Usage of Multiplex Polymerase Chain Reaction (PCR) Panels at a Single Tertiary Hospital in Manila: Most Common Pathogens Detected and Implications on Antimicrobial Therapy

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ABSTRACT

Background and Objectives. Multiplex Polymerase Chain Reaction (PCR) is an emerging diagnostic tool used to rapidly detect infectious disease pathogens, aiding decision-making in antimicrobial therapy. This study aims to describe the usage of multiplex PCR (BioFire® FilmArray®) panels, namely Respiratory Panel, Pneumonia Panel, Gastrointestinal (GI) Panel and Meningitis/Encephalitis (ME) Panel in adult patients in a tertiary hospital in Manila, from 2020 to 2023 (2021 to 2024 for Meningitis/Encephalitis Panel); to identify the most common pathogens detected for each panel; and to determine the implications on antimicrobial therapy.

Methods. Panel results were retrieved with assistance from the laboratory staff through the hospital information systems (HIS), and patient demographics and antimicrobial therapy from the HIS. Descriptive analysis was done.

Results. Among adult patients subjected to Multiplex PCR panels in Asian Hospital and Medical Center in the period specified above, the most requested was the Respiratory panel, followed by the Pneumonia panel, GI panel, and ME panel. Pneumonia panel has the highest positivity rate at 61%, followed by the GI panel at 42.1%, the ME panel at 40%, and the respiratory panel at 18.3%. The majority of the respiratory panel pathogens were community-acquired, with SARS-CoV-2, Influenza A, and Rhinovirus/Enterovirus as the most commonly detected pathogens. Pneumonia panel pathogens were mostly hospital-acquired, with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (with resistance genes CTX-M and NDM) being the most commonly detected. The sample size is much smaller for the remaining panels. The most commonly found pathogens in the GI panel were the Enteroaggregative and Enteropathogenic *Escherichia coli* and *Clostridium difficile*, and for the ME panel, *Streptococcus agalactiae* and Varicella zoster virus. Overall, awareness of the etiologies of infectious diseases through multiplex PCR panels led to starting of new antimicrobials and the continuation of the present antimicrobials, more than shifting or stopping antimicrobials.

Conclusion. This study determined the most common pathogens detected in Multiplex PCR panels in Asian Hospital and Medical Center as above. Awareness of the etiologic agents for all 4 panels appeared useful in the decision-making on antimicrobial therapy. In the respiratory panel, the majority resulted in starting new antimicrobials (32.4%), followed by continuing antimicrobials (23.1%). In the pneumonia panel, it seemed like the majority had already started the appropriate empiric therapy since most continued with the present antibiotics (51.7%), followed by starting (22.8%) and

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shifting (18.3%) antimicrobials. In the GI panel, most also continued with the current antibiotics (29.5%), closely followed by starting (17.6%), remaining without antimicrobials (17.6%), and shifting antimicrobials (11.8%). It is in the ME panel that most started with new antimicrobials (44.5%) and stopped (33.3%) specific antimicrobials. The study underscores the value of multiplex PCR in enhancing diagnostic accuracy and guiding antimicrobial decision-making.

Keywords: multiplex polymerase chain reaction, respiratory panel, pneumonia panel, gastrointestinal panel, meningitis/encephalitis panel, antimicrobial therapy, most common pathogens detected

INTRODUCTION

Polymerase chain reaction (PCR) plays an immense role in the diagnosis of infectious diseases. PCR testing is a fast and accurate way to detect certain pathogens, whether bacterial, viral, fungal, and/or parasitic, by amplifying a small amount of genetic material or DNA in a sample. Aside from infectious disease diagnostic testing, it has also been used in genetic testing and even forensics.

To improve the diagnostic capacity of PCR, a variant of the test has been developed: *multiplex polymerase chain reaction*.[1] Multiplex PCR, in comparison to a single-primer-set PCR, is used to detect more than one infectious agent in a single test. It does so by amplifying more than one target sequence and including more than one pair of primers, which are the short pieces of single-stranded DNA that are complementary to the target sequence where the polymerase begins to synthesize new DNA in the reaction. The use of multiplex PCR reduces cost, time, and effort within the laboratory and has been successfully applied in many areas of nucleic acid diagnostics.[1] In the field of infectious disease diagnosis, it has been used to identify pathogens (and detect antimicrobial resistance genes) in the following, but not limited to respiratory infections, gastroenteritis, and central nervous system infections.

Multiplex PCR assays are proving to be very useful diagnostic tools during this modern age of medicine. Conventional cultures, if done, would take at least two days for initial results, hence making it difficult to initiate earlier targeted antibiotic therapy. Delay in administering appropriate therapy may lead to devastating outcomes, especially in the critically ill, highlighting the importance of these PCR panels, wherein results come in just a few hours, bringing about an early initiation of effective, definitive therapy.

A study by Lagamayo and Rusia-Uy in 2021 reviewed a 2-year use of all available FilmArray® panels (when the tests were first introduced in the Philippines) used in both adult and pediatric patients in a tertiary hospital in Manila pre-COVID pandemic in 2017 to 2019.[2] Of 547 tests, the most requested FilmArray® panel was the Gastrointestinal panel with 48%, followed by the Meningitis/Encephalitis Panel with 26%, the Respiratory panel with 23% and lastly, the blood culture identification panel with 3%. The most commonly isolated pathogens for the GI panel for single pathogen gastroenteritis were *Escherichia coli*, *Clostridium difficile,* and *Cyclospora cayetansis*. The most common pathogens in the respiratory panel were Influenza A, Human Rhinovirus/Enterovirus, and Influenza B; for Meningitis/Encephalitis panels, the *Streptococcus pneumoniae, E. coli,* and Enterovirus; for the blood culture identification panel, *Staphylococcus (mec A gene)*, *Neisseria meningitidis,* and *Enterobacteriaceae (Klebsiella)*. The positivity rates of the gastrointestinal, respiratory, and blood culture identification panels were impressive at 60%, 57%, and 83%, respectively.[2] As expected, a high percentage of pathogens from the respiratory panel were viruses. The lowest positivity rate was seen with the meningitis/encephalitis panel at 14%, though still a good number, since these viruses could not have grown in the usual CSF bacterial culture. In conclusion, multiplex PCR panels provide early patient diagnosis and identify common pathogens that may have been missed by traditional culture methods.[2]

Several more studies outside of the Philippines have analyzed the usage of multiplex PCR. A study by Webber et al. in Missouri evaluated the diagnostic yield and accuracy of the multiplex PCR panel, BioFire® FilmArray® pneumonia panel (BFPP) for identification of pathogens in lower tract specimens.[3] Specimens (sputum, bronchial alveolar lavage/bronchial washing, tracheal aspirate) were taken from patients 18 years of age and above in the ED and the ICU in 2018. The bacterial and viral detection rate was 117 of 200, or 58.5%. Results showed the most common bacteria detected by BFPP were *Staphylococcus aureus* (22%) and *Haemophilus influenzae* (14%). The most common viruses were rhinovirus/enterovirus (4.5%), influenza A virus (3%), and respiratory syncytial virus (2%). There was strong agreement between BFPP and standard methods for the detection of viruses (99.2%) and bacteria (96.8%). Most bacteria (60 of 61 or 98.4%) detected by standard methods were also identified by BFPP. However, the study stated that the limitation of the BFPP assay is the absence of fungal targets and *Stenotrophomonas maltophilia*, which were detected in 26 and 4 of 200 specimens, respectively, via standard methods. Overall, the study concluded that the BFPP was a rapid and accurate method for detecting pathogens for lower respiratory tract infections.[3]

Studies on lower respiratory tract infection multiplex PCR, more than other infectious processes, are more popular. Kosai et al. analyzed the targets in lower respiratory tract specimens (sputum, tracheal aspirate, BAL) using a multiplex PCR pneumonia panel and compared the detection results with those of bacterial culture methods and antimicrobial susceptibility testing.[4] Of 57 samples in Nagasaki University Hospital, the most frequently detected by multiplex PCR were *P. aeruginosa*, followed by *S. aureus*, *K. pneumoniae*, *A. calcoaceticus baumannii* complex, *E. cloacae* complex, and

H. influenzae; with the most common resistance markers being mecA/mecC and MREJ, and the CTX-M. The overall positive and negative percent agreements between the PN panel and culture methods for bacterial detection were 100.0% and 92.9%, respectively. It concluded that the multiplex PCR enhanced the detection of pathogens and antimicrobial resistance markers in lower respiratory tract specimens.[4] However, it recommended further studies to clarify its effects on patient management cost-effectiveness.

A systematic review and meta-analysis by Clark et al. in 2023 included 27 studies with 17,321 patients in order to evaluate the impact of multiplex PCR testing in patients with acute respiratory tract infection in the hospital setting. It demonstrated that its use reduced time to results by 22.24 hours and hospital length of stay for patients by 0.82 days.[5]

Furthermore, the potential for antimicrobial stewardship was demonstrated by Buchan et al in 2022. The study used bronchoalveolar lavage as a specimen for a multiplex PCR pneumonia panel in eight US clinical centers.[6] It showed a combined 96.2% positive percent agreement (PPA) and 98.1% negative percent agreement (NPA) for the qualitative identification of 15 bacterial targets compared to routine bacterial culture. On review of patient medical records, the potential for antibiotic adjustment in 70.7% of patients based on the pneumonia panel result, including discontinuation or de-escalation in 48.2% of patients, resulted in an average savings of 6.2 antibiotic days per patient.[6]

In the Philippines, due to the inherent disadvantage of cost, the use of multiplex PCR panels was limited before the COVID-19 pandemic. It was during the pandemic that more, but still quite a few, hospitals carried these test kits. Currently, there is limited local data on the usage of these tests.

METHODS

This study is a descriptive study conducted from June to October 2024. It aimed to determine the most frequently detected pathogens detected in the four Multiplex PCR (BioFire® FilmArray®) panels used on adult patients in a tertiary hospital in Manila, the Asian Hospital and Medical Center, from the time they were first used in the institution from June 2020 until December 2023 for the Pneumonia panel; from October 2020 until December 2023 for the Respiratory panel and the Gastrointestinal (GI) panel; and from February 2021 until May 2024 for the Meningitis (ME) panel. The information gained from this might be used to estimate the prevalence of infections by certain organisms in the community. Determining the causative agents of infectious diseases, whether viral, bacterial, fungal, or parasitic, would be advantageous, in that an appropriate and targeted antimicrobial therapy can be decided, as well as may reduce the unnecessary use of antibiotics.

Moreover, this study also sought to describe the baseline characteristics of the study participants according to age, gender, type of patient whether inpatient or outpatient), and the presence or absence of symptoms; to determine the most common pathogens detected in multiplex PCR panels stratified by the diagnosis of community-acquired infections versus hospital-acquired infections; and to determine the changes in decision-making after a positive result (or any organism detected) as follows: 1) continuing antimicrobials, 2) starting antimicrobials, 3) shifting antimicrobials, 4) stopping antimicrobials, or 5) no change or remaining without antimicrobials.

Selection and Description of Participants

The study included adults 19 years old and above subjected to Multiplex PCR (BioFire® FilmArray®) Pneumonia panel, Respiratory panel, GI panel, and ME panel, both inpatients and outpatients, and symptomatic and asymptomatic patients. In admitted patients who were tested more than once in a single admission with a positive test result, all positive tests were included. Those with incomplete documentation of details in the hospital information systems (HIS), such as the presence or absence of symptoms and the use of antimicrobial treatment, were still included and were well-noted. Data on the patients' comorbidities were not included. Symptomatic subjects were, for Pneumonia panel and Respiratory panel, those with any of the following respiratory symptoms: cough, rhinorrhea, difficulty of breathing, shortness of breath, sore throat, desaturation, fever, loss of appetite, fatigue; for GI panel, those with any of the following gastrointestinal symptoms: diarrhea/loose stools, bloody stools, abdominal pain/cramps, loss of appetite, fatigue and fever; for ME panel, those with any of the following CNS infection symptoms: headache, stiff neck, vomiting, seizures, altered sensorium, behavioral changes, loss of appetite, fatigue, fever. Patients were stratified presumptively as having community-acquired infections if with symptoms already present during the time of hospital admission and multiplex PCR done within 48 hours from admission; and presumptively as having hospital-acquired infections if symptoms developed only 48 hours from the time of admission and multiplex PCR done beyond 48 hours from admission – to include patients who were already symptomatic upon admission, but with the multiplex PCR done beyond 48 hours from admission.

Data Collection

Panel results were retrieved from the Molecular Diagnostics laboratory staff and the HIS, and patient demographics and antimicrobial therapy from the HIS. Data was collected only after the study was approved by the Research Ethics Committee (REC) of the institution, ensuring that the patients' identities were protected. The researchers did not use informed consent since the study was descriptive, with only a chart review done. There was no patient interaction. No external source of funding was used for this study.

Data was merged into a single dataset using Microsoft Excel. Data cleaning included verification of entries, standardization of variable names, and checking for missing values. Patients with incomplete documentation were retained but appropriately labeled as "N/A" (not available) in the analysis. Each patient was assigned a number code to maintain anonymity and data integrity. Only the two authors have access to the raw files. The finalized dataset without patient identifiers was then submitted to the statistician for importing into SPSS version 20 for descriptive analysis.

Statistics

Data analysis was primarily descriptive. Frequencies and percentages were used to summarize categorical variables, including demographic characteristics (age group, gender, inpatient/outpatient status, presence/absence of symptoms), timing of testing (community- or hospital-acquired), and changes in antimicrobial management (continuation, initiation, shifting, discontinuation, or no change). Continuous data were described using mean, standard deviation, median, and range. Tabulations were used to depict the characteristics of the patients who had a positive result, all pathogens detected in each panel stratified presumptively to community- or hospital-acquired, and changes in antimicrobial therapy. In addition, graphical representations with bar graphs were created to highlight the most common pathogens and the most changes seen in antimicrobial therapy. All analyses were performed using SPSS Statistics Version 20.

RESULTS

Among adult patients subjected to Multiplex PCR (BioFire® FilmArray®) panels in Asian Hospital and Medical Center from June 2020 to December 2023 (Pneumonia panel), October 2020 to December 2023 (Respiratory and GI panel) and February 2021 to May 2024 (ME panel), the most frequently requested was the Respiratory panel with 3,805 tests done, followed by the Pneumonia panel with 272 tests, the GI panel with 38 tests and the ME panel with 15 tests. Table 1 shows the positivity rate for each panel, showing that the pneumonia panel has the highest positivity rate at 61%, followed by the GI panel at 42.1%, the ME panel at 40%, and the respiratory panel at 18.3%.

Table 1. Positivity Rate of Multiplex PCR (BioFire® FilmArray®) Panels Done at Asian Hospital and Medical Center

Multiplex PCR Panel	Positive	Negative	Total	Positivity Rate (%)
Respiratory Panel	698	3107	3805	18.3
Pneumonia Panel	166	106	272	61.0
Gastrointestinal Panel	16	22	38	42.1
Meningitis/ Encephalitis Panel	6	9	15	40.0

Patient Characteristics

Among adult patients subjected to Multiplex PCR (BioFire® FilmArray®) panels in Asian Hospital and Medical Center, there were 886 patients with positive results: 698 for the respiratory panel, 166 for the pneumonia panel, 16 for the GI panel, and 6 for the ME panel. Table 2 displays the participants' characteristics, showing that overall, there was an almost equal number of patients belonging to the younger and older age groups, but separately, a greater percentage at 79.5% and 56.3% of patients were 60 years of age and older for the pneumonia panel and the GI panel, respectively.

Table 2. Demographic Characteristics of Patients

Characteristics	Respiratory		Pneumonia		GI		ME		Total	
	(n=698)	%	(n=166)	%	(n=16)	%	(n=6)	%	(n=886)	%
Age (years)										
19-59	409	58.6	34	20.5	7	43.8	4	66.7	454	51.2
60 and above	289	41.4	132	79.5	9	56.3	2	33.3	432	48.8
Gender										
Male	323	46.3	106	63.9	8	50.0	4	66.7	441	49.8
Female	375	53.7	60	36.1	8	50.0	2	33.3	445	50.2
Patient type										
Outpatient	213	30.5	5	3.0	2	12.5	0	0	220	24.8
Inpatient	485	69.5	161	97.0	14	87.5	6	100	666	75.2
Symptoms										
Within 48h of admission	373	53.4	132	79.5	14	87.5	6	100	525	59.3
Beyond 48h of admission	6	0.9	22	13.3	0	0.0	0	0	28	3.1
N/A* and Asymptomatic	319	45.7	12	7.2	2	12.5	0	0	333	37.6
Timing of test										
Within 48h of admission	458	65.6	36	21.9	11	68.8	6	100	511	57.7
Beyond 48h of admission	22	3.2	127	76.4	3	18.7	0	0	152	17.1
N/A*	218	31.2	3	1.7	2	12.5	0	0	223	25.2

**N/A = Presence/absence of symptoms not documented in the hospital records; outpatient*

In contrast, for the respiratory panel and the ME panel, more patients were below 60 years of age at 58.6% and 66.7%, respectively. Overall, there was also an almost equal proportion of males and females, but for the pneumonia panel and the ME panel, most were males at 63.9% and 66.7%, respectively.

Overall, 75% of patients were admitted patients – 485 (69.5%) of 698 patients for the respiratory panel, 161 (97%) of 166 for the pneumonia panel, 14 (87.5%) of 16 for the GI panel, and all 6 of 6 patients for the ME panel.

The majority were symptomatic patients at 62%. For the respiratory panel, 458 (65.6%) of 698 positive patients were stratified presumptively as having community-acquired infection based on the presence of symptoms and the timing of testing done within 48 hours of admission. In contrast, for the pneumonia panel, 127 (76.4%) of 166 positive patients were stratified presumptively as having hospital-acquired infection. Included also in the hospital-acquired group were patients with symptoms on admission or within 48 hours from admission, but with the test done beyond 48 hours.

Respiratory Panel

There were 18 organisms detected in the Respiratory panel in Asian Hospital and Medical Center from October 2020 to December 2023. Overall, the most common pathogen was SARS-CoV-2 at 317 (41.3%) of 767, Influenza A at 149 (19.4%), and Rhinovirus/Enterovirus at 139 (18.1%) (Table 3). Most were stratified as community-acquired at 477 (62.2%) of 767. Twenty-two (2.9%) were hospital-acquired. The rest cannot be stratified because they were either outpatients with no documentation in the HIS of the presence of symptoms, or were asymptomatic. For community-acquired, SARS-CoV-2 was still the most commonly detected at 208 (43.6%) of 477, followed by Rhinovirus/Enterovirus at 81 (17%), and Influenza A at 74 (15.5%). For hospital-acquired infections, the same pathogens were also the most commonly detected, with SARS-CoV-2 in 12 (54.5%) of 22 and Rhinovirus/Enterovirus at 7 (31.8%). Other pathogens also frequently detected in the respiratory panel were Adenovirus, Influenza B, Human Metapneumovirus, Respiratory Syncytial Virus, and Coronavirus. Figure 1A shows the top 10 pathogens in the respiratory panel stratified into community-acquired and hospital-acquired, while Figure 1B shows the overall top 10.

Pneumonia Panel

There were 22 pathogens detected in the Pneumonia panel in Asian Hospital and Medical Center from June 2020 to December 2023. In contrast to the respiratory panel, the pneumonia panel was more frequently requested to determine hospital-acquired infections. Most turned out to be hospital-acquired at 249 (75.6%) of 329. But, in both hospital- and community-acquired infections, the most common pathogens were *Pseudomonas aeruginosa* (52 or 20.9% of 249 for hospital-acquired and 9 or 12.3% of 73 for community-acquired) and *Klebsiella pneumoniae* (51 or 20.5% of 249 for hospital-acquired and 13 or 17.8% of 73 for community-acquired). Other frequently detected pathogens included *Acinetobacter calcoaceticus baumannii* complex, *Enterobacter cloacae* complex, *Staphylococcus aureus*, and

Table 3. Organisms Detected and Frequency of Detection in the Respiratory Panel (n=767)

Respiratory Panel Pathogens	Hospital-acquired (n=22)		Community-acquired (n=477)		Not stratified (n=268)		Total (n=767)	
	No.	%	No.	%	No.	%	No.	%
SARS-CoV-2	12	54.5	208	43.6	97	36.2	317	41.3
Influenza A	1	4.5	74	15.5	74	27.6	149	19.4
Rhinovirus/Enterovirus	7	31.8	81	17.0	51	19.0	139	18.1
RSV	1	4.5	29	6.1	7	2.6	37	4.8
Adenovirus	0	0.0	17	3.6	6	2.2	23	3.0
Influenza B	0	0.0	14	2.9	6	2.2	20	2.6
Human Metapneumovirus	1	4.5	16	3.4	2	0.7	19	2.5
Coronavirus OC43	0	0.0	11	2.3	4	1.5	15	2.0
Parainfluenza Virus 3	0	0.0	10	2.1	5	1.9	15	2.0
Coronavirus HKU1	0	0.0	3	0.6	8	3.0	11	1.4
Coronavirus 229E	0	0.0	4	0.8	3	1.1	7	0.9
Coronavirus NL63	0	0.0	3	0.6	3	1.1	6	0.8
Parainfluenza Virus 4	0	0.0	4	0.8	0	0.0	4	0.5
Parainfluenza Virus 1	0	0.0	0	0.0	1	0.4	1	0.1
Parainfluenza Virus 2	0	0.0	0	0.0	1	0.4	1	0.1
Bordetella parapertussis	0	0.0	1	0.2	0	0.0	1	0.1
Chlamydophila pneumoniae	0	0.0	1	0.2	0	0.0	1	0.1
Mycoplasma pneumoniae	0	0.0	1	0.2	0	0.0	1	0.1

Rhinovirus/Enterovirus (Table 4A). Figure 2A shows the 10 most commonly detected pathogens in the pneumonia panel stratified into community-acquired versus hospital-acquired, while Figure 2B shows the overall most commonly detected.

Aside from detecting the pathogens, the Pneumonia panel was also used to identify resistance genes. Overall, the most common was CTX-M (an extended-spectrum beta-lactamase gene marker) at 21 (35.6%) of 59; NDM and VIM (both carbapenemase gene markers) at 17 (28.8%) and 8 (13.6%), respectively; and MecA/C and MREJ (Methicillin resistance gene markers) at 5 (8.5%) (Table 4B).

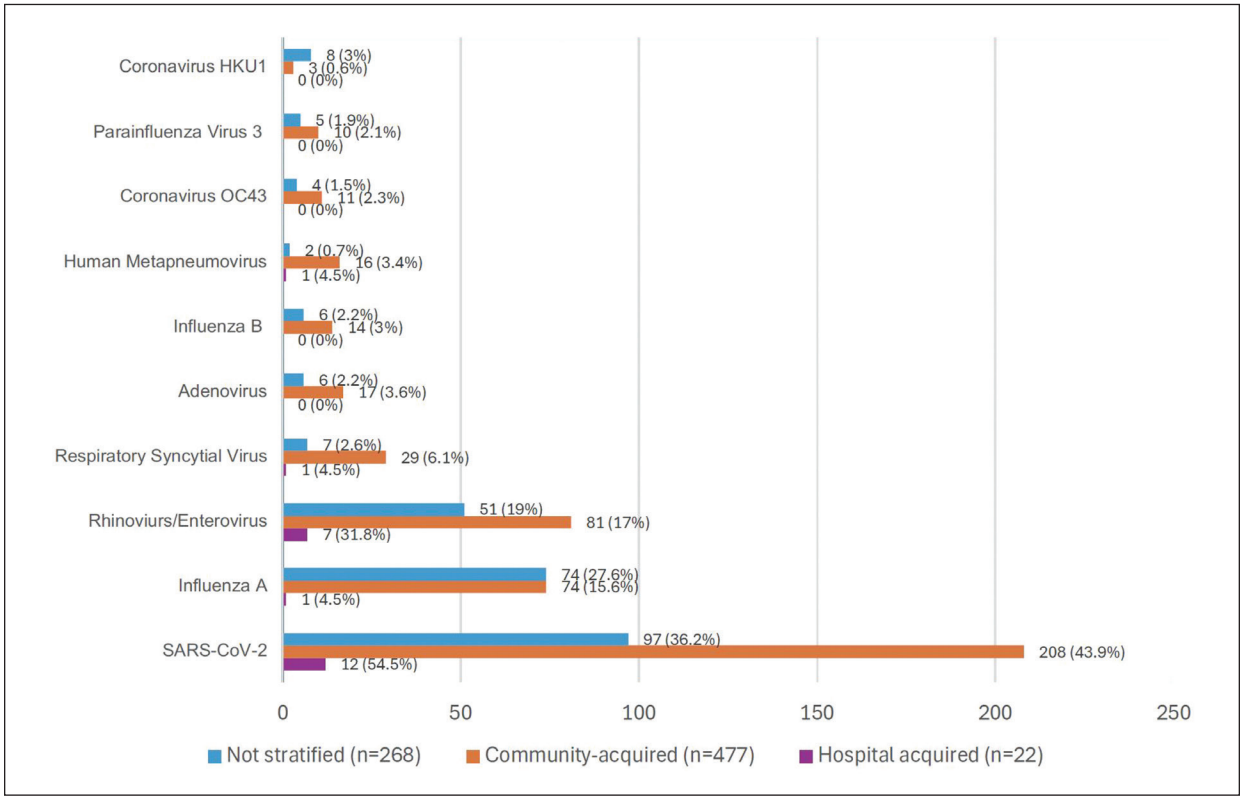

Figure 1A. Frequency distribution of the 10 most commonly detected Respiratory Panel pathogens, community- versus hospital-acquired, in Asian Hospital and Medical Center from October 2020 to December 2023 (n=767).

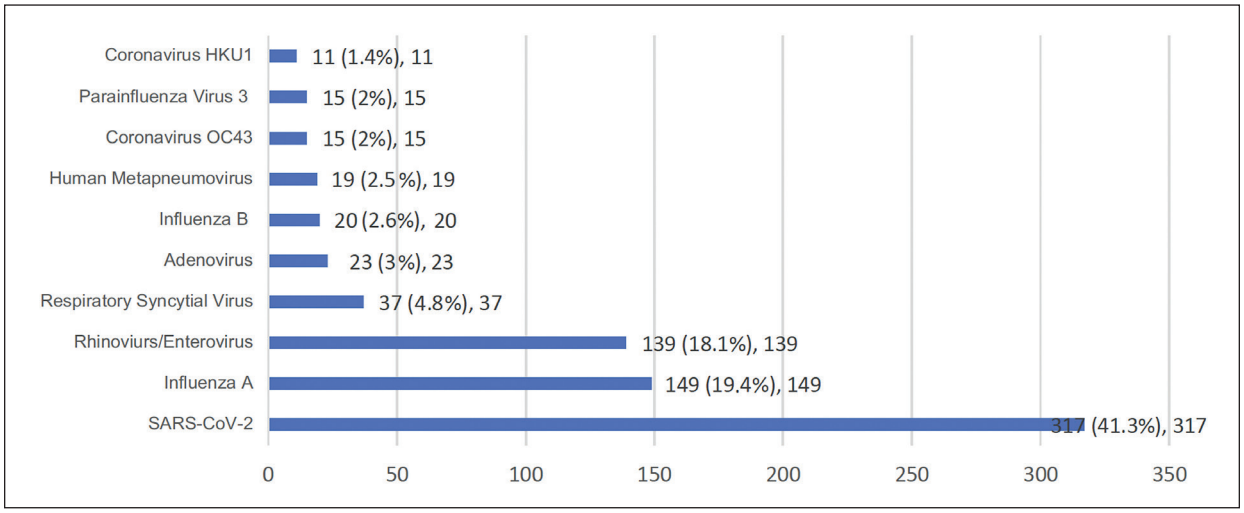

Figure 1B. Frequency distribution of the 10 most commonly detected Respiratory Panel pathogens overall, in Asian Hospital and Medical Center from October 2020 to December 2023 (n=767).

Gastrointestinal Panel

There were 11 pathogens detected in the GI panel in the Asian Hospital and Medical Center from October 2020 to December 2023. Most were community-acquired at 20 (80%) of 25. Among the community-acquired pathogens, the most common were Enteroaggregative *E. coli* (EAEC) and Enteropathogenic *E. coli* (EPEC), both at 5 (25%) of 20. For the hospital-acquired pathogens, equally detected were *Clostridium difficile* toxin A/B, *Plesiomonas shigelloides*, and Salmonella, each at 33.3% (Table 5). Figure 3A shows the most commonly detected pathogens in the GI panel stratified into community-acquired and hospital-acquired, while Figure 3B shows overall.

Meningitis/Encephalitis Panel

There were four pathogens detected in the ME panel in the Asian Hospital and Medical Center from February 2021 to May 2024. All were community-acquired. The pathogens were *Streptococcus agalactiae* and Varicella zoster virus (each at 33.3%), followed by *Cryptococcus neoformans/gatti* and Herpes simplex virus-1 (Table 6 and Figure 4).

Implications on Antimicrobial Therapy

Knowledge of the pathogens detected by multiplex PCR panels resulted in mostly starting new antimicrobials at 274 (30.3%) of 904; continuation of present antimicrobials at 261 (28.9%); and no change at 120 (13.3%) overall across all 4 multiplex PCR panels. For the respiratory panel alone, majority at 226 (32.4%) of 698 were started on antimicrobials, particularly due to starting of antiviral therapy for SARS-CoV-2 (as the most commonly detected pathogen), as well as other viral illnesses such as Influenza; 117 (16.7%) had no change or remained without antimicrobials; 189 (27.1%) were not stratified due to lack of documentation, and less than 1% had antimicrobials shifted or stopped. For the pneumonia panel alone, 93 (51.7%) of 180 had present antimicrobials continued, 41 (22.8%) had new antimicrobials started, 33 (18.3%) had antimicrobials shifted, 9 (5%) stopped antimicrobials, and 4 (2.2%) had no change. For the GI panel alone, 5 (29.5%) of 17 resulted in continuation of antimicrobials, 3 (17.6%) each started new antimicrobials and had no change or no antimicrobials. For the ME panel alone, 4 (44.5%) of 9 resulted to starting of antimicrobials, followed by 3 (33.3%) stopping of antimicrobials and 2 (22.2%) continuing of antimicrobials (Table 7, Figures 5A and 5B).

Table 4A. Organisms Detected and Frequency of Detection in the Pneumonia Panel (n=329)

Pneumonia Panel Pathogens	Hospital-acquired (n=249)		Community-acquired (n=73)		Not stratified (n=7)		Total (n=329)	
	No.	%	No.	%	No.	%	No.	%
Klebsiella pneumoniae group	51	20.5	13	17.8	1	14.3	65	19.8
Pseudomonas aeruginosa	52	20.9	9	12.3	1	14.3	62	18.8
Acinetobacter calcoaceticus baumannii complex	21	8.4	2	2.7	0	0.0	23	7.0
Staphylococcus aureus	14	5.6	8	11	1	14.3	23	7.0
Enterobacter cloacae complex	17	6.8	3	4.1	0	0.0	20	6.1
Human Rhinovirus/Enterovirus	17	6.8	3	4.1	0	0.0	20	6.1
Haemophilus influenza	7	2.8	7	9.6	1	14.3	15	4.6
Escherichia coli	10	4.0	3	4.1	0	0.0	13	4.0
RSV	9	3.6	3	4.1	0	0.0	12	3.6
Streptococcus agalactiae	9	3.6	2	2.7	1	14.3	12	3.6
Influenza A	2	0.8	6	8.2	0	0.0	8	2.4
Parainfluenza virus	3	1.2	4	5.5	1	14.3	8	2.4
Serratia marcescens	7	2.8	0	0.0	0	0.0	7	2.1
Klebsiella oxytoca	6	2.4	1	1.4	0	0.0	7	2.1
Streptococcus pneumoniae	6	2.4	0	0.0	1	14.3	7	2.1
Human Metapneumovirus	6	2.4	1	1.4	0	0.0	7	2.1
Coronavirus	4	1.6	2	2.7	0	0.0	6	1.8
Moraxella catarrhalis	2	0.8	3	4.1	0	0.0	5	1.5
Klebsiella aerogenes	3	1.2	1	1.4	0	0.0	4	1.2
Influenza B	1	0.4	1	1.4	0	0.0	2	0.6
Proteus spp.	1	0.4	1	1.4	0	0.0	2	0.6
Adenovirus	1	0.4	0	0.0	0	0.0	1	0.3

DISCUSSION

Among adult patients subjected to Multiplex PCR (BioFire® FilmArray®) panels in Asian Hospital and Medical Center from June 2020 to December 2023 (Pneumonia panel), October 2020 to December 2023 (Respiratory and GI panel) and February 2021 to May 2024 (ME panel), the most frequently requested was the Respiratory panel with 3,805 tests done, followed by the Pneumonia panel with 272 tests, the GI panel with 38 tests and the ME panel with 15 tests. It is important to note that the study period included the COVID-19 pandemic period, which is why the respiratory panel was frequently requested. It was done even to patients without respiratory symptoms as a requirement for admission to the above institution. Also, it was often preferred over the oropharyngeal swab RT-PCR (single PCR) COVID test because of the shorter turnaround time.

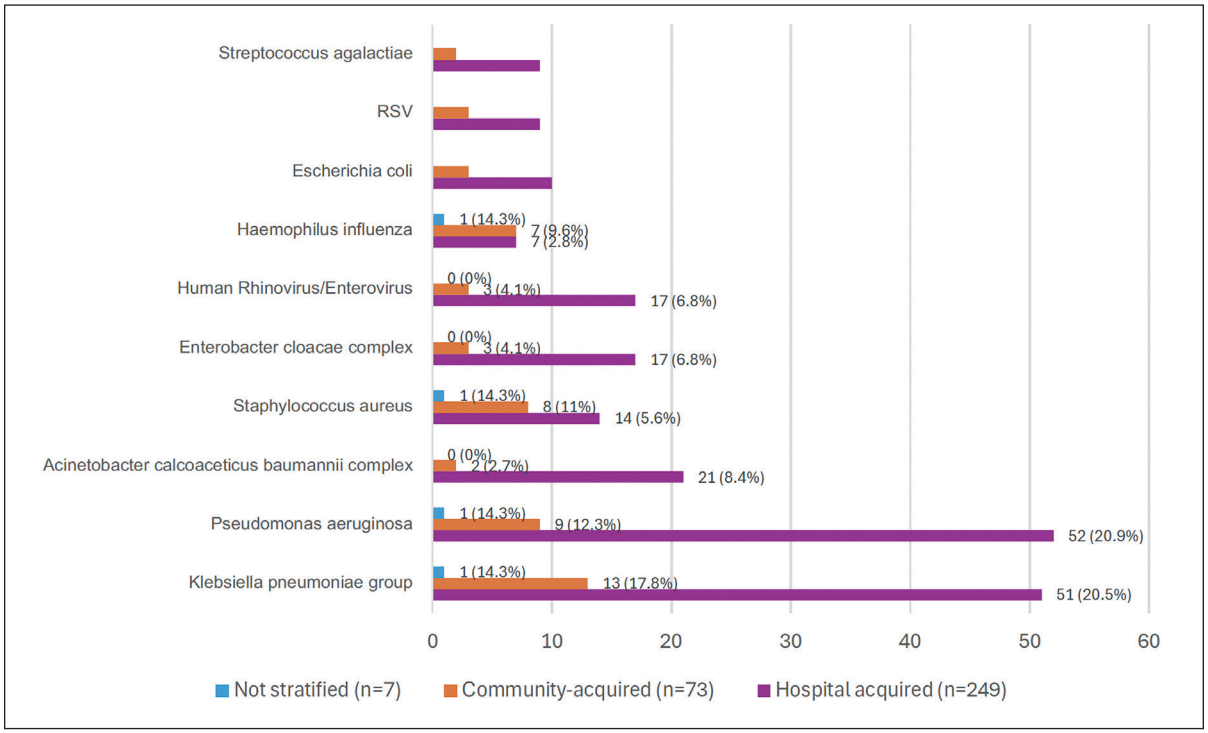

Figure 2A. Frequency distribution of the 10 most commonly detected Pneumonia Panel pathogens, community- versus hospital-acquired, in Asian Hospital and Medical Center from June 2020 to December 2023 (n=329).

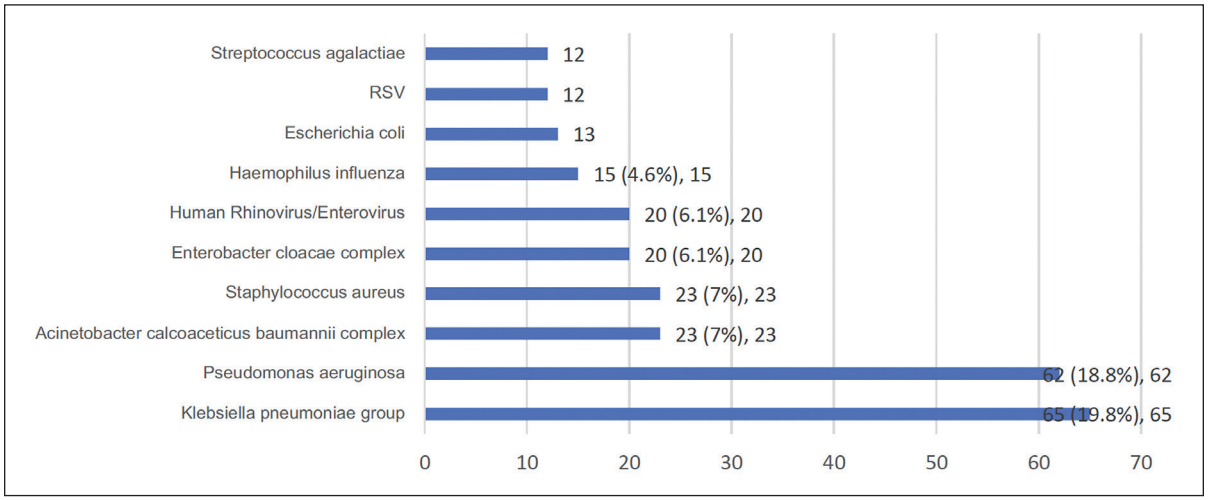

Figure 2B. Frequency distribution of the 10 most commonly detected Pneumonia Panel pathogens overall, in Asian Hospital and Medical Center from June 2020 to December 2023 (n=329).

Table 4B. Resistance Genes and Frequency of Detection via the Pneumonia Panel in Asian Hospital and Medical Center from June 2020 to December 2023 (n=59)

Resistance Genes	Hospital-acquired (n=55)		Community-acquired (n=4)		Not stratified (n=0)		Total (n=59)	
	No.	%	No.	%	No.	%	No.	%
CTX-M	19	34.5	2	50	0	0	21	35.6
NDM	15	27.3	2	50	0	0	17	28.8
VIM	8	14.5	0	0	0	0	8	13.6
MecA/C and MREJ	5	9.1	0	0	0	0	5	8.5
IMP	4	7.3	0	0	0	0	4	6.8
OXA-48-like	4	7.3	0	0	0	0	4	6.8

Table 5. Organisms Detected and Frequency of Detection in the Gastrointestinal Panel (n=25)

GI Panel Pathogens	Hospital-acquired (n=3)		Community-acquired (n=20)		Not stratified (n=2)		Total (n=25)	
	No.	%	No.	%	No.	%	No.	%
Enteroaggregative E. coli (EAEC)	0	0.0	5	25	0	0	5	20
Enteropathoogenic E. coli (EPEC)	0	0.0	5	25	0	0	5	20
Clostridium difficile toxin A/B	1	33.3	2	10	1	50	4	16
Norovirus GI/GII	0	0.0	2	10	1	50	3	12
Salmonella	1	33.3	1	5	0	0	2	8
Campylobacter	0	0.0	1	5	0	0	1	4
Plesiomonas shigelloides	1	33.3	0	0	0	0	1	4
Enterotoxigenic E. coli) (ETEC)	0	0.0	1	5	0	0	1	4
Shiga-like toxin-producing Escherichia (STEC)	0	0.0	1	5	0	0	1	4
Shigella/Enteroinvasive E. coli (EIEC)	0	0.0	1	5	0	0	1	4
Cyclospora cayetanensis	0	0.0	1	5	0	0	1	4

The pneumonia panel has the highest positivity rate at 61%, followed by the GI panel at 42.1%, the ME panel at 40%, and the respiratory panel at 18.3%. Though with the lowest positivity rate, the respiratory panel has the most positive results (any organism detected) at 698 (78.8%), owing to the larger number of tests done, followed by the pneumonia panel at 166 (18.7%), the GI panel at 16 (1.8%), and the ME panel at 6 (0.7%). The study by Lagamayo and Rusia-Uy in 2021, reviewing the 2-year use of FilmArray® panels in adult and pediatric patients in Manila before the COVID pandemic in 2017-2019, showed impressive positivity rates, particularly for the gastrointestinal (60%), respiratory (57%), and blood culture identification panels (83%).[2] These findings reflect the growing diagnostic role of multiplex PCR in detecting both community- and hospital-acquired infections in the local setting. Our study generally confirms the positivity rate of the GI panel, but it appears lower for the respiratory panel. The practice of doing a respiratory panel only if a previous rapid antigen test for SARS-CoV-2 in COVID-19 suspects turns out positive may have contributed to this, since a negative rapid antigen test may increase the chances of a negative respiratory panel test when used primarily to detect SARS-CoV-2 (unless symptomatic).

This study included all adult patients subjected to these panels with a positive result or any organism detected. For the patient characteristics, a greater percentage at 79.5% and 56.3% of patients, were 60 years of age and older for the pneumonia panel and the GI panel, respectively. In contrast, for the respiratory panel and the ME panel, more patients were below 60 years of age at 58.6% and 66.7%, respectively. As for the gender, most were males at 63.9% and 66.7% for the pneumonia panel and the ME panel, respectively. Seventy-five percent were admitted patients. Those with symptoms starting prior to admission and within 48 hours of admission, and testing done within 48 hours of admission, were stratified presumptively as community-acquired. Those with symptoms starting and testing done beyond 48 hours from admission were stratified presumptively as hospital-acquired, including patients who were already symptomatic upon admission, but with the pneumonia panel done beyond 48 hours from admission.

Results showed that for the respiratory panel pathogens, most were community-acquired (62% of 767), with the most frequently detected pathogen being SARS-CoV-2 (43.6%), which was expected because the study covered the period of the COVID-19 pandemic from 2020 to 2023. The next most commonly detected respiratory panel pathogens were influenza A and Rhinovirus/Enterovirus, which were also the most commonly detected pathogens pre-COVID pandemic in Manila in the study by Lagamayo and Rusia-Uy, and in

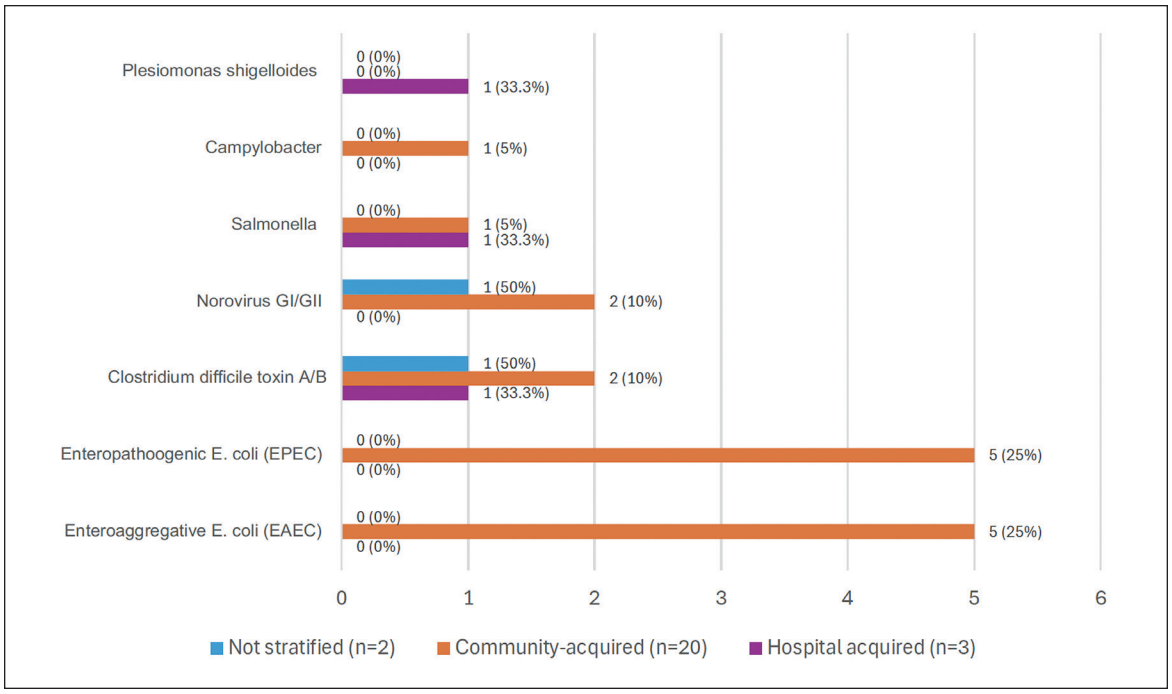

Figure 3A. Frequency distribution of the most commonly detected GI Panel pathogens, community- versus hospital-acquired, in Asian Hospital and Medical Center from October 2020 to December 2023 (n=25).

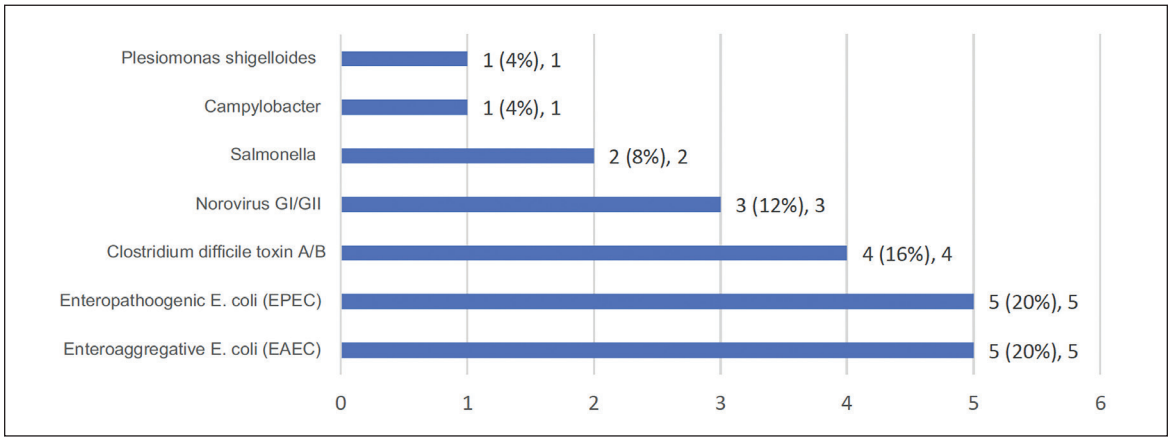

Figure 3B. Frequency distribution of the most commonly detected GI Panel pathogens overall, in Asian Hospital and Medical Center from October 2020 to December 2023 (n=25).

Missouri in the study of Webber et al.[2,3] This may suggest that, except for SARS-CoV-2, the community-acquired respiratory pathogens are similar, before and during the COVID-19 pandemic.

On the other hand, for the pneumonia panel pathogens, most were hospital-acquired (75.6% of 329). Compared to the respiratory panel, the pneumonia panel has more bacterial targets; it was more frequently requested beyond 48 hours from admission to detect hospital-acquired or nosocomial pneumonia, particularly for patients who have already spent a significant time admitted in the hospital, and for more complicated cases. For both community- and hospital-acquired infection, the most commonly detected pathogens were *Pseudomonas aeruginosa* (18.8%) and *Klebsiella pneumoniae* (19.8%). The most commonly detected resistance genes were CTX-M, an extended beta-lactamase gene marker (35.6%), and NDM, a carbapenemase gene marker (28.8%). These results align with the Department of Health (DOH) Research Institute for Tropical Medicine 2024 Annual Report of the Antimicrobial Resistance Surveillance Program (ARSP), which stated that the most common pathogens among bacteriologically-confirmed respiratory tract infections were *Klebsiella pneumoniae* ss. *pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, with elevated resistance rates,

Table 6. Organisms Detected and Frequency of Detection in the Meningitis/Encephalitis (n=6)

ME Panel Pathogens	Hospital-acquired (n=0)		Community-acquired (n=6)		Not stratified (n=0)		Total (n=6)	
	No.	%	No.	%	No.	%	No.	%
Streptococcus agalactiae	0	0	2	33.3	0	0	2	33.3
Varicella zoster virus (VZV)	0	0	2	33.3	0	0	2	33.3
Cryptococcus neoformans/gatti	0	0	1	16.7	0	0	1	16.7
Herpes Simplex Virus (HSV-1)	0	0	1	16.7	0	0	1	16.7

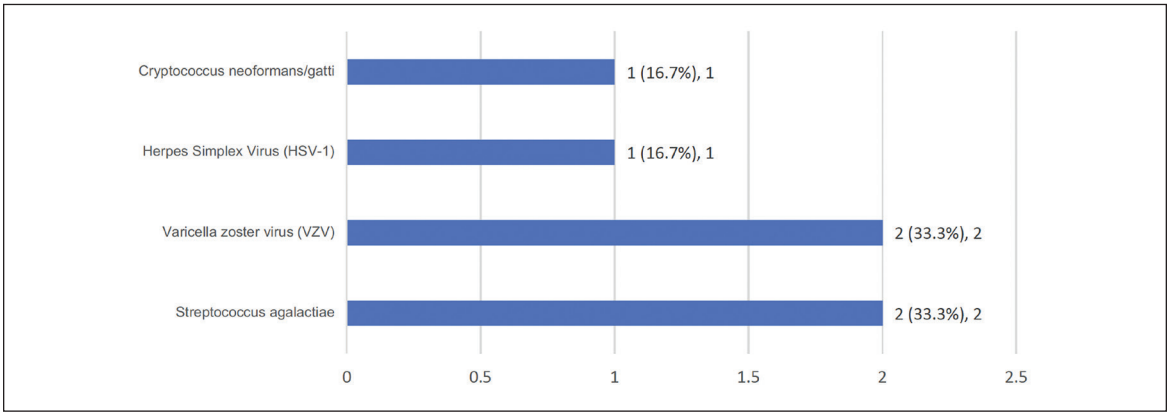

Figure 4. Frequency distribution of the most commonly detected ME Panel pathogens (all community-acquired) in Asian Hospital and Medical Center from February 2021 to May 2024 (n=6).

particularly concerning ESBL and carbapenem resistance.[7] In addition to possibly detecting nosocomial pathogens that would otherwise have been missed by culture in those already on antibiotics, the detection of resistance genes by the pneumonia panel appeared somewhat useful, as shown in the proportion of patients who had antimicrobials that were either stopped or shifted because of the panel results.

For the GI panel, 80% of the 25 was community-acquired, with the most frequent pathogens being *Escherichia coli* (enteroaggregative and enteropathogenic). The other detected pathogens were *Clostridium difficile* toxins A/B, Norovirus GI/GII, Salmonella, Campylobacter, and *Plesiomonas shigelloides*. However, the 2024 ARSP showed that the most common pathogens among bacteriologically-confirmed diarrheal diseases were *Salmonella* sp., *Vibrio cholerae*, and *Shigella* sp., which remained to have low resistance rates locally (except increasing Cotrimoxazole resistance for Vibrio).[7] The ARSP is culture-based, in contrast to the multiplex PCR results reviewed in our study. Because of the limited number of positive samples, our study adds information not on the most common GI pathogens, but rather the pathogens that may be going around in the community, causing gastrointestinal disease that may not always be detected by culture.

For the ME panel, all (however, only 6 positive results) were community-acquired, with the most commonly detected being *Streptococcus agalactiae*, Varicella zoster virus, Herpes simplex virus-1, and *Cryptococcus neoformans/gatti*. In contrast, the study done by Lagamayo and Rusia-Uy showed the most common gastrointestinal pathogens detected by multiplex PCR were *Streptococcus pneumoniae*, *E. coli*, and Enterovirus; while the 2024 ARSP showed that the most common pathogens among bacteriologically-confirmed CSF infections were *Pseudomonas* sp., *Acinetobacter baumannii*, and *Klebsiella pneumoniae* ss. *pneumoniae*.[2,7] Similarly, the interpretation of the results is limited by the small number of positive cases identified in this study, and the ARSP is culture-based, while our data demonstrates some of the pathogens that otherwise would not have been detected by culture, especially the viral pathogens.

Awareness of the etiologic agents for all four panels appeared useful in starting new antimicrobials and in continuing existing antimicrobials. But it is in the respiratory panel that the majority resulted in starting new antimicrobials (32.4%) – particularly due to starting antiviral therapy for SARS-CoV-2 and other common viral illnesses, such as Influenza, followed by continuing antimicrobials (23.1%) and remaining without antimicrobials (16.7%). In the pneumonia panel, it seemed like the majority had already started the appropriate empiric therapy since most continued with the present antimicrobials (51.7%), followed by starting (22.8%) and shifting (18.3%) antimicrobials. In the GI panel, the majority also continued with the current antibiotics (29.5%), closely followed by starting (17.6%), remaining without (17.6%), and shifting (11.8%) antimicrobials. It is in the ME panel that most started with new antimicrobials (44.5%) and stopped (33.3%) specific antimicrobials.

Table 7. Implications of Multiplex PCR Positive Results on Antimicrobial Therapy

Panel	Continue anti-microbials		Start anti-microbials		Shift anti-microbials		Stop anti-microbials		No change/ No antimicrobials		N/A*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Respiratory Panel (n=698)	161	23.1	226	32.4	3	0.4	2	0.3	117	16.7	189	27.1
Pneumonia Panel (n=180)	93	51.7	41	22.8	33	18.3	9	5	0	0.0	4	2.2
GI Panel (n=17)	5	29.5	3	17.6	2	11.8	1	5.9	3	17.6	3	17.6
ME Panel (n=9)	2	22.2	4	44.5	0	0	3	33.3	0	0.0	0	0.0
Total (n=904)	261	28.9	274	30.3	38	4.2	15	1.7	120	13.3	196	21.6

**N/A = details of antimicrobial therapy not documented in the hospital records*

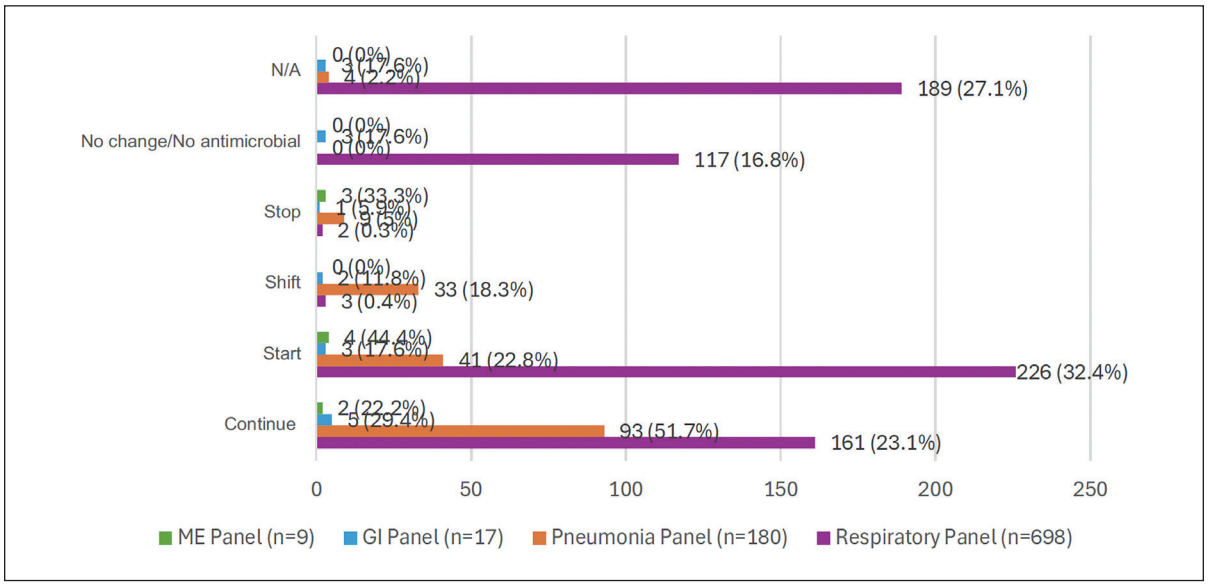

Figure 5A. Implication of Positive Multiplex PCR panel results (per panel) on the Antimicrobial therapy of patients in Asian Hospital and Medical Center from June 2020 to December 2023 (Pneumonia panel), October 2020 to December 2023 (Respiratory and GI panel), and February 2021 to May 2024 (ME panel).

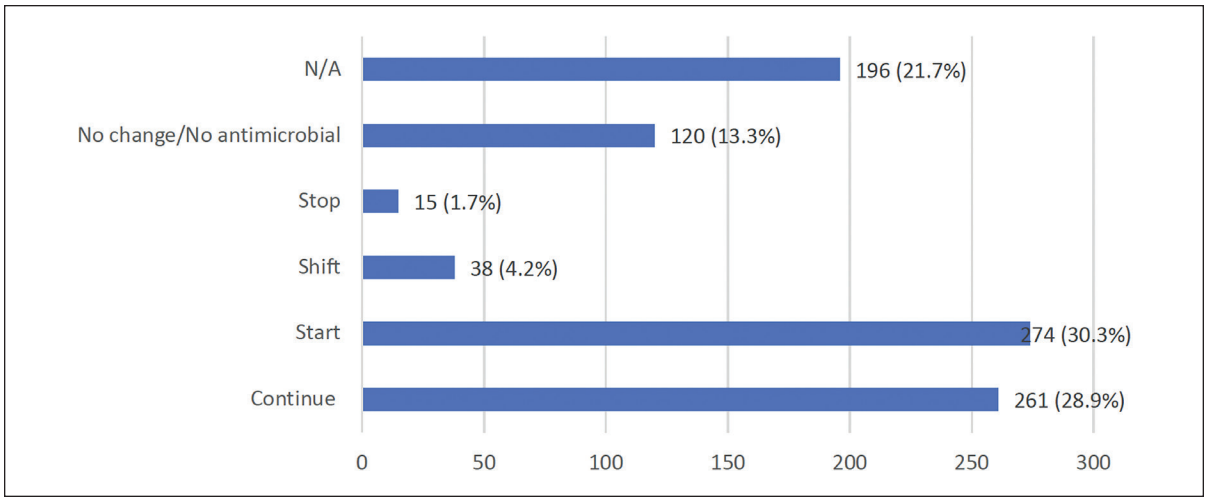

Figure 5B. Implication of Positive Multiplex PCR panel results (overall) on the Antimicrobial therapy of patients in Asian Hospital and Medical Center from 2020/2021 to 2023/2024.

CONCLUSION

Overall, the study underscores the value of multiplex PCR in enhancing diagnostic accuracy and guiding antimicrobial decision-making. The rapid identification of pathogens and resistance markers may facilitate earlier initiation of targeted therapy, reduce unnecessary antibiotic use, and support antimicrobial stewardship efforts – an urgent need in the Philippines, where empiric broad-spectrum antibiotic prescribing remains common. The observed trend of clinicians continuing, starting, shifting, or stopping antimicrobials after PCR results suggests that such diagnostic tools can meaningfully influence therapeutic choices.

The limitations of this study include its retrospective design, with data collected in a single tertiary center in Manila in 2020-2023 (for Respiratory, Pneumonia, and GI panels) and in 2021-2024 (for ME panel). The samples were stratified presumptively as either community- or hospital-acquired based on a 48-hour cut-off. The smaller sample sizes for GI and ME panels are limitations as well. The kind of antimicrobial therapy was not specified (e.g., antiviral, antibacterial, etc.). Lastly, the results of conventional cultures done simultaneously with the multiplex PCR panels were not taken into consideration – these would also have implications on antimicrobial therapy.

Because of the above limitations, future studies may consider expanding the scope of this research by including multiple tertiary and secondary hospitals in our country to allow comparison of pathogen profiles across different settings. Establishing ongoing surveillance of multiplex PCR results would help monitor emerging infectious trends and antimicrobial resistance patterns over time. Longitudinal studies linking results with patient outcomes could further clarify the clinical and economic impact of these diagnostic tools. These efforts may provide a more robust evidence base to guide antimicrobial stewardship and inform national guidelines on infectious disease management. Ultimately, the continued and judicious use of multiplex PCR testing can support timely, targeted therapy, improving patient outcomes and contributing to better public health.

Statement of Authorship

Both authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

Both authors declared no conflicts of interest.

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