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Improgo LVF, Delos Reyes CA. Diagnostic Accuracy of a Pneumonia Panel Multiplex RT-PCR Assay for Detection of Respiratory Bacterial Pathogens Among Pediatric Patients Admitted for Pneumonia at a Tertiary Hospital in Manila, Philippines
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ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF PNEUMONIA PANEL MULTIPLEX RT-PCR ASSAY FOR THE DETECTION OF RESPIRATORY BACTERIAL PATHOGENS AMONG PEDIATRIC PATIENTS ADMITTED FOR PNEUMONIA AT A TERTIARY HOSPITAL IN MANILA, PHILIPPINES

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ABSTRACT

Background: Pneumonia in children causes significant morbidity and mortality especially in those less than 5 years of age. Accuracy of multiplex diagnostic tests for rapid identification of microbial etiology is not well-established. This study was undertaken to determine the diagnostic accuracy of a pneumonia panel multiplex RT-PCR assay in the detection of respiratory pathogens among pediatric patients with pneumonia.

Methodology: This retrospective analytic cross-sectional study included children less than 19 years old diagnosed with pneumonia between June 2022 to June 2023 with respiratory samples for bacterial culture and pneumonia panel multiplex RT PCR assay.

Results: A total of 211 pediatric patients were included in the study. Of these, 72% were diagnosed with community acquired pneumonia, 95% of them had endotracheal aspirate specimens. Bacterial pathogens were detected in 131 patients (61%). Of the 172 patients who had positive detections, 51 (30%) had at least one antibiotic-resistant gene identified. There was moderate agreement between the two methods (72.51%, kappa = 0.4632). Pneumonia panel multiplex RT-PCR assay showed acceptable discriminative ability (0.74) with accuracy at 72.51% and high sensitivity (88.42%), but specificity was only 59.48%. Its use resulted to the escalation of antimicrobial therapy in 15% of patients.

Conclusion: The pneumonia panel multiplex RT PCR assay is a valid diagnostic aid in the detection of respiratory pathogens for children with pneumonia given its good discriminative ability, high accuracy, and sensitivity.

KEYWORDS: *Pneumonia, Pneumonia Panel Multiplex RT-PCR Assay, Pediatrics*

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INTRODUCTION

Pneumonia is linked to significant morbidity and mortality. According to the World Health Organization[1], pneumonia is responsible for the deaths of 740, 180 children under the age of 5 in 2019, accounting for 14% of overall mortality in this age group. In Southeast Asia, the population of the Philippines suffer from particularly high pneumonia mortality rates with 92.28 deaths per 100,000 people. [2] Pneumonia is a leading cause of death in both under-5-year-old and older than 70-year-old populations in the country, with 76 child deaths per 100,000 children recorded in 2019.[3] Furthermore, pneumonia was the second leading cause of mortality among Filipino children aged one to four years with the number of deaths caused by the disease approximately at 17%.[3]

Diagnosis of pneumonia depends largely to clinical signs and symptoms. However, diagnostic aids are recommended, especially for severe cases, to determine the etiology and the need for antibiotic treatment. Bacterial culture, though not recommended to be routinely done, is still considered the gold standard in establishing diagnosis and for definitive antibiotic therapy.[4] Challenges to utilizing cultures include lengthy period for recovery and identification of potential pathogens typically ranging from 48 to 96 hours, low yield, isolation of colonizers and contaminants, as well as the impact of prior antibiotic use limiting it clinical utility.[5,6]

To address the limitations identified with standard bacterial culture, rapid diagnostic tests were developed for early and accurate identification of microbial etiology. The pneumonia panel multiplex RT-PCR assay is a molecular device that can rapidly identify viruses, bacteria and antimicrobial resistance genes in sputum, tracheal aspirate and bronchoalveolar lavage specimens obtained from individuals with signs of a lower respiratory tract infection.[6,7] It can identify 15 typical bacteria (4 gram-positive and 11 gram-negative), 3 atypical bacteria, and 9 viruses, along with 7 antibiotic-resistant targets (1 for extended-spectrum β-lactamases (ESBLs), 5 for carbapenem-resistant Enterobacteriaceae (CRE), and 1 for methicillin-resistant *Staphylococcus aureus* (MRSA) within one hour. International studies have evaluated its potential for rapid determination of lower respiratory tract pathogens and aid in immediate targeted treatment, with sensitivity ranging from 75 to 100% and specificity from 76.5 to 100%, comparable to standard culture methods.[7,8,9] However, to our knowledge, a local comprehensive review of the pneumonia panel multiplex RT-PCR assay's accuracy, agreement with standard bacterial culture, and impact on antibiotic therapy, especially among pediatric patients, is currently lacking. This study, therefore, aims to determine the diagnostic accuracy of the pneumonia panel multiplex RT-PCR assay in detecting respiratory bacterial pathogens among pediatric patients admitted for pneumonia in a tertiary hospital. Furthermore, this study aims to identify respiratory pathogens and antibiotic resistance detected by both the pneumonia panel multiplex RT-PCR assay and bacterial culture. It also seeks to determine the level of agreement between these two methods in the detection of respiratory pathogens.

MATERIALS AND METHODS

This is a retrospective analytic cross-sectional study that included all pediatric patients, 0 to 18 years of age, diagnosed with pneumonia of any type and severity at the Philippine General Hospital with respiratory samples sent for both pneumonia panel multiplex RT-PCR assay and bacterial culture from June 1, 2022, to June 30, 2023. This diagnostic accuracy study assessed the validity of the pneumonia panel multiplex RT-PCR assay specifically for respiratory bacterial pathogens. Atypical organisms and viral pathogens were also identified by the pneumonia panel multiplex RT-PCR assay. However, they were not compared to viral or specialized cultures due to difficulty in isolating such organisms including the high cost, labor-intensive procedures, and lengthy time to obtain results it

entails.[10] Sample size was calculated using the formula for sensitivity by Hajian-Tilaki.[11] Parameters were based on a previous study[8] specifying a sensitivity of the pneumonia panel multiplex RT-PCR assay equal to 98.50%, prevalence of pneumonia at 65.70%, maximum marginal error of 5%, and alpha set at 0.05, a minimum of 35 patients are needed.

$$= \frac{Z_{\frac{\alpha}{2}}^2 \widehat{Se}\,(1-\widehat{Se})}{d^2\, x\, Prev}$$

Where:
n=total sample size
Se=sensitivity
d=maximum marginal error
Prev= prevalence

$$n = \frac{1.96^2\ 0.9850\ (1-0.9850)}{0.05^2\ x\ 0.6570}$$
$$n = 35$$

This study's main focus is on the sensitivity of the multiplex assay to determine if it can be used for screening purposes (minimize false negatives); thus, for the sample size computation, this parameter was prioritized instead of the specificity. Even if the minimum sample size was calculated, the researcher did a total enumeration technique, a type of purposive sampling, wherein all eligible cases will be included in the study.

Patients who already received antibiotics before their specimen was collected, as well as those with underlying conditions like immunosuppression, malignancy, heart disease, or lung disease, were still included in the study as long as they met the specified inclusion criteria. Pediatric patients with lower respiratory tract specimen submitted for either the pneumonia panel multiplex RT-PCR assay or bacterial culture only, those with nasopharyngeal specimens submitted, those with unavailable medical charts and invalid culture results (i.e. unfit or inadequate sample) were excluded.

The study followed the ethical considerations set out by the Declaration of Helsinki, National Ethics Guidelines for Health Research and Data Privacy Act of 2012 and Good Clinical Practice. It was approved by the University of the Philippines – Research Ethics Board (UPMREB). Permission was obtained from the Microbiology Department and Medical Records Section prior to data collection with strict compliance to the Data Privacy Act of 2012. The study utilized data gathered only from the Microbiology logbooks and review of patients' charts. There was no contact with the families of the participants nor persons involved in the care of the patient. Hence, a waiver for informed consent was requested from UPMREB since (1) the research procedures entail not more than minimal risk and anonymity can be maintained and information is considered non-sensitive, (2) the waiver will not adversely affect the rights and welfare of the participants, (3) the research cannot be practicably carried out without the waiver and (4) the participants will be provided with additional pertinent information after their participation (whenever appropriate) in accordance to the National Ethical Guidelines for Health and Health Related Research 2017. There is no financial, professional or propriety conflict of interest in the conduct of this study.

All information was strictly kept confidential and no names of the participants appeared in the data collection tools. A code was used and assigned to each patient record. A master list linking the code number and subject identity was kept separately from the research data. Only primary investigators had access to the list. The completed data collection forms were compiled and collated in sealed brown envelope. Data was encoded in a Microsoft Excel Sheet and saved in a password protected external hard drive. These were made available to the biostatistician for data analysis.

As part of the descriptive statistics to show the patient's characteristics, clinical data included age, gender, comorbidities, type of pneumonia, use of empiric antibiotic and place of collection were extracted from the their electronic medical records. Pneumonia panel multiplex RT-PCR results were obtained from the pneumonia panel logbook and bacterial culture results were obtained from the

respiratory culture logbook of the Microbiology Laboratory department.

Data were encoded in MS Excel by the researcher. Stata MP version 17 software was used for data processing and analysis. Continuous variables were presented as median (interquartile range/ IQR) due to the non-normal data distribution. Shapiro Wilk's test was used to assess normality of data. Categorical variables were expressed as frequencies and percentages.

Kappa statistics was used to determine the agreement between the results of the pneumonia panel multiplex RT-PCR assay and bacterial culture in diagnosing pneumonia and detecting each specific pathogen. Kappa statistics was interpreted as follows: poor <0, slight 0-0.20, fair 0.21-0.40, moderate 0.41-0.60, substantial 0.61-0.80, and almost perfect >0.80.[12] Factors such as antibiotic administration, timing of sample collection, and the intrinsic properties of the organisms were not accounted for, as the study did not include subgroup analysis.

To test for diagnostic validity, bacterial culture served as the gold standard, and test results were considered as: (a) true positive, if both pneumonia panel multiplex RT-PCR assay (regardless if multiple detections) and bacterial culture identified the same organism, (b) false positive, if pneumonia panel multiplex RT-PCR assay but not bacterial culture detected an organism, (c) true negative, if neither pneumonia panel multiplex RT-PCR assay and bacterial culture detected an organism, and (d) false negative, if bacterial culture but not pneumonia panel multiplex RT-PCR assay detected an organism. Overall sensitivity and specificity as well as sensitivity and specificity for each bacterial pathogen were determined.

A Receiver Operating Characteristic (ROC) curve was created for the computation of the discriminative ability (based on Area Under the Curve or AUC) of the pneumonia panel multiplex RT-PCR assay. The following diagnostic accuracy parameters were also calculated: accuracy, sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios.

DEFINITION OF TERMS

1. Bacteriologically-confirmed pneumonia - pneumonia with presence of bacterial growth in culture regardless of colony forming units and hours of incubation, obtained from the final laboratory report.
2. Pneumonia Panel Multiplex RT PCR result - results obtained from the pneumonia panel multiplex RT-PCR assay which is a molecular, multiplex device that identifies 15 typical bacteria, 3 atypical bacteria, 9 viruses and 7 antibiotic resistance genes.
3. Antibiotic Resistance Gene - detected by the pneumonia panel multiplex RT PCR assay that indicates resistance to either extended spectrum beta lactamases (ESBL) or carbapenemases (CRE) for gram negative isolates and resistance to methicillin for gram positive isolates.
4. Antibiotic Resistance by culture - antimicrobial resistance identified on bacterial culture isolate. This would also include the antibiotic sensitivity pattern:
    (a) Multidrug-resistant - non-susceptibility to at least one agent in three or more antimicrobial categories
    (b) Extensively drug-resistant - non-susceptibility to at least one agent in all but two or fewer antimicrobial categories
    (c) Pandrug-resistant - non-susceptibility to all agents in all antimicrobial categories
5. Comorbidities - other illnesses at the time of pneumonia diagnosis based on medical charts:
    (a) Immunosuppression - any condition that weakens the immune system whether drug induced or due to

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chronic diseases such as HIV, genetic disorders or malnutrition other than malignancy
   (b) Malignancy - any type of cancer either blood or solid organ tumors
   (c) Heart disease - any type of cardiac condition whether congenital or acquired
   (d) Lung disease - any type of respiratory condition other than pneumonia
6. Type of pneumonia - pneumonia based on final diagnosis in medical charts
   (a) Community-acquired - signs and symptoms of lower respiratory tract infection present/acquired outside the hospital setting
   (b) Hospital-acquired - signs and symptoms of pneumonia developing after at least 48 hours of hospitalization; including ventilator-associated pneumonia with symptoms noted 48–72 hour incubation time-period after endotracheal intubation
7. Lower respiratory tract specimen - sputum, endotracheal aspirate or bronchoalveolar lavage specimen submitted and analyzed via bacterial culture and pneumonia panel multiplex RT-PCR assay
8. Antibiotic escalation - any broadening of antimicrobial spectrum, which includes a change or addition of a new antibiotic
9. Antibiotic de-escalation - any narrowing of antimicrobial spectrum, which could include a change in agent with narrower spectrum or discontinuation of an antibiotic in a multidrug regimen

RESULTS

A total of 211 patients were included in the study. Table 1 shows the characteristics and clinical profile of the patients. The median age was 1 year, and most were males (56%). Majority were diagnosed with community-acquired pneumonia (72%). Most of the samples submitted were endotracheal aspirates (95%). Immunosuppression was the most common comorbidity affecting a fourth of the patients and all patients received empiric antibiotics.

Table 1. Clinico-demographic profile of pediatric patients with pneumonia that had bacterial culture and pneumonia panel multiplex RT PCR assay (n=211)

	n = 211 (%)
Age (in years), median	1 [IQR: 0.3-6]
< 1 year old	102 (48)
1 – 10 years old	75 (36)
> 10 years old	34 (16)
Sex	
Male	119 (56)
Female	92 (44)
Comorbidities, % yes	
Immunosuppression	53 (25)
Malignancy	9 (4)
Heart disease	35 (17)
Lung disease	28 (13)
Empiric antibiotics	
Yes	211 (100)
No	0
Type of pneumonia	
Community acquired	152 (72)
Hospital acquired	59 (28)
Specimen, %yes	
Sputum	10 (5)
Endotracheal aspirate	201 (95)
Bronchoalveolar lavage	0
Place of Collection	
Emergency Room	159 (75)
Pediatric Ward	42 (20)
Pediatric Intensive Care Unit	3 (1)
Neonatal Intensive Care Unit	7 (3)

The pneumonia panel multiplex RT-PCR assay showed a positive result for bacteria, atypical bacteria, or virus in 172 (82%) of patients. 131 (62%) patients were positive for bacteria and of these patients, 62 were positive for one organism, 41 were

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positive for two organisms, 20 were positive for three organisms, 4 for four organisms and 4 were positive for five organisms. The most common bacteria were *Haemophilus influenzae* (23%), followed by *Moraxella catarrhalis* (15%), *and Staphylococcus aureus* (15%). Among the 172 patients who were positive to bacteria, 51 (30%) had at least one antibiotic-resistant gene detected. 12% were positive for ESBL resistance gene, while 22% for carbapenemase resistant gene. Of the 31 patients who tested positive for *Staphylococcus aureus,* mecA/MREJ gene was detected in 58% of cases.

Ninety-five patients (45%) had positive bacterial cultures. The most common isolate was *Klebsiella pneumoniae* (10%), followed by *Pseudomonas aeruginosa* (9%) and *Staphylococcus aureus* (8%). Furthermore, several isolates were detected via bacterial culture which was not included in the panel hence considered as false negative. These organisms were *Elizabethkingia meningoseptica, Stenotrophomonas maltophilia, Diphtheroids, Citrobacter freundii, Citrobacter werkmanii, Citrobacter koseri, Achromobacter xylosidans, Corynebacterium diphtheriae* and *Acinetobacter iwofii.*

Among the 95 patients with bacterial isolates on culture, 37 (39%) exhibited antibiotic resistance, 14 % of which were classified as extensively drug resistant (see Table 2).

Table 2. Respiratory pathogens detected by the pneumonia panel multiplex RT PCR assay and bacterial culture amongst pediatric patients admitted for pneumonia

	Pneumonia panel multiplex RT-PCR n(%)	Bacterial culture n(%)
BACTERIA, %yes		
Any bacteria	131 (62)	95 (45)
Acinetobacter calcoaceticus-baumannii complex	23 (11)	9 (4)
Enterobacter cloacae	11 (5)	6 (3)
Escherichia coli	9 (4)	7 (3)
Haemophilus influenzae	49 (23)	11 (5)
Klebsiella aerogenes	0	0
Klebsiella oxytoca	0	0
Klebsiella pneumoniae group	22 (10)	22 (10)
Moraxella catarrhalis	32 (15)	0
Proteus spp.	4 (2)	0
Pseudomonas aeruginosa	27 (13)	19 (9)
Serratia marcescens	5 (2)	5 (2)
Staphylococcus aureus	31 (15)	17 (8)
Streptococcus agalactiae	2 (1)	0
Streptococcus pneumoniae	23 (11)	5 (2)
Streptococcus pyogenes	2 (1)	0
Eliizabethkingia	-	1 (1)
Stenotrophomonas maltophilia	-	3 (1)
Diphtheroids	-	1 (1)
Citrobacter freundii	-	1 (1)
Achromobacter xylosoxidans	-	1 (1)
Citrobacter werkmanii	-	1 (1)
Corynebacterium diphtheriae	-	1 (1)
Citrobacter koseri	-	2 (1)
Acinetobacter lwofii	-	1 (1)
ATYPICAL BACTERIA, %yes		
Any atypical bacteria	2 (1)	-
Legionella pneumophilia	0	-
Mycoplasma pneumoniae	2 (1)	-
Chlamydia pneumoniae	0	-
VIRUS, % yes		-
Any virus	111 (53)	-
Influenza A	6 (3)	-
Influenza B	4 (2)	-
Adenovirus	8 (4)	-
Coronavirus	4 (2)	-
Parainfluenza virus	20 (9)	-
Respiratory syncytial virus	13 (6)	-
Human Rhinovirus/ Enterovirus	73 (35)	-
Human Metapneumovirus	5 (2)	-
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0	-
RESISTANCE GENE, %yes [n=172]		-
ESBL		-
CTX-M	26 (12)	-
CRE	39 (22)	-
IMP	5 (3)	-
KPC	4 (2)	-
NDM	22 (13)	-
OXA-48 like	3 (2)	-
VIM	5 (3)	-
MRSA [n=31]		-
mecA/C and MREJ	18 (58)	-
ANTIMICROBIAL RESISTANCE PATTERN, %yes [n=95]		
CRE	-	9 (9)
ESBL	-	9 (9)
MRSA	-	9 (9)
MDR	-	6 (6)
XDR	-	13 (14)
PDR	-	1 (1)
AMPC	-	3 (3)
HLARE	-	0
VRE	-	0

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For the succeeding agreement and validity analysis, only bacterial isolates (excluding atypical) were considered. Table 3 shows the agreement between the two methods which was 72.51%. Kappa statistics indicate moderate agreement in detecting any respiratory bacterial pathogen. For specific respiratory pathogens, the agreement ranged from 80.09 to 99.05%, and based on kappa, a slight to substantial agreement was observed. Kappa statistics was highest for *Pseudomonas aeruginosa*.

Table 3. Agreement of the pneumonia panel multiplex RT-PCR assay with bacterial culture

	% Agreement	Kappa	Interpretation
Any respiratory pathogen	72.51%	0.4632	Moderate
Specific respiratory pathogen			
○ *Acinetobacter calcoaceticus-baumannii complex*	90.52%	0.3342	Fair
○ *Enterobacter cloacae*	96.68%	0.5725	Moderate
○ *Escherichia coli*	95.26%	0.3508	Fair
○ *Haemophilus influenzae*	80.09%	0.2348	Fair
○ *Klebsiella aerogenes*	-	-	-
○ *Klebsiella oxytoca*	-	-	-
○ *Klebsiella pneumoniae group*	92.42%	0.5940	Moderate
○ *Moraxella catarrhalis*	84.83%	0.0000	Slight
○ *Proteus spp.*	98.10%	0.0000	Slight
○ *Pseudomonas aeruginosa*	92.42%	0.6111	Substantial
○ *Serratia marcescens*	97.16%	0.3854	Fair
○ *Staphylococcus aureus*	89.57%	0.4884	Moderate
○ *Streptococcus agalactiae*	99.05%	0.0000	Slight
○ *Streptococcus pneumoniae*	91.47%	0.3311	Fair
○ *Streptococcus pyogenes*	99.05%	0.000	Slight

Kappa statistics was interpreted as follows: poor <0, slight 0-20, fair 0.21-0.40, moderate 0.41-0.60, substantial 0.61-0.80, and almost perfect >0.80.

The pneumonia panel multiplex RT-PCR assay showed acceptable discriminative ability (0.74), accuracy (72.99%) and high sensitivity (88.42%), though specificity was modest at 59.548% (see Table 4). Across all organisms, the discriminative ability was acceptable, and accuracy was high (80.09% - 97.16%). The sensitivity greatly varied, ranging from 40 to 100%. Notably, the pneumonia panel multiplex RT-PCR assay showed sensitivity below 60% for *Escherichia coli* and *Serratia marcescens.* Specificity values were all 80% or above with the lowest observed for *Haemophilus influenzae* (80%) (see Table 5).

Table 4. Diagnostic Accuracy of the pneumonia panel multiplex RT-PCR assay in detecting any respiratory bacterial pathogen

Parameter	
Discriminative ability AUC (95% CI)	0.74 (0.68-0.79)
Accuracy	72.51%
Sensitivity	88.42%
Specificity	59.48%
Positive Predictive Value (PPV)	64.12%
Negative Predictive Value (NPV)	86.25%
Likelihood ratio positive (LR+)	2.18
Likelihood ratio negative (LR-)	0.19

Table 5. Diagnostic Accuracy of the pneumonia panel multiplex RT-PCR assay in detecting specific respiratory pathogen

	Discriminative ability AUC (95% CI)	Accuracy	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Acinetobacter calcoaceticus-baumannii complex	0.79 (0.63-0.96)	90.52%	66.67%	91.58%	26.09%	98.40%	7.92	0.36
Enterobacter cloacae	0.90 (0.74-1.00)	96.68%	83.33%	97.07%	45.45%	99.50%	28.47	0.17
Escherichia coli	0.70 (0.50-0.90)	95.26%	42.86%	97.06%	33.33%	98.02%	14.57	0.59
Haemophilus influenzae	0.81 (0.69-0.93)	80.09%	81.82%	80%	18.37%	98.77%	4.09	0.23
Klebsiella aerogenes	-	-	-	-	-	-	-	-
Klebsiella oxytoca	-	-	-	-	-	-	-	-
Klebsiella pneumoniae group	0.80 (0.69-0.90)	92.42%	63.64%	95.77%	63.64%	95.77%	15.03	0.38
Moraxella catarrhalis	-	-	-	-	-	-	-	-
Proteus spp.	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	0.86 (0.77-0.96)	92.42%	78.95%	93.75%	55.56%	97.83%	12.63	0.22
Serratia marcescens	0.69 (0.45-0.93)	97.16%	40%	98.54%	40%	98.54%	27.47	0.61
Staphylococcus aureus	0.84 (0.73-0.94)	89.57%	76.47%	90.72%	41.94%	97.78%	8.24	0.26
Streptococcus agalactiae	-	-	-	-	-	-	-	-
Streptococcus pneumoniae	0.96 (0.94-0.98)	91.47%	100%	91.26%	21.74%	100%	11.44	0
Streptococcus pyogenes	-	-	-	-	-	-	-	-

In Table 6, the use of the pneumonia panel multiplex RT-PCR assay led to the escalation of antimicrobial therapy in 32 (15%) patients. However, among patients with viral detections only, antibiotics were not discontinued.

Table 6. Impact of the pneumonia panel multiplex RT-PCR assay results on antimicrobial therapy

Antibiotic modification	n(%)
Escalation	32 (15)
De-escalation	0
Discontinuation	0
No change	179 (85)

DISCUSSION

In this study, the diagnostic accuracy of the pneumonia panel multiplex RT-PCR assay was compared to bacterial culture in detecting respiratory pathogens in 211 pediatric patients diagnosed with pneumonia. The pneumonia panel multiplex RT-PCR assay was able to identify pathogens in more cases (131 patients, 62%) compared to bacterial culture (95 patients, 45%). Furthermore, the pneumonia panel multiplex RT-PCR assay had a high sensitivity at 88% determined by dividing the true positive results (85) by the total number of true positive and false negative results (95). Yoo et. al[8] and Ginocchio et. al[9] showed a higher positive bacterial detection using the pneumonia panel with a sensitivity above 85%. As a PCR-based diagnostic test, the pneumonia panel multiplex RT-PCR assay can detect both viable and nonviable organisms. Thus, these findings may represent a higher sensitivity to detect very small levels of nucleic acids from organisms that are challenging to culture or from nonviable organisms. However, the pneumonia panel multiplex RT-PCR assay in this study was found to be less specific consistent also with the findings of earlier research.[8,9] One contributing factor may be the low sample size to compute for specificity.

Cojuc Konisberg[13] evaluated the diagnostic accuracy of the pneumonia panel multiplex RT-PCR assay in adult covid patients with ventilator associated pneumonia. The pneumonia panel multiplex RT-PCR assay still detected substantially more pathogens compared to culture with a high sensitivity at 95%. Additionally, the agreement between the pneumonia panel multiplex RT-PCR assay and bacterial culture had a moderate agreement by Cohen's kappa, consistent with our study. However, compared to a U.S. study done on adult hospitalized patients with pneumonia, agreement values obtained in this study were lower.[14] Notably, the results of our study revealed that 22% of the samples were positive on pneumonia panel multiplex RT-PCR but were negative by bacterial culture posing the question of whether they were false positives or whether the panel's performance was more accurate than culture. One significant reason for this, is that the pneumonia panel multiplex RT-PCR assay distinguishes samples by identifying genetic material.[15] Residual nucleic acids, which may not indicate active bacterial replication because of antimicrobials, can still flag as positive on such panels. While this aspect of molecular assays can still facilitate rapid diagnosis and timely treatment initiation, it is essential to conduct careful clinical correlation and interpretation to address any inconsistencies.[16]

The most common gram-negative organisms detected by the pneumonia panel multiplex RT-PCR assay in this study were *Haemophilus influenzae* and *Moraxella catarrhalis.* Both detected pathogens are in line with a previous study done in Norway that included 72 patients with community acquired pneumonia.[17] *Haemophilus influenzae* was considered a relevant cause of pneumonia especially in the pediatric age group. Apart from gram negative bacteria, the pneumonia panel multiplex RT-PCR assay also detected gram positive organisms, particularly *Streptococcus pneumoniae* and *Staphylococcus aureus,* important pathogens especially among critically ill and immunocompromised patients.[18] Detection of these pathogens, particularly *Haemophilus influenzae* and *Streptococcus pneumoniae* is consistent with global data where bacterial etiology is found to have higher percentage in lower to middle income countries like the Philippines.[19,20]

Out of the 172 patients that had positive detections by the pneumonia panel multiplex RT-PCR assay, 111 (53%) tested positive for a viral pathogen. Viral detections must not be underemphasized especially in the pediatric age group were majority of the cases of pneumonia are viral in etiology.[21] The most common identified virus was human rhinovirus/enterovirus (35%). Furuse et al [22], on his study on the epidemiological and clinical characteristics of children with acute respiratory viral

infection in the Philippines from 2014 to 2016, rhinovirus was most frequently detected followed by respiratory syncytial virus (RSV) among the 4735 samples collected. However, in contrast to this study, Javier et al [23] found that RSV was the most prevalent pathogen detected using a respiratory multiplex RT-PCR assay among hospitalized children with acute respiratory infections. Similarly, a local surveillance conducted in 2016 identified RSV as the predominant organism responsible for hospitalizations. Additionally, RSV infections were most prevalent from July to October.[24]

The pneumonia panel multiplex RT-PCR assay did not detect certain clinically relevant pathogens that were isolated in bacterial cultures, such as *Elizabethkingia meningoseptica, Stenotrophomonas maltophilia, and Citrobacter spp.* These organisms are not included in the assay's detection panel, presenting a potential limitation.[7,8,9] Studies have highlighted this limitation[7,8,9,25], noting that these pathogens can also cause significant morbidity and mortality.[26-28] Therefore, prompt identification and targeted antibiotic treatment for these organisms are crucial and will be missed by the pneumonia panel.

This study also found that 30% that were positive for a respiratory pathogen had at least one antibiotic-resistant gene detected, majority of which were either extended spectrum beta-lactamase (ESBL) or carbapenemase (CRE). In addition, more than half of detected *Staphylococcus aureus* had mecA/MREJ gene indicative of methicillin resistance. In a multicenter evaluation involving 904 bronchioalveolar and 925 sputum specimens from patients with lower respiratory tract infections in 8 United States clinical sites, ESBL and CRE resistance genes were also found to be the most predominant, specifically CTX-M and KPC.[29] On review of medical charts, most of the changes in antibiotic therapy were based on the presence of resistance genes detected by the pneumonia panel multiplex RT PCR assay. These findings are in contrast with the study by Sogaard et. al where detection of resistance genes had minimal clinical relevance.[30] However, it was conducted in a setting with low MDR prevalence. In our setting where multidrug resistant (MDR) organisms are prevalent, the detection of these antibiotic resistance genes have a greater potential to impact initial therapeutic management.

De-escalation and discontinuation of antibiotics were not observed in this study. Most patients presented with severe pneumonia necessitating intravenous broad-spectrum antibiotics even when only viral pathogens were detected by the pneumonia panel multiplex RT-PCR assay. The possibility of a concomitant bacterial infection was considered in the presence of clinical signs and symptoms with any of the following parameters: elevated white blood cell count, C-reactive protein, procalcitonin, or imaging findings of alveolar infiltrates, lung consolidation, air bronchograms, and/or pleural effusion.[4] Moreover, in the absence of these ancillary parameters, the decision to initiate empirical antibiotic treatment relied on the clinician's assessment and informed judgment. In this study, 71 patients had both virus and bacteria detected by the pneumonia panel multiplex RT-PCR assay. Epidemiological studies have shown that viral-bacterial co-infections are more common in children with severe pneumonia. [31,32] Several mechanisms have been proposed to explain the synergistic relationship between viruses and bacterial colonization and invasion, which increases the burden of morbidity and mortality.[32,33]

To our knowledge, there are no locally published studies that evaluated the diagnostic accuracy of the pneumonia panel multiplex RT-PCR assay in the pediatric population. The findings from this study were comparable to existing global literature and can contribute to the current information about the use of the pneumonia panel multiplex RT-PCR assay. However, this study also had several important limitations. The retrospective cross sectional design is one limitation wherein data were obtained from microbiology logbooks and diagnosis of pneumonia were reviewed from medical charts, rather than a prospective assessment. Standard

collection, transport, and storage of specimens were also not controlled and directly observed by the researcher in real-time. The study focused on bacterial pathogens but viruses detected were enumerated; however evaluation of their concordance to viral culture was not done. All pediatric patients diagnosed with pneumonia regardless of an underlying condition, comorbidity and antibiotic history were also included which may affect results.

CONCLUSION AND RECOMMENDATIONS

The pneumonia panel multiplex RT-PCR assay is a valid diagnostic tool for detecting respiratory bacterial pathogens due to its good discriminative ability, high accuracy, and sensitivity. It can aid clinical decision-making by enabling rapid etiologic diagnosis and timely initiation of targeted treatment. *Haemophilus influenzae*, a significant cause of pneumonia in both pediatric and adult populations [19,20], was the most prevalent bacterial pathogen detected.

However, a potential drawback of the pneumonia panel multiplex RT-PCR assay is its inability to identify emerging drug-resistant pathogens such as *Elizabethkingia meningoseptica* and *Stenotrophomonas maltophilia*, which are associated with significant morbidity and mortality. Additionally, the pneumonia panel multiplex RT-PCR assay detected bacterial targets that have at least one antibiotic-resistant gene, most commonly extended-spectrum beta-lactamase (ESBL) or carbapenemase (CRE). The identification of these resistance genes has the potential to significantly influence initial therapeutic management strategies.

A prospective study with a larger sample size can be pursued to provide a more comprehensive data. Further research on the correlation between quantitative bacterial culture results and the number of copies of genetic material detected by the pneumonia panel multiplex RT-PCR assay to determine the most clinically significant isolate is also recommended. Finally, further investigations into the impact of targeted therapy guided pneumonia panel multiplex RT-PCR results on clinical outcomes, length of stay and costs in contrast to patients receiving empiric antibiotics would be highly beneficial.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Pneumonia in Children [Online]. World Health Organization. 2022 [cited 2023 Jan 28]. Available from https://www.who.int/news-room/fact-sheets/detail/pneumonia
2. Dadonaite B, Roser M. Pneumonia [Online]. Our World in Data. 2022 [cited 2023 Jan 28]. Available from https://ourworldindata.org/pneumonia
3. Philippines Profile [Online]. UNICEF. 2021 [cited 2023 Feb 11]. Available from https://data.unicef.org/countdown-2030/country/Philippines/1/
4. Jalandoni-Cabahug MaV, Capeding MR, Cruz KAD, Castellano MJ, De Leon MN, Padua JR. 2021 Clinical practice guidelines in the evaluation and management of pediatric community-acquired pneumonia. PIDSP Journal [Internet]. 2023 Apr 3 [cited 14 May 2024];24(1):121–75. Available from: https://doi.org/10.56964/pidspj202324010
5. Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. Clinical Infectious Diseases. 2011 May 1;52(suppl_4):S296-304.
6. The BioFire® FilmArray® Pneumonia (PN) Panel [Online]. Biomerieux. [cited 2023 Aug 13]. Available from https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/
7. Murphy CN, Fowler R, Balada-Llasat JM, Carroll A, Stone H, Akerele O, et al. Multicenter Evaluation of the BioFire FilmArray Pneumonia/Pneumonia Plus Panel for Detection and Quantification of Agents of Lower Respiratory Tract Infection. J Clin Microbiol [Internet]. 2020 Jun 24 [cited 2023 Jan 28];58(7):e00128-20. Available from

https://pubmed.ncbi.nlm.nih.gov/32350043 DOI: 10.1128/JCM.00128-20.

8. Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFire FilmArray Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. Int J Infect Dis [Internet]. 2020 Jun [cited 2023 Jan 28];95:326-331. Available from https://pubmed.ncbi.nlm.nih.gov/32179139 DOI: 10.1016/j.ijid.2020.03.024.
9. Ginocchio CC, Garcia-Mondragon C, Mauerhofer B, Rindlisbacher C; and the EME Evaluation Program Collaborative. Multinational evaluation of the BioFire® FilmArray® Pneumonia plus Panel as compared to standard of care testing. Eur J Clin Microbiol Infect Dis [Internet]. 2021 Aug [cited 23 Jan 28];40(8):1609-1622. Available from https://pubmed.ncbi.nlm.nih.gov/33655440 DOI: 10.1007/s10096-021-04195-5.
10. Hodinka RL. Point-CounterPoint: Is the era of viral culture over in the Clinical Microbiology laboratory? Journal of Clinical Microbiology [Internet]. 2013 Jan 1 [cited 13 May 2024];51(1):2–8. Available from: https://doi.org/10.1128/jcm.02593-12
11. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. Journal of Biomedical Informatics [Internet]. 2014 Apr [cited 2 Oct 2024];48(1):193–204. Available from https://pubmed.ncbi.nlm.nih.gov/24582925/ DOI
12. Watson PF, Petrie A. Method agreement analysis: a review of correct methodology. Theriogenology [Internet]. 2010 Jun 1 [cited 2023 Aug 29];73(9):1167-79. Available from https://pubmed.ncbi.nlm.nih.gov/2013835/DOI:10.1016/j.theriogenology.2010.01.003.
13. Cojuc-Konigsberg G, Moscona-Nissan A, Guijosa A, Mireles Dávalos CD, Martínez MEJ, Mújica Sánchez MA, et al. Diagnostic accuracy of the BioFire® FilmArray® pneumonia panel in COVID-19 patients with ventilator-associated pneumonia. BMC Infectious Diseases [Internet]. 2023 Aug [cited 2023 Aug 29] 2023;23(1):1-9. Available from https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-023-08486-4. DOI: 10.1186/s12879-023-08486-4.
14. Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, et al. Practical comparison of the BioFire FilmArray pneumonia panel to routine diagnostic methods and potential impact on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. Journal of Clinical Microbiology [Internet]. 2020 Jun 24 [cited 2023 Jan 28];58(7):e00135-20. Available from https://pubmed.ncbi.nlm.nih.gov/32350045/ DOI: 10.1128/JCM.00135-20.
15. Poritz MA, Lingenfelter B. Multiplex PCR for Detection and Identification of Microbial Pathogens. Advanced Techniques in Diagnostic Microbiology. 2018;475–93.
16. Crémet L, Gaborit B, Bouras M, Drumel T, Guillotin F, Poulain C, et al. Evaluation of the FilmArray® Pneumonia Plus Panel for Rapid Diagnosis of Hospital-Acquired Pneumonia in Intensive Care Unit Patients. Frontiers in Microbiology. 2020 Aug 25;11.
17. Serigstad S, Markussen D, Grewal HMS, Ebbesen M, Kommedal Ø, Heggelund L, et al. Rapid syndromic PCR testing in patients with respiratory tract infections reduces time to results and improves microbial yield. Sci Rep [Internet]. 2022 [cited 2023 Aug 29];12:326. Available from https://pubmed.ncbi.nlm.nih.gov/3501335/DOI: 10.1038/s41598-021-03741-7.
18. Kamel NA, Alshahrani MY, Aboshanab KM, El Borhamy MI. Evaluation of the BioFire FilmArray pneumonia panel plus to the conventional diagnostic methods in determining the microbiological etiology of hospital-acquired pneumonia. Biology [Internet]. 2022 Feb 27 [cited 2023 Feb 11];11(3):377. Available from https://pubmed.ncbi.nlm.nih.gov/3533675/DOI: 0.3390/biology11030377.
19. Marangu D, Zar HJ. Childhood pneumonia in low-and-middle-income countries: an update. Paediatric respiratory reviews. 2019 Nov 1;32:3-9.
20. Santos, J. A review of pneumonia in the Philippines. PIDSP Journal [Internet]. 2022 [cited 2023 Sep 11]; 22(2):6-11. Available from https://www.pidsphil.org/home/wp-content/uploads/2021/09/003_vol-22-no-2_SANTOS_PNEUMONIA.pdf.
21. Ebeledike C, Ahmad T. Pediatric pneumonia [Internet]. 2023 [Cited 2023 August]. Available from https://www.ncbi.nlm.nih.gov/books/NBK536940/
22. Furuse Y, Tamaki R, Suzuki A, Kamigaki T, Okamoto M, Saito-Obata M, et al. Epidemiological and Clinical Characteristics of Children with Acute Respiratory Viral Infections in the Philippines: a Prospective Cohort Study. Clin Microbiol Infect [Internet]. 2020 Sep 17 [cited 2023 Sep 11]. Available from: https://pubmed.ncbi.nlm.nih.gov/32950713 DOI:10.1016/j.cmi.2020.09.017
23. Javier K, Navoa-Ng JA, Cotoco-Chu N. Rapid Detection of Respiratory Pathogens Using a Multiplex PCR Assay Among Hospitalized Children with Acute Respiratory Infection. PIDSP Journal [Internet]. 2021 Sep 7[cited 13

May 2024];22(2):55–65. Available from: https://doi.org/10.56964/pidspj20212202008

24. Severe Acute Respiratory Infection Surveillance 2015-2016 [Internet]. [cited 2023 Sep 11]. Available from https://www.pidsphil.org/home/wp-content/uploads/2018/09/New-Respiratory-Pathogens-Dr.-Rosario-Capeding.pdf
25. Crotty MP, Meyers S, Hampton N, Bledsoe S, Ritchie DJ, Buller RS, et al. Impact of antibacterials on subsequent resistance and clinical outcomes in adult patients with viral pneumonia: an opportunity for stewardship. Critical Care [Internet]. 2015 Dec [cited 2023 Aug 28];19(1):1-1. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4650137/ DOI: 10.1186/s13054-015-1120-5.
26. Webber DM, Wallace MA, Burnham CAD, Anderson NW. Evaluation of the BioFire FilmArray Pneumonia Panel for detection of viral and bacterial pathogens in lower respiratory tract specimens in the setting of a Tertiary Care Academic Medical Center. Journal of Clinical Microbiology [Internet]. 2020 Jun 24 [cited 13 May 2024];58(7). Available from: https://doi.org/10.1128/jcm.00343-20
27. Kanderi T, Shrimanker I, Mansoora Q, Shah K, Yumen A, Komanduri S. Stenotrophomonas maltophilia: An Emerging Pathogen of the Respiratory Tract. the American Journal of Case Reports [Internet]. 2020 May 21[cited 13 May 2024];21. Available from: https://doi.org/10.12659/ajcr.921466
28. Moore LSP, Owens DS, Jepson A, Turton JF, Ashworth S, Donaldson H, et al. Waterborne Elizabethkingia meningoseptica in Adult Critical Care1. Emerging Infectious Diseases [Internet]. 2016 Jan 1[cited 13 May 2024];22(1):9–17. Available from: https://doi.org/10.3201/eid2201.150139
29. Murphy CN, Fowler R, Balada-Llasat JM, Carroll A, Stone H, Akerele O et al. Multicenter evaluation of the BioFire FilmArray pneumonia/pneumonia plus panel for detection and quantification of agents of lower respiratory tract infection. Journal of Clinical Microbiology [Internet]. 2020 Jun 24 [cited 2023 Jan 28];58(7):e00128-20. Available from https://pubmed.ncbi.nlm.nih.gov/32350043/ DOI: 10.1128/JCM.00128-20
30. Søgaard KK, Hinic V, Goldenberger D, Gensch A, et al. Evaluation of the clinical relevance of the Biofire© FilmArray pneumonia panel among hospitalized patients. Infection [Internet]. 2023 Aug 12 [cited 2023 Aug]; 52:173-181.Available from https://link.springer.com/article/10.1007/s15010-023-02080-1. DOI: 10.1007/s15010-023-02080-1
31. Rodrigues CMC, Groves H. Community-Acquired Pneumonia in Children: the Challenges of Microbiological Diagnosis. Journal of Clinical Microbiology [Internet]. 2018 Mar 1 [cited 14 May 2024];56(3). Available from: https://doi.org/10.1128/jcm.01318-17
32. Meskill SD, O'Bryant SC. Respiratory virus co-infection in acute respiratory infections in children. Current Infectious Disease Reports [Internet]. 2020 Jan 1[cited 14 May 2024];22(1). Available from: https://doi.org/10.1007/s11908-020-0711-8
33. Bosch A a. TM, Biesbroek G, Trzcinski K, Sanders E a. M, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. PLOS Pathogens [Internet]. 2013 Jan 10[cited 14 May 2024];9(1):e1003057. Available from: https://doi.org/10.1371/journal.ppat.1003057