American Journal of Infectious Diseases

Research Article

Impact of Multiplex PCR Panel on Reducing Broad-Spectrum Antibiotic Use in Ventilator-Associated Pneumonia

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Article history
Received: 23-01-2025
Revised: 11-04-2025
Accepted: 09-05-2025

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Abstract: The extended use of broad-spectrum antimicrobials contribute to the growing threat of antimicrobial resistance. This study evaluated the impact of a multimodal strategy aiming at reducing the duration of broad-spectrum antimicrobial therapy in Ventilator-Associated Pneumonia (VAP). Conducted in a single Intensive Care Unit (ICU), this quasi-experimental, retrospective study compares a pre-intervention period (08/01/2018-07/01/2019) with an intervention period (08/01/2022-07/01/2023). Adult patients receiving antimicrobials for suspected VAP with positive respiratory cultures were included, 35 and 53 VAP events in the pre-intervention and intervention period, respectively. The intervention combined a diagnostic and treatment algorithm with a multiplex PCR Pneumonia Panel (PnP), education for the ICU staff, and enhanced communication with a multidisciplinary consultation group. The median time from antibiotic prescription to modification decreased from 76 h (IQR 63-100) to 21 h (IQR 8-31), a reduction of 55 hours (95% CI -67, -42; p<0.001). The time difference for each of the most used broad spectrum antimicrobials, vancomycin, carbapenems and colistin, was not statistically significant (-28 hours [95% CI-64, 8; p = 0.129], -3.4 hours [95% CI -51 , 44; p = 0.888] and -25 hours [95% CI-56, 5; p = 0.104] respectively).The PnP demonstrated high specificity (100% [95% CI 92.3-100]) and negative predictive value (98.2%) for methicillin-resistant Staphylococcus aureus with a sensitivity of 83.3% (95% CI 35.9-99.6), and moderate performance for extended-spectrum beta-lactamase detection with a sensitivity of 62.5% (95% CI 24.5-91.5), specificity of 84.1% (95% CI 69.9 - 93.4) and negative predictive value of 88.2%. This study demonstrates that a multimodal strategy with a rapid diagnostic method, education and improved communication can significantly reduce the duration of empirical broad-spectrum antimicrobials in critical patients treated for VAP.

Keywords: Antimicrobial Stewardship, Pneumonia, Pneumonia Panel, Rapid Diagnostic Tests, Multiplex

Introduction

Antimicrobial Resistance (AMR) is recognized by the World Health Organization (WHO) as a critical global health threat (Lee *et al.*, 2016). A recent study published in The Lancet Regional Health Americas estimated 569,000 deaths associated with and 141,000 deaths directly attributable to, bacterial AMR across 35 countries within the WHO Region of the Americas in 2019 (Antimicrobial Resistance Collaborators, 2023). Lower respiratory tract infections, particularly Ventilator-Associated Pneumonia (VAP), are significant contributors to this burden, driving substantial antimicrobial consumption in Intensive Care Units (ICUs) (Antimicrobial Resistance Collaborators, 2023; European Centre for Disease Prevention and Control,

2023). VAP accounts for nearly 50% of antimicrobial use in critical care, often necessitating broad-spectrum antibiotics, particularly during empirical therapy, which further promotes the development of AMR (Bergmans *et al.*, 1997; De Waele *et al.*, 2018).

Although AMR poses a universal threat, its impact is particularly severe in critically ill patients, who face higher mortality rates due to bacterial resistant infections (Brusselaers *et al.*, 2011). Rapid diagnostic methods have emerged as promising tools to optimize antimicrobial use by facilitating early pathogen identification and enabling timely antimicrobial de-escalation. However, the impact of these methods on antimicrobial use remains inconclusive, as current evidence has failed to demonstrate a statistically significant difference in antibiotic use in ICU patients with pneumonia in a real life setting (Miller *et al.*, 2023). Furthermore, evidence from resource-constrained settings, such as Latin America, is limited. The effectiveness of these methods likely depends on their integration into an Antimicrobial Stewardship Program (ASP) involving close collaboration between microbiologists, pharmacists, physicians and Infectious Disease (ID) specialists. Evaluating the impact of these diagnostic tools is essential to determine their role in reducing broad-spectrum antimicrobial use, particularly in low- and middle-income countries where AMR rates are higher (Antimicrobial Resistance Collaborators, 2023).

