resistance genes) and provides pathogen identification in a much shorter timeframe which allows clinicians to optimize targeted pathogen-specific therapy sooner. In this study, we aimed to evaluate the diagnostic performance of the BioFire® FilmArray® pneumonia multiplex PCR panel against a composite reference standard for the detection of pneumonia causing microorganisms in sputum obtained from HIV infected individuals.

MATERIALS AND METHODS

Study design and population

A diagnostic cross-sectional study was conducted between November 2019 and February 2020 among adult HIV positive patients accessing clinical care at a Mulago hospital in Uganda. Patients meeting the inclusion criteria (aged ≥ 18 years, cough <2 weeks, fever, dyspnea, gene expert negative for pulmonary Tuberculosis and willing to provide informed consent for study participation were purposively sampled, then health educated on how to collect quality sputum samples for analysis.

Study procedures

Two sputum samples from each patient were collected in a clean wide mouth container, well packaged, labelled with patient's particulars then transported to the laboratories for analysis. Sputum samples sent to the microbiology laboratory were subjected to Bartletts grading to determine the quality of samples prior to further processing using Gram staining and culture. Samples with a satisfactory Bartletts score (+1,+2) were processed further while those with unsatisfactory Bartletts were discarded. All the 88 sputum samples were Bartletts satisfactory and processed further using Gram staining and culture methods on appropriate bacteria culture media. Sputum samples sent to the molecular laboratory were prepared into aliquots, coded and assigned a study number then analysed on the Bio Fire Film Array machine using the pneumonia panel. Laboratory results arising from the tests were sent to the study investigator who could then relay the results to the clinicians treating the patients.

Statistical Analysis

All data were double entered in Epi Info and exported to STATA version 14 software (STATA, College Station, Texas) for statistical analysis. The composite reference standard was defined as positive for pneumonia using the all-positive rule i.e., positive in all three methods (Gram staining, sputum culture and chest x-ray), and also negative for pneumonias by all the three methods. This gives the method 100% hypothetical sensitivity, specificity, and positive and negative predictive values.

Descriptive continuous variables were summarized as medians and interquartile ranges while categorical variables were summarized as percentages to see their distribution. Using 2 × 2 contingency table and Receiver Operator Characteristic curves, the primary outcomes of the study i.e., sensitivity, specificity, area under curve and predictive values of multiplex PCR were calculated by comparing to a composite reference gold standard generated from three diagnostic tests.

Ethics Statement

Ethical approval to carry out the study was sought from the Department of Medicine Makerere University, the School of Medicine Research and Ethics Committee, and the Kiruddu, Naguru hospital ethics committee and UNCST. Written informed consent was obtained from all participants before screening and before collecting sputum samples.

RESULTS

Characteristics of study population

Between November 2019 and February 2020, 88 adult HIV positive patients accessing clinical care at a Ugandan tertiary hospital were enrolled in the study. The median age of those enrolled was 45 (IQR 35-53). 43.2% (38/88) of the patients had been treated for opportunistic infections within the past 2 years prior to hospital visit and oral candidiasis was the common opportunistic infection treated. The median absolute CD4 cell count was 125 cells/µl (IQR 92-330), 21.6% (23/88) had a current CD4 T cell count <100 cells/µl and 14.8% (13/88) had taken Highly Active Antiretroviral Treatment (HAART) for a short duration lasting <6 months. Of the 79 (89.7%) taking antibiotics prior to hospital visit, majority 45.5% (36/88) had taken Ampiclox antibiotics. Characteristics of patients in our study population at time of enrolment are shown in Table 1.

Table 1: Baseline characteristics of study patients.

Variable		Statistic
N		88
Sex, n%	Female	
	Male	47(53.4%)
		41(46.6%)
Age (years), median (IQR)		45(35-53)
Age range (years), n (%)	18-30	
	31-45	18(20.5%)
	45-54	
	>55	30(34.1%)
		12(13.6%)
		28(31.8%)
Hospital admission, n (%)	Inpatients	33(37.5%)
	Outpatients	55(62.5%)
CD4 T-cell count (cells/µl), median (IQR)		125(92-330)
CD4 T-cell count range (cells/µl), n (%)	<100	
	100-200	23(26.1%)
	201-400	
	>400	36(40.9%)

		19(21.6%)
		10(11.4%)
Duration on HAART (months), n (%)	3-6	
	7-12	13(14.8%)
	13-24	
	>24	23(26.1%)
		30(34.1%)
		22(25.0%)
Antibiotic use prior to hospitalisation, n (%)	Yes	
	No	79(89.8%)
		09(10.2%)

Diagnostic performance of Multiplex PCR compared to Composite reference standard

Using a composite reference standard (Gold standard) generated by combining 3 diagnostic tests, 23 patients were identified as truly diseased with pneumonia while 65 patients were identified as not diseased with pneumonia. Multiplex PCR and the composite reference standard were concordant in identifying 21 patients as positive for pneumonia disease and 29 patients as negative for pneumonia disease, giving a per cent agreement of 56.8%, Cohens Kappa of 0.23 (p-value 0.0015) as shown in table 2.

Multiplex PCR test when compared to the composite reference standard (Gold standard) yielded a Sensitivity of 91.3%, Specificity of 44.6%, PPV 37% and NPV of 93.5%.

Data presented are the numbers, percentages, numerator/ denominator, and 95% confidence interval. N=number of observations, PPV=Positive Predictive Value, NPV=Negative Predictive Value, AUC=Receiver-Operator Characteristic (ROC) area under the curve for sensitivity and specificity. % agreement (Accuracy)=overall probability that a patient is correctly classified.

Respiratory organisms detected by multiplex PCR

Multiplex PCR was able to detect respiratory organisms in 57 sputum samples out of the 88 samples analysed. 26 (45.6%) samples had bacterial organisms as the only agents detected, 3 (5.3%) samples had atypical bacterial organisms as the only agents detected and 28 (49.1%) samples had both bacterial and viral organisms.

Streptococcus Pneumoniae, Hemophilus Influenzae and Klebsiella Pneumoniae were the most common bacterial organisms detected in sputum samples of HIV positive patients, while *Humanmeta Pneumo virus* and *corona virus* were the most common viruses detected by Multiplex PCR. Microorganisms' identification was on average 4.5 hours faster with Multiplex PCR than with sputum culture, likewise the turn-around time for detection of antimicrobial resistance markers by Multiplex PCR was shorter by 50.2-hour reduction when compared to antimicrobial susceptibility determination by standard phenotypic methods using sputum culture. The distribution of bacterial and viral microorganisms detected in the 57 sputum samples is shown in figures 1 and 2.

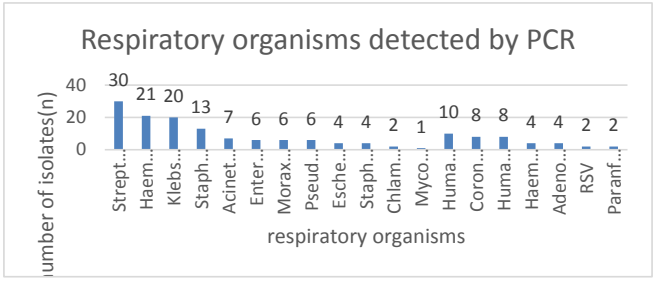

Figure 1: Showing distribution of bacterial organisms detected by multiplex PCR.

Resistance gene markers detected by Multiplex PCR

Multiplex PCR was able to detect four resistance genes patterns i.e., CTX-M, OXA-48, NIDM and VIM resistance gene patterns. CTX-M resistance gene patterns were the most detected with about 17/57 (29.8%) sputum samples of HIV positive patients with pneumonia having CTX-M resistance gene pattern. Bacterial organisms commonly detected in sputum samples with CTX-M resistance gene patterns were *Klebsiella Pneumonia and Hemophilus Influenza*. Resistance gene patterns detected by Multiplex PCR are shown in Figure 2.

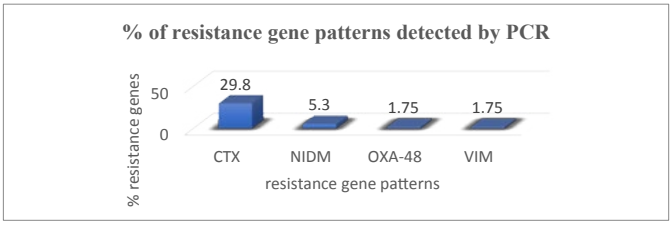

Figure 2: Showing percentage of resistance gene patterns detected by PCR test.

DISCUSSION

This study demonstrated that the multiplex PCR is a rapid and sensitive test for identifying pneumonia microorganisms and resistance markers in patients with suspected pneumonia. The test had a sensitivity of 91.3%, which is a high enough sensitivity for a diagnostic test. However, the test had a poor specificity. This makes it a good screening tool but poor confirmatory tool. According to [11], a good screening test should have a high enough sensitivity to minimize the occurrence of false positives since it is able to correctly identify a proportion of individuals with a positive test result among those with actual disease. Results of the study are similar to findings by [12-14] whose study findings demonstrated a 90% sensitivity of molecular PCR tests in detecting respiratory microorganisms in sputum samples. The findings however contrast with findings by [15], where molecular PCR had a low (72%) sensitivity. The findings could partly be explained by PCR modalities utilizing a selected ply target in their operating modality that increases their sensitivity in detecting genes associated with pneumonia microbes, as documented by [16-19].

The specificity in the study was at 44.6% and this could have

Table 2: Comparison between Multiplex PCR and Composite Reference standard (GOLD) in detection of pneumonia micro-organisms.

Test	Sample	N	FP	FN (%)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	% Agreement (95%CI)	Kappa (p-value)	AUC (95%CI)
Multiplex	Sputum	88	36	2	91.30%	44.60%	36.80%	93.60%	56.80%	0.23	0.68
PCR			-40.90%	-2.30%	(71.9-98.9)	(32.3-57.5)	(31.2-42.9)	(78.9-98.3)	(45.8-67.3)	-0.0015	(0.59-0.76)

affected the PPV in the study. The results are similar to studies by [13], and contrast with studies done by Ozlem A et al. (2013). We however cannot fully rely on the comparisons and contrasts with other studies because unlike sensitivity and specificity, predictive values are largely dependent on disease prevalence in examined population. i.e., the higher the prevalence the higher the positive predictive value. Therefore, predictive values from one study should not be transferred to some other setting with a different prevalence of the disease in the population.

On the other hand, the NPV was 93.5%, which tells us that of the 31 patients testing negative with PCR, 93.5% actually do not have pneumonia disease. A high NPV value of Multiplex PCR justifies the utility of this test as a diagnostic test especially in screening for serious illnesses like pneumonia, as documented by [11], who contends that negative predictive value is more useful in choosing screening tests for serious illnesses because high NPV minimizes false negatives. The findings contend with study findings by [12-14]. Multiplex PCR test had a diagnostic odds ratio of 8.5. The diagnostic odds ratio tells us how much high the odds are of getting a positive test result in a person with pneumonia disease compared to a person without pneumonia disease, and a value greater than one is appropriate enough as a diagnostic test. In the study, Multiplex PCR had a value of 8.5, hence a satisfactory diagnostic test. The findings contend with those of Stalin et al. (2006) where diagnostic odds ratio of molecular PCR was >1. Area under curve (AUC) was 0.68, which value reveals a sufficient diagnostic accuracy and indicates that 68% of the patients in the study were classified appropriately in their diseased and non-diseased group. Findings compare with a study by [14].

Improved detection of many bacterial and viral micro-organisms associated with pneumonia was observed with the use of Multiplex PCR [20]. The advantage of Multiplex PCR test is its ability to detect bacterial organisms even after initiation of antibiotic treatment [21]. In this study, Multiplex PCR detected a significant number of potential micro-organisms in 65% of sputum samples. This is in line with studies by [22-24] were PCR detected micro-organisms in 60%-79% of sputum samples.

This study is one of the first to evaluate the performance of the Multiplex PCR pneumonia panel among HIV positive patients with suspected pneumonia. The limitations of this work relate to its moderate sample size, lack of a defined gold standard for pneumonia and non-probability sampling method which reduces generalizability.

CONCLUSIONS

In light of the above diagnostic performance characteristics displayed by Multiplex PCR test, we can make conclusions that the test has demonstrated a high sensitivity which makes the test a rapid screening test for detection of pneumonia microorganisms in sputum.

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