ACIN
ASOCIACIÓN COLOMBIANA DE INFECTOLOGÍA

infectio

ARTÍCULO ORIGINAL

Performance of the FilmArray® Pneumonia Panel compared to bacterial culture in patients who were requested both diagnostic methods

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Abstract

Introduction: Pneumonia is one of the most frequent infections with the highest mortality and morbidity rates, and the aim of this study was to evaluate the diagnostic utility of the FilmArray® Pneumonia Panel (FPP) in the detection of a wide range of pathogens, including 15 bacterial species, in samples obtained from bronchoalveolar lavage (BAL) and orotracheal secretions (OTS).

Materials and methods: A total of 190 respiratory samples were collected from patients who underwent both FPP testing and culture. Standard laboratory procedures, including Gram staining and quantitative bacterial counting, were performed on various agar media. Microorganisms were identified using the Vitek-MS system and susceptibility testing was conducted according to the guidelines of the Clinical and Laboratory Standards Institute.

Results: The most frequently detected microorganisms were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. FPP demonstrated high sensitivity (95%) but lower specificity (48%) than culture. The panel detected more pathogens, including *Acinetobacter calcoaceticus-baumannii* and *Streptococcus agalactiae*, and identified antibiotic resistance genes KPC and VIM, providing valuable information on pathogen resistance.

Discussion: The FPP is a promising diagnostic tool for identifying respiratory pathogens and antimicrobial resistance genes in pneumonia patients. FPP demonstrated high sensitivity, which can significantly reduce the time required for diagnosis and treatment decisions. However, its lower specificity indicates that it should be used in conjunction with culture to confirm results and prevent overdiagnosis.

Keywords: Pneumonia; FilmArray Pneumonia panel; Resistance Genes; Culture

Rendimiento del Panel de Neumonía FilmArray® comparado con Cultivo Bacteriano en pacientes a los que se les solicitaron ambos métodos de diagnóstico

Resumen

Introducción: La neumonía es una de las infecciones más frecuentes con mayor tasa de mortalidad y morbilidad, y el objetivo de este estudio fue evaluar la utilidad diagnóstica del FilmArray® Pneumonia Panel (FPP) en la detección de una amplia gama de patógenos, incluidas 15 especies bacterianas, en muestras obtenidas de lavado broncoalveolar (BAL) y secreciones orotraqueales (OTS).

Materiales y métodos: Se recogieron 190 muestras respiratorias de pacientes a los que se les realizó tanto la prueba de FPP como el cultivo. Se realizaron procedimientos de laboratorio estándar, incluida la tinción de Gram y el recuento bacteriano cuantitativo, en varios medios de agar. Los microorganismos se identificaron utilizando el sistema Vitek-MS y se realizaron pruebas de susceptibilidad de acuerdo con las directrices del Clinical and Laboratory Standards Institute.

Resultados: Los microorganismos detectados con mayor frecuencia fueron *Pseudomonas aeruginosa*, *Staphylococcus aureus* y *Klebsiella pneumoniae*. El FPP demostró una alta sensibilidad (95%) pero una menor especificidad (48%) que el cultivo. El panel detectó más patógenos, incluidos *Acinetobacter calcoaceticus-baumannii* y *Streptococcus agalactiae*, e identificó los genes de resistencia a los antibióticos KPC y VIM, lo que proporcionó información valiosa sobre la resistencia a los patógenos.

Discusión: La prueba FPP es una herramienta de diagnóstico prometedora para identificar patógenos respiratorios y genes de resistencia a los antimicrobianos en pacientes con neumonía. La prueba FPP demostró una alta sensibilidad, lo que puede reducir significativamente el tiempo necesario para el diagnóstico y las decisiones de tratamiento. Sin embargo, su menor especificidad indica que debe usarse junto con el cultivo para confirmar los resultados y prevenir el sobrediagnóstico.

Palabras claves: Neumonía; Panel de neumonía FilmArray; Genes de resistencia; Cultivo

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Recibido: 05/08/2024; Aceptado: 28/01/2025

Cómo citar este artículo: J.C. Trejos-Valencia, *et al.* Performance of the FilmArray® Pneumonia Panel compared to Bacterial Culture in patients who were requested both diagnostic methods. Infectio 2025; 29(2): 86-94

Introduction

Pneumonia is one of the most frequent infections with the highest mortality and morbidity rates[1]. According to the Global Burden of Diseases (GBD) data in 2019, globally, 489 million people had lower respiratory tract infections, and 2.4 million died from this cause[2].

According to the National Administrative Department of Statistics (DANE, by its name in Spanish), pneumonia was the fifth leading cause of death in Colombia in 2020, with 5,043 deaths[3].

Owing to the morbidity and mortality associated with pneumonia, an adequate microbiological diagnosis is necessary to identify the etiology and guide the selection of appropriate and timely antibiotic treatment for the patient. The performance of conventional microbiological tests, such as bacterial culture, presents various challenges and limitations, including the time required to obtain results, the low specificity of cultures due to the presence of colonizing microbiota in the sample, and the administration of empirical antibiotic treatments prescribed by healthcare personnel or self-medication by the patient before sample collection[4].

To reduce the identification time and guide timely optimal clinical decision-making, molecular biology techniques have been implemented, which have improved the diagnostic performance in patients with pneumonia. These new polymerase chain reaction (PRC)-based tests are fast, simple, versatile, and can detect multiple pathogens within a few hours and identify genes encoding antimicrobial resistance mechanisms.

The FilmArray® Pneumonia Panel (FPP) is one of the available tests that identifies bacteria and viruses, as well as detects some microbial resistance mechanisms, in significantly less time than conventional microbiological tests. It can also detect microorganisms that routine methods may miss[5,6].

Our study aimed to evaluate the performance of FPP by comparing it with bacterial culture to detect bacterial agents and resistance genes in bronchoalveolar lavage (BAL) and orotracheal secretion (OTS) samples in patients with clinical presentation of pneumonia.

Materials and Methods

Study design and population

A diagnostic accuracy study was conducted based on the microbiology laboratory records of the Fundación Valle del Lili from June 2020 to June 2021, including patients of any age range, of both sexes, in-hospital, and out-of-hospital.

Respiratory tract samples

The study included 190 respiratory samples: A convenience sampling method was used to collect samples from patients undergoing both FPP and bacterial culture within 48 hours, ensuring that they met the inclusion criteria. The samples were collected according to the microbiological sample collection instructions of Fundación Valle del Lili. In the case of BAL, a pulmonologist was responsible for the procedure, and in the case of OTS samples, a respiratory physiotherapist was responsible. The samples were refrigerated (4-8 degrees Celsius) and received directly at the Microbiology Laboratory, where they were kept under the same conditions until processing.

Routine microbiology techniques

Samples were processed following standard laboratory procedures to detect respiratory pathogens. Gram staining was performed on the OTS samples to assess sample quality. Semiquantitative counting techniques were employed with different dilutions, based on the type of sample cultured. Respiratory samples were cultured on blood agar, chocolate agar, and MacConkey agar and incubated at 35°C in a CO2 atmosphere. Growth was monitored every 24 h for 72 h. Significant bacterial growth was defined as OTS samples ≥10^5 CFU/mL and BAL samples ≥10^4 CFU/mL. Microbiological analysis of a positive culture, growth of primary pathogens, number of identified microorganisms, bacterial count, and correlation with Gram staining were considered, following the recommendations of the American Society for Microbiology (ASM)[7].

The microorganisms were identified using a Vitek MS mass spectrometry system (bioMérieux). Minimum inhibitory concentrations for various antimicrobials were determined using Vitek-2 (bioMérieux) or MicroScan (Beckman-Coulter) following the Clinical and Laboratory Standards Institute (CLSI) guidelines[8]. If a susceptibility test showed resistance to carbapenems, the presence of carbapenemases was confirmed using the Rapidec® Carba NP test (bioMérieux). Positive results were further tested to determine the carbapenemase type using NG-Test CARBA5® (Ng Biotech).

FilmArray® Pneumonia Panel

The FPP utilizes PRC methodology for the simultaneous detection and identification of multiple respiratory viral and bacterial nucleic acids, as well as specific antibiotic resistance genes. The FilmArray TORCH system (bioMérieux) was used, according to the manufacturer's instructions. The panel menu offers the detection of 15 bacteria, three fastidious bacteria, eight viruses, and seven antimicrobial resistance genes. The bacterial reports were semi-quantitative, providing an approximation of genome copies/mL of 10^4, 10^5, 10^6, ≥10^7. The results for the resistance genes have been qualitatively reported.

Statistical analysis

The analysis was conducted using the R statistical software version 4.2.1 (R Foundation for Statistical Computing). The identification of bacterial targets by the panel was compared with the reference standard bacterial culture to calculate the sensitivity and specificity, along with the 95% confidence interval (CI) for each variable. Antibiotic resistance genes detected by the panel were compared with phenotypic methods in the culture, which required a positive concordance percentage. Results from the panel and culture were considered concordant when they identified the same microorganism. Categorical variables are described as absolute frequencies and percentages.

Ethical aspects
Approved by the Institutional Review Board/Biomedical Research Ethics Committee (Protocol No. 1904), it uses anonymous data, exempting it from informed consent per Colombia's Resolution 8430 of 1993.

Results

The study evaluated 190 lower respiratory tract samples, with 115 (60.5%) being OTS specimens and 75 (39.5%) BAL specimens. The most frequently detected microorganisms in the culture were *Pseudomonas aeruginosa* (n=27, 11.1%), *Staphylococcus aureus* (n=25, 10.3%) and the *Klebsiella pneumoniae* group (n=20, 8.2%). Other microorganisms detected in the culture included *Acinetobacter Iwoffii*, *Acinetobacter gyllenbergii*, *Burkholderia cepacia*, *Morganella morganii*, *Pseudomonas alcaligenes*, *Pseudomonas fluorescens*, *Pseudomonas mosselii*, *Stenotrophomonas maltophilia*, *Klebsiella ozaenae*, and *Pasteurella multocida*.

In the FPP, *S. aureus*, *P. aeruginosa* and the *K. pneumoniae* group were the most frequently detected microorganisms, similar to the culture. The panel detected more microorganisms than the culture, including *Acinetobacter calcoaceticus-baumannii* complex, *Moraxella catarrhalis*, and *Streptococcus agalactiae,* which were not isolated from the culture. The FPP demonstrated an overall sensitivity of 95% (CI: 89 – 99) and a specificity of 48% (CI: 38 – 59) compared with culture. (Table 1).

Next, we describe the results according to sample type.

Bronchoalveolar lavage:
FPP had 100% (CI: 84 – 100) sensitivity and 57% (CI: 42 – 70) specificity for identifying bacteria in BAL samples. The culture and the panel had concordance for the following microorganisms: *Enterobacter cloacae* complex, *Escherichia coli*, *Haemophilus influenzae*, *K. pneumoniae* group, *Proteus* spp, *P. aeruginosa*, *S. aureus* and *Streptococcus pneumoniae*.

In the culture, one bacterium was detected in 17 (77.3%) samples and two bacteria were detected in five (22.7%) samples. In contrast, FPP detected one bacterium in 28 (63.6%) samples, two bacteria in 11 (25%) samples, three bacteria in three (6.8%) samples, and four or more bacteria in one sample (2.3%). The panel detected all bacteria from the BAL cultures within its menu, and no microorganisms absent in the panel's menu were isolated from the cultures.

Forty strains were detected in the molecular panel, but not in the bacterial culture. The microorganisms detected by the panel and not isolated in the culture were: *A. calcoaceticus-*

Table 1. Performance of the FilmArray® Pneumonia Panel vs. Culture in OTS and BAL samples

Pathogen	Culture + / Panel +	Culture + / Panel -	Culture - / Panel +	Culture - / Panel -	Performance, % (95% CI)	
					Sensitivity	Specificity
Gram positive bacteria						
Staphylococcus aureus	25	0	28	137	100 (86, 100)	83 (76, 88)
Streptococcus agalactiae	0	0	13	177	-	93 (89, 96)
Streptococcus pneumoniae	4	1	7	178	80 (28, 99)	96 (92, 98)
Streptococcus pyogenes	0	1	0	189	0 (0, 97)	100 (98, 100)
Gram negative bacteria						
Haemophilus influenzae	7	0	9	174	100 (59, 100)	95 (91, 98)
Enterobacterales						
Enterobacter cloacae complex	2	1	4	183	67 (9, 99)	98 (95, 99)
Escherichia coli	3	1	10	176	75 (19, 99)	95 (90, 97)
Klebsiella aerogenes	1	1	5	183	50 (1, 99)	97 (94, 99)
Klebsiella oxytoca	2	0	3	185	100 (16, 100)	98 (95, 100)
Klebsiella pneumoniae group	20	0	25	145	100 (83, 100)	85 (79, 90)
Moraxella catarrhalis	0	0	1	189	-	99 (97, 100)
Proteus spp	2	0	6	182	100 (16, 100)	97 (93, 99)
Serratia marcescens	9	0	8	173	100 (66, 100)	96 (91, 98)
Non – Fermenting						
Acinetobacter complex calcoaceticus/baumanii	0	0	4	186	-	98 (95, 99)
Pseudomonas aeruginosa	27	0	22	141	100 (87, 100)	87 (80, 91)
Overall result	**82**	**4**	50	47	95 (89, 99)	48 (38, 59)

CI: Confidence interval; OTS: orotracheal secretion; BAL: bronchoalveolar lavage

baumannii complex (n=1), *S. agalactiae* (n=2), *H. influenzae* (n=5), *E.coli* (n=3), *S. aureus* (n=7), *S. pneumoniae* (n=2), *K. pneumoniae* group (n=3), *P. aeruginosa* (n=14) and *Serratia marcescens* (n=3). (Table 2).

The panel detected the following antibiotic resistance genes in the 75 BAL samples analyzed, in order of frequency: KPC (n=10, 13.3%), VIM (n=6, 8.0%), NDM (n=4, 5.3%), CTX-M (n=4, 5.3%), and mecA/C (n=1, 1.3%). No IMP genes were detected. Of the eight samples with significant colony growth in the culture, one sample detected a strain producing one of the carbapenemases detectable by the panel, which was reported by the test under study (*P. aeruginosa* with a VIM-type carbapenemase). In another sample with a positive culture, oxacillin-sensitive *S. aureus* was isolated, and the panel detected the same bacterium but with the presence of the mecA/C gene. In the remaining six positive culture samples: *P. aeruginosa* (n=3), *P. mirabilis* (n=2), and *K. pneumoniae* (n=1), no resistance mechanisms studied were established; either because the tests used to determine carbapenemase production and type were negative, or because the analysis of the susceptibility test results did not warrant their performance. In all these samples, the panel detected the presence of at least one gene encoding some of the resistance mechanisms detectable according to its menu, distributed among the following enzyme types: KPC (n=6), VIM (n=2), NDM (n=2), and CTX-M (n=3). (Table 3).

The panel's quantification in copies/mL was compared to the culture reports in colony-forming units (CFU). Of the 68 bacteria detected and semi-quantitatively reported by the panel, 27 (39.7%) showed a growth of ≥ 10,000 CFU/mL in the bacterial culture. The percentage of bacterial isolation in the culture increased with the number of copies/mL detected by the panel, as follows: 0% (10^4 copies/mL), 33.3% (10^5 copies/mL), 60% (10^6 copies/mL), and 88% (≥10^7 copies/mL), respectively. (Table 4).

Orotracheal secretion:

The sensitivity of FPP for identifying bacteria in OTS samples was 94% (CI: 85–98), and the specificity was 39% (CI: 24–55). Sensitivity varied from 50% to 100% by bacterial type. The culture and the panel had positive concordance for the following microorganisms: *E. cloacae* complex, *E. coli*, *H. influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *K. pneumoniae* group, *P. aeruginosa*, *S. marcescens*, *S. aureus* and *S. pneumoniae*.

In the culture, one bacterium was isolated from 50 (70.4%) samples, two bacteria from 18 (25.4%) samples, and three bacteria from three (4.2%) samples. In contrast, FPP detected one bacterium in 42 (46.2%) samples, two bacteria in 24 (26.4%) samples, three bacteria in 15 (16.5%) samples, four bacteria in eight (8.8%) samples, and five bacteria in two (2.2%) samples. The following microorganisms were isolated in the culture but were not in the panel's menu: *Pseudomonas fluorescens*, *Stenotrophomonas maltophilia*, *Morganella morganii*, *Klebsiella ozaenae*, *Acinetobacter gyllengergii*, *Pseudomonas mosselii*, *Pasteurella multocida*, and *Burkholderia cepacia*.

The panel detected 104 strains that were not isolated from the bacterial cultures. The microorganism with the greatest difference between the panel and the culture was *K. pneumoniae* (n=22, 19.1%). (Table 5).

Table 2. Performance of the FilmArray® Pneumonia Panel vs. Culture in BAL

Pathogen	Culture + / Panel +	Culture + / Panel -	Culture - / Panel +	Culture - / Panel -	Performance, % (95% CI)	
					Sensitivity	Specificity
Acinetobacter complex calcoaceticus/ baumanii	0	0	1	74	-	99 (93, 100)
Enterobacter cloacae complex	1	0	0	74	100 (2, 100)	100 (95, 100)
Escherichia coli	1	0	3	71	100 (2, 100)	96 (89, 99)
Haemophilus influenzae	2	0	5	68	100 (16, 100)	93 (85, 98)
Klebsiella aerogenes	0	0	0	75	-	100 (95, 100)
Klebsiella oxytoca	0	0	0	75	-	100 (95, 100)
Klebsiella pneumoniae group	4	0	3	68	100 (40, 100)	96 (88, 99)
Moraxella catarrhalis	0	0	0	75	-	100 (95, 100)
Proteus spp	2	0	0	73	-	97 (91, 100)
Pseudomonas aeruginosa	8	0	14	53	100 (63, 100)	79 (67, 88)
Serratia marcescens	0	0	3	72	-	96 (89, 99)
Staphylococcus aureus	8	0	7	59	100 (63, 100)	88 (78, 95)
Streptococcus agalactiae	0	0	2	73	-	97 (91, 100)
Streptococcus pneumoniae	1	0	2	72	100 (2, 100)	97 (91, 100)
Streptococcus pyogenes	0	0	0	75	-	100 (95, 100)
Overall result	21	0	23	30	100 (84, 100)	57 (42, 70)

CI: Confidence interval; BAL: bronchoalveolar lavage

Table 3. Comparison of Detection of Resistance Mechanisms and Genes: FilmArray® Pneumonia Panel vs. Culture in BAL

Culture result	DPC[1] test	DTC[2] test	panel
P. aeruginosa carbapenem resistant	Positive	VIM	VIM
S. aureus OXA sensitive	N/A	N/A	mecA/C
P. aeruginosa carbapenem resistant	Negative	Negative	KPC + VIM
P. mirabilis IMI resistant (Natural resistance)	Not done	Negative	KPC NDM VIM
P. aeruginosa IMI, MEM resistant (ceftazidime sensitive)	Not done	Not done	KPC
P. mirabilis IMI resistant (Natural resistance)	NR[3]	NR[3]	VIM
P. aeruginosa sensitive	N/A	N/A	KPC + CTX-M
K. pneumoniae sensitive	N/A	N/A	NDM

N/A: not applicable; BAL: bronchoalveolar lavage.
[1] – Carbapenemase production determination.
[2] - Carbapenemase type determination.
[3] – Natural resistance.

The panel detected the following antibiotic resistance genes in the 115 tracheal aspirate samples analyzed, in order of frequency: KPC (n=10, 8.7%), VIM (n=6, 5.2%), NDM (n=9, 7.8%), CTX-M (n=6, 5.2%), and IMP (n=3, 2.6%). No mecA/C genes were detected.

In five samples that showed significant colony growth in the OTS, a carbapenemase-producing strain was isolated according to the test used for its detection. In all of these cases, FPP also detected at least one carbapenemase. In two of these strains, immunochromatographic tests were performed to determine the type of carbapenemase, which yielded results for IMP and NDM. The results matched the panel's detection of NDM in a *K. pneumoniae* strain. For the IMP present in a *P. aeruginosa* isolate, in addition to detecting this gene, the presence of KPC and VIM was also detected in the sample. In the other three strains where the panel detected carbapenemase, the test to determine its type was not performed on the isolated strain from the culture, as it was clinically considered that the patients did not have a bacterial superinfection. The panel detected carbapenemases in two samples (KPC and NDM), whereas in the bacteria isolated from the cultures (*K. pneumoniae* and *S. marcescens*), susceptibility tests did not establish resistance to carbapenems. In another sample, the isolated *P. aeruginosa* strain showed resistance to carbapenems, but the test to determine the presence of carbapenemases was negative. (Table 6).

Correlation between the panel results and the bacterial count in the culture

The results expressed in copies/mL from the panel were compared with the quantification category of the cultures reported in CFU. Of the 178 bacteria detected and semi-quantitatively reported by the panel, 74 (41.6%) showed a growth of ≥ 100,000 CFU/mL in the bacterial culture. The percentage of bacterial isolation in the culture increased with the number of copies/mL detected by the panel, as follows: 3.1% (10^4 copies/mL), 17.9% (10^5 copies/mL), 34.2% (10^6 copies/mL), and 76.8% (≥10^7 copies/mL), respectively. (Table 7).

Discussion

Respiratory infections, including pneumonia, are caused by various pathogens, making it crucial to accurately and promptly identify causative agents to ensure appropriate

Table 4. Percentage of bacteria detected by the FilmArray® Pneumonia Panel according to number of Copies/mL vs. Bacterial Isolation in BAL Culture ≥ 10,000 CFU/mL

Copies/mL bacteria	Panel (n)	Culture (n)	%	Microorganisms' growth in culture ≥ 10.000 CFU/mL
10^4 copies/mL	27	0	0%	No
10^5 copies/mL	9	3	33%	*Enterobacter cloacae complex* *Pseudomonas aeruginosa* *Staphylococcus aureus*
10^6 copies/mL	15	9	60%	*Staphylococcus aureus* *Klebsiella pneumoniae group* *Haemophilus influenzae* *Pseudomonas aeruginosa*
≥ 10^7 copies/mL	17	15	88%	*Klebsiella pneumoniae group* *Escherichia coli* *Staphylococcus aureus* *Proteus spp.,* *Pseudomonas aeruginosa* *Streptococcus pneumoniae* *Haemophilus influenzae*
Total	68	27		

BAL: bronchoalveolar lavage.

Table 5. Performance of FilmArray® Pneumonia Panel vs. Culture in OTS

Pathogen	Culture + / Panel +	Culture + / Panel -	Culture - / Panel +	Culture - / Panel -	Performance, % (95% CI)	
					Sensitivity	Specificity
Acinetobacter complex *calcoaceticus/baumanii*	0	0	3	112	-	97 (93, 99)
Enterobacter cloacae complex	1	1	4	109	50 (1, 99)	96 (91, 99)
Escherichia coli	2	1	7	105	67 (9, 99)	94 (88, 97)
Haemophilus influenzae	5	0	4	106	100 (48, 100)	96 (91, 99)
Klebsiella aerogenes	1	1	5	108	50 (1, 99)	96 (90, 99)
Klebsiella oxytoca	2	0	3	110	100 (16, 100)	97 (92, 99)
Klebsiella pneumoniae group	16	0	22	77	100 (79, 100)	78 (68, 86)
Moraxella catarrhalis	0	0	1	114	-	99 (95, 100)
Proteus spp	0	0	6	109	-	95 (89, 98)
Pseudomonas aeruginosa	19	0	8	88	100 (82, 100)	92 (84, 96)
Serratia marcescens	9	0	5	101	100 (66, 100)	95 (89, 98)
Staphylococcus aureus	17	0	20	78	100 (80, 100)	80 (70, 87)
Streptococcus agalactiae	0	0	11	104	-	90 (84, 95)
Streptococcus pneumoniae	3	1	5	106	75 (19, 99)	95 (90, 99)
Streptococcus pyogenes	0	1	0	114	-	100 (97, 100)
Overall result	**61**	**4**	**27**	**17**	**94 (85, 98)**	**39 (24, 55)**

CI: Confidence interval; OTS: orotracheal secretion.

treatment that benefits the patient. Etiological diagnosis is essential, and cannot be performed without laboratory tests. Conventional methods such as bacterial culture and Gram staining have limitations in sensitivity and specificity owing to the polymicrobial nature and normal microbiota of respiratory samples[9]. To overcome these limitations, molecular techniques have been introduced, such as FPP, which allows for the identification and detection of microbial resistance mechanisms in significantly less time than traditional microbiological tests[10].

Our study evaluated 190 samples by comparing the culture results with those of FPP. The most frequently detected microorganisms (by both the panel and culture) *P. aeruginosa* (11.1%), *S. aureus* (10.3%) and the *K. pneumoniae* group (8.2%). In a 2021 study comparing the standard diagnostic test with the panel under study, the most frequently detected bacteria were *S. aureus* (21.15%), *H. influenzae* (19.69%) and *P. aeruginosa* (15.63%). The results differ in our study, although there are similarities in the detection of certain pathogens[11].

According to our results, the overall sensitivity and specificity of the FPP were 95% and 48%, respectively; in BAL samples, they were 100% and 57%, respectively; and in the OTS samples, the figures were 94% and 39%, respectively. In a study in 2020, Yoo et al. evaluated FPP in 31 sputum samples and 69 endotracheal aspirate samples and found an overall sensitivity of 98.5% and specificity of 76.5%[12]. Another study, in 2020, conducted by Edin et al. on lower respiratory tract specimens (n=88) found that the FPP had 100% positive concordance, with two false negatives of uncertain clinical significance[13]. In 2021, prospective studies in South Africa and France assessed the panel's effectiveness; South Africa reported a sensitivity of 92.0% and specificity of 93.8% in 59 lower respiratory tract specimens, while France found a positive agreement of 94.4% and a negative agreement of 96.0% in 217 endotracheal aspirate samples and 240 bronchoalveolar lavage samples[14,15] Srivastava et al. analyzed 162 respiratory samples (sputum, endotracheal aspirate, and bronchoalveolar lavage) in northern India, finding results very similar to those found in our study: positive concordance of 100% and a negative concordance of 47.8%[16].

Table 6. Comparison of detection of Resistance Mechanisms and Genes: FilmArray® Pneumonia Panel vs. OTS Culture

Culture growth	DPC[1] test	DTC[2] test	Panel
K. pneumoniae carbapenem resistant	Positive	NDM	NDM
P. aeruginosa carbapenem resistant	Positive	IMP	IMP + KPC+ VIM
K. pneumoniae carbapenem resistant	Positive	Not done	CTXM+KPC
K. pneumoniae carbapenem resistant	Positive	Not done	CTXM+KPC
P. aeruginosa carbapenem resistant + *P. mirabilis* + *K. ozaenae* sensitive	Positive	Not done	CTXM + KPC+ VIM + NDM
P. aeruginosa carbapenem resistant	Negative	Not done	NDM
K. pneumoniae carbapenem sensitive	N/A	N/A	KPC
S. marcescens carbapenem sensitive	N/A	N/A	NDM

N/A: not applicable; OTS: orotracheal secretion.
[1] – Carbapenemase production determination.
[2] - Carbapenemase type determination.

Table 7. Percentage of Bacteria Detected by the FilmArray® Pneumonia Panel according to the number of Copies/mL vs. Bacterial Isolation in OTS
Culture ≥ 100,000 CFU/mL

Copies/mL bacteria	Panel (n)	Culture (n)	%	Microorganisms' growth in culture ≥ 100.000 CFU/mL
10^4 copies/mL	32	1	3.1%	*Klebsiella pneumoniae* group
10^5 copies/mL	39	7	17.9%	*Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Serratia marcescens* *Pseudomonas aeruginosa*
10^6 copies/mL	38	13	34.2%	*Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae group* *Serratia marcescens* *Staphylococcus aureus* *Proteus spp.* *Pseudomonas aeruginosa* *Escherichia coli*
≥ 10^7 copies/mL	69	53	76.8%	*Enterobacter cloacae complex* *Klebsiella pneumoniae group* *Haemophilus influenzae* *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Streptococcus pneumoniae*
Total	178	74		

In our study, we only considered microorganisms present in the panel menu. Fifty-four specimens showed discrepant results between the culture and FPP, with some bacterial strains being detected only by the molecular method. Some bacterial isolates, such as *S. agalactiae* (n=13), *M. catarrhalis* (n=1), and *A. calcoaceticus/baumanii complex* (n=4), were detected in the panel but did not grow in the culture. These findings account for the low specificity observed in this study. The opposite also occurred with the recovery of strains in the culture that were not detected by the panel because they were not included in the menu. We must consider that the samples analyzed using the panel were not subjected to a dilution process, as was done when using the bacteriological culture, as recommended by the Lower Respiratory Tract Infection Diagnosis Guidelines[17], with the aim of reducing the probability of isolating and reporting bacteria that could be part of the normal respiratory microbiota and not responsible for the infectious syndrome.

The FPP semi-quantitatively reports bacterial pathogens (excluding fastidious bacteria, misleadingly termed "atypical") in copies/mL, ranging from 10^4 to ≥ 10^7. The test manufacturer does not specify a threshold value above which bacterial detection can be interpreted as potentially clinically relevant and/or below which it might be part of the normal respiratory microbiota. The test insert states: "Semi-quantitative range results (copies/mL) generated by the FilmArray® Pneumonia Panel are not equivalent to CFU/mL and are not consistently correlated with the amount of bacterial analytes compared to CFU/mL. For samples with multiple detected bacteria, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria determined by culture (CFU/mL). Clinical correlation is advised to determine the importance of the semi-quantitative range (copies/mL) for clinical management"[18]. To contribute to the knowledge related to this topic, we calculated the percentage of bacteria detected by the FPP based on the number of copies/mL reported versus the isolation of bacteria in the culture by sample type, expressed in CFU/mL. As expected, the probability of isolating bacteria in the culture increased as the number of copies/mL detected by the panel increased. For the BAL and OTS samples, 88% and 76.8% of bacteria detected by the panel from 10^7 copies/mL were isolated in the culture with a count of ≥ 10,000 CFU/mL and ≥ 100,000 CFU/mL, respectively. Yoo et al. revealed that 88.2% of the identified bacteria (67/76) with ≥10^6 copies/mL also yielded positive culture results with significant quantities of bacteria[12]. Kamel et al. reported that the total number of bacteria detected by the panel at concentrations of 10^4, 10^5, 10^6, and 10^7 copies/mL were 36, 13, 15, and 33, respectively, whereas culture methods revealed a total of 2, 8, 7, and 29 bacteria at concentrations of 10^3, 10^4, 10^5, and 10^6 CFU/mL, respectively[19]. Buchan et al. reported that all 27 bacterial isolates with > 10^5 CFU/mL in the culture were reported as 10^5, 10^6, or 10^7 copies/mL by the panel (100% concordance), with 85.2% (23/27) reported as 10^7 genomic copies[10]. Kosai et al. noted that the panel semi-quantitatively detected higher copies (≥ 10^6 copies/mL) of bacterial targets when they were positive using the culture method[20]. A 2023 meta-analysis examined the relationship between the

detection thresholds of the FPP in copies/mL and bacterial culture results in CFU/mL. Some studies have explored this correlation and observed that higher thresholds of the panel (10^6, 10^7) were associated with elevated levels of bacteria in cultures with similar thresholds. However, for the lower thresholds of the panel (10^4, 10^5), the panel's detection level was often approximately one logarithm higher than that in the culture [21]. Distinguishing between colonizing organisms and pathogens persists because bacterial levels below the culture threshold can result in false positives in the FPP. Further research is needed to establish a consensus threshold for clinical interpretation to avoid unnecessary antibiotic treatments.

Owing to the limited number of strains isolated and the possible resistance mechanisms and genes that the panel could detect, statistically significant conclusions were not possible. In the three strains with phenotypically established carbapenemases, the panel correctly detected the type. In other cases, there was no concordance, or the presence of a resistance mechanism was not evaluated, either because the strain did not exhibit a resistance pattern suggesting the presence of carbapenemase or because it was not attempted to identify it, considering the bacterium to be a colonizer based on the patient's clinical conditions.

In 2020, Yoo et al. found that the panel detected 25 resistance genes in 22 specimens[12]. Murphy et al. confirmed that this panel could provide preliminary antimicrobial susceptibility data by detecting genes such as mecA/mecC and CTX-M-type extended-spectrum beta-lactamases (ESBL) [22]. Camélé-na et al. detected resistance markers such as mecA/C, CTX-M, and VIM[23]. It is important to mention that the panel could not directly link a detected resistance gene to a specific strain. The gene may belong to an undetectable strain or be unexpressed, leading to negative sensitivity test results.

It is clear that the main "uncertainty" related to the interpretation of FFP results stems from bacteria detected in the 10^4 or 10^5 copies/mL ranges. In one of the studies reviewed, it was mentioned that the semi-quantitative values (copies/mL) from the Pneumonia Panel are on average approximately 1 log 10 higher than the values reported in culture (CFU/mL). Therefore, bacteria detected at a concentration of 10^4 copies/mL could represent counts of 10^3 CFU/mL in culture, being non-representative according to current guidelines[10]. Based on this, the results would be significant in the case of LBA samples, with counts ≥ 10^5 copies/mL, and in SOT samples with counts ≥ 10^6 (if counts ≥10^5 CFU/mL are considered significant) or ≥10^7 (in case counts ≥10^6 CFU/mL are considered significant). We clarify that this is only a proposal since there is no research to date to support it. We must consider that, as mentioned in the research on the subject, the interpretation of "false positive" results, understood as those in which the FPP detected a microorganism not present in the culture, may be due to various factors, ranging from the administration of antibiotics before taking the sample to the process of proper interpretation of the culture. In addition, we must consider comparing a more sensitive test (PCR) with another with a lower level of sensitivity (culture). An individualized analysis is recommended, sometimes involving health professionals responsible for patient's care [22].

The main limitations of the study were its small sample size and retrospective nature. The condition of prior empirical antibiotic treatment before sample collection was not established, which can significantly alter the sensitivity of bacterial culture but not detection by the molecular panel.

Further research is required to determine the clinical significance of bacteria detected at 10^4 or 10^5copies/mL but not isolated in culture, to guide antibiotic treatment decisions.

In conclusion, the current diagnostic strategies use various tests to identify pathogens. While bacterial culture is the "gold standard," its results depend on sample quality, are resource-intensive, and can take days. Its specificity is also questionable, owing to the sample type. The FPP can detect key bacterial agents and resistance genes simultaneously, reducing the reporting time from days to hours, making it valuable for the diagnosis and timely treatment of lower respiratory infections.

The FilmArray® Pneumonia panel demonstrated high sensitivity for detecting both bacteria and antimicrobial resistance genes in BAL and OTS samples. The low specificity suggests that the test may produce false positives, potentially leading to incorrect diagnoses and unnecessary treatment. Semi-quantitative results were expressed in copies/mL, which is useful for identifying the burden of microorganisms. It is essential to analyze the results of the panel in conjunction with those obtained from the cultures to evaluate the relative importance of the bacteria isolated and/or detected, and the results of phenotypic susceptibility and/or resistance genes detected.

Ethical considerations

Protection of persons. The authors state that no human or animal experiments were performed in this article.

Protection of vulnerable populations. The authors declare that there is no vulnerable population in this research.

Confidentiality. The authors declare that they have followed their facility's protocols on the publication of patient data.

Privacy. The authors declare that no patient data appear in this article.

Financing. This work did not receive funding from any public, private, commercial or non-profit institution.

Conflict of interests. The authors declare that they have no conflict of interest.

Acknowledgments. The authors thank Centro de Investigaciones Clínicas, microbiology lab personnel, and the patients and their families. The authors would also like to thank Dr. Juan P. Díaz-Solórzano M.D., for providing language editing for this manuscript.

Authors´ contribution. JC and JA contributed to the research conceptualization, JL and HJ performed the data analysis, and JC, JA and HJ wrote the draft and final manuscript. All authors contributed to read and approved the version of the submitted manuscript.

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