The integration of rapid diagnostic methods within Antimicrobial Stewardship Programs (ASPs) holds considerable promise. For instance, Buchan *et al.* (2020) estimated that empirical antimicrobial regimens could be modified in 70% of cases, resulting in escalation or discontinuation in 48.2% of those cases and a reduction of 6.2 antibiotic days per patient. In another study conducted in a pediatric ICU, while test results suggested potential antibiotic changes in 80% of cases, changes were only implemented in 46% of cases, with escalation being more frequent than de-escalation or discontinuation (Plattner *et al.*, 2024). A study by Soloaga *et al.* further highlighted the utility of Pneumonia Panels (PnPs), demonstrating antibiotic modifications in 74.6% of 194 respiratory samples tested, although the time to antimicrobial adjustment was not specified (Soloaga *et al.*, 2021). Many of these studies have focused on the potential benefits of rapid diagnostic methods but have not demonstrated a statistically significant reduction in the duration of exposure to broad-spectrum antimicrobials.

This study aims to assess the impact of a multimodal strategy incorporating rapid diagnostic methods, educational interventions and enhanced interdisciplinary communication, on the duration of empirical broad-spectrum antimicrobial therapy in VAP patients in an ICU setting. We hypothesized that these interventions would reduce the duration of broad-spectrum antimicrobial use during empirical treatment in patients with VAP.

Materials and Methods

Study Design

This quasi-experimental study was conducted in a 38 bed ICU of an acute care hospital. It compared two time periods: Pre-intervention (Miller *et al.*, 2023) and intervention (Miller *et al.*, 2023). Data from the COVID-19 pandemic period (March 2020–March 2022) was excluded due to disruptions in diagnostic and treatment protocols. The intervention included a new diagnostic and treatment algorithm for suspected VAP, featuring the FilmArray multiplex PCR system, an educational program and enhanced communication with a multidisciplinary consultation group.

Study Population

The study enrolled adult ICU patients with suspected VAP and positive respiratory cultures. Inclusion criteria were: Mechanical ventilation ≥48 h, antibiotic use for VAP during ≥72 h and criteria consistent with VAP (a new pulmonary infiltrate or progression of a previous infiltrate and at least two of the following: Fever [>38°C], leukocytosis [>12,000/mm³], leukopenia [<4,000/mm³], purulent sputum, or impaired gas exchange or increased oxygen requirement or ventilatory demand).

VAP did not represent the primary or present reason for admission (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9-CM], code [481-486, 997.31]); this approach was used to prevent inclusion of subjects with community-onset pneumonia. The study included patients with VAP episodes. In some cases, patients were included more than once if they experienced another VAP episode during the study period.

Patients with community-acquired pneumonia, those under 18 years old, or those treated for infections other than VAP were excluded, as well as those under palliative care and restriction of therapeutic measures.

Procedures and Data Collection

The hospital's microbiology laboratory operates on a 24 h on-call service, processing respiratory samples daily. Preliminary culture results are reported to the ID team within 48 h, while definitive results are available within 96-144 h, depending on sample complexity, particularly in polymicrobial cases. Respiratory specimens are excluded from culture if they contain <25 leukocytes and ≥10 epithelial cells per field. The BioFire FilmArray Pneumonia Panel (PnP), a multiplex PCR diagnostic method used in this study, detects genetic material from 26 microorganisms and 7 antimicrobial resistance markers, facilitating rapid identification of

pathogens and resistance mechanisms for therapeutic decision-making in pneumonia cases. An initial verification process was conducted to validate the multiplex PCR assay, as described herein. Due to institutional limitations in access to alternative viral diagnostic methods, direct comparative analysis for viral targets was not feasible. However, bacterial detection was validated through parallel culture testing.

The hospital's ASP ensures appropriate use of antimicrobials in accordance with hospital guidelines for the treatment of infectious diseases. This program is led by the Infectious Diseases (ID) team and incorporates daily educational interventions to address deviations from these guidelines.

In the critical care units, patients prescribed broad-spectrum antimicrobials classified as "Watch" or "Reserve" under the WHO AWaRe classification are actively monitored (World Health Organization, 2023). These antimicrobials include 4th-generation cephalosporins, vancomycin, carbapenems, ceftazidime-avibactam, ceftolozane-tazobactam, ceftaroline, colistin and linezolid. The ID team conducts in-person consultations for 40 h per week, during weekdays, to review and optimize antimicrobial therapy. Prior to this study, in the time frame outside these hours, consultations were facilitated through a paging system.

The hospital’s empirical antimicrobial guidelines for Ventilator-Associated Pneumonia (VAP), based on local microbiological data, during the study period, outlined the following: Ampicillin-sulbactam for VAP within ≤3 days since admission, cefepime plus colistin for 4–6 days since admission, meropenem plus colistin for ≥7 days since admission.

As part of a comprehensive multimodal strategy, the study implemented several targeted interventions to improve the management of suspected VAP: Development of a diagnostic and treatment algorithm aimed at standardizing diagnostic workflows and initiating antimicrobial therapy promptly and appropriately (Appendix 1). Integration of a rapid diagnostic method, the PnP, enabling early identification of causative pathogens and resistance markers to guide therapy. Establishment of a permanent, multidisciplinary consultation group, accessible 24/7 via a mobile application. This team included microbiologists, pharmacists, intensive care physicians and ID specialists.

To facilitate adoption, the study group conducted an educational meeting with the ICU team in July 2022 to present the algorithm and discuss its practical implementation. The interventions were overseen and executed by a multidisciplinary team comprising infectious disease specialists, intensive care physicians and microbiologists. The Case Report Form (CRF) captured baseline and demographic characteristics of the study participants, including age, gender, Charlson Comorbidity Index score at ICU admission and immunosuppression status (Appendix 2). Microbiological findings were recorded for both the Pneumonia Panel (PnP) and standard or conventional methods such as cultures. The conventional practice included mass spectrometry through the VITEK MS platform (BioMerieux). The team of microbiologists used a variety of methods to determine the sensitivity of microorganisms to antimicrobials, including the Vitek 2C system (Biomerieux, Marcy, l'Etoile, France) and the agar diffusion technique (Bauer & Kirby technique).

The Infectious Diseases (ID) team interpreted the rapid diagnostic test (PnP) results, with a threshold of $\geq 10^4$ copies/mL considered indicative of a relevant pathogen load. Results below this threshold were interpreted as colonization or low-level presence, potentially of clinical significance. ID physicians integrated these results with clinical correlation, considering patient symptoms and signs, radiographic findings, hypoxemia, fraction of inspired oxygen and other laboratory data (e.g., white blood cell count). Following the interpretation of the results, the Infectious Diseases (ID) team provided their recommendation to the ICU physicians. Broad-spectrum antimicrobials were discontinued and changed, if feasible and according to the PnP results, to the narrowest-spectrum antimicrobial approved by facility guidelines for Ventilator-Associated Pneumonia (VAP). These modifications, implemented by the ID team in adherence to institutional and Antimicrobial Stewardship Program (ASP) recommendations, were deemed appropriate.

The prescribed antimicrobial treatments were categorized as broad-spectrum for VAP, as outlined in the study methodology. Additionally, data points were collected on critical time intervals: Initiation of each antimicrobial, sample collection, laboratory check-in, preliminary and definitive microbiological results and subsequent antimicrobial de-escalation, escalation, or discontinuation.

The study team also assessed adherence to the intervention protocol among patients in the intervention period. This evaluation included compliance with the ID suggestions for antimicrobial change for those with positive respiratory bacterial cultures and antimicrobial prescriptions for suspected VAP. Mortality outcomes were analyzed for both the pre- and intervention periods to identify any potential changes.

All study data were retrieved from the hospital’s centralized, single Electronic Health Record (EHR) system. This system consolidates a comprehensive range of patient information, including administrative data (e.g., test requests, appointment scheduling, drug usage) and clinical records (e.g., diagnoses, medical progress notes, laboratory results). Patient health problems and comorbidities were automatically coded using a terminology server integrated with a local thesaurus,

which maps and encodes data with SNOMED CT to ensure standardized representation.

Outcomes

The primary outcome was the time to discontinuation of broad-spectrum antimicrobials for VAP when applying a new diagnostic and treatment algorithm. Secondary outcomes included time to discontinuation of specific broad-spectrum antimicrobials (vancomycin, meropenem, colistin), concordance between PnP and cultures for Methicillin Resistant *Staphylococcus Aureus* (MRSA) and Extended-Spectrum Beta-Lactamases (ESBLs), adherence to the protocol and mortality rates in both study periods.

Data Analysis

Baseline characteristics were described using medians and Interquartile Ranges (IQRs) for quantitative variables, based on their observed distribution. Categorical variables were summarized as absolute frequencies and percentages. Comparisons of qualitative variables were conducted using the Chi-square test, while quantitative variables were analyzed using either the 2-sample t-test or the Mann-Whitney U test, depending on their distribution. Statistical significance was set at a two-tailed p-value of <0.05.

For the primary outcome, median times between the two cohorts were analyzed using a mixed-effects linear regression model. This model accounted for repeated measures among patients with multiple VAP episodes. Due to the data's inherent hierarchical structure, the study's objectives and the model's capacity to manage clustered and correlated data, a mixed-effects linear regression model was employed to analyze the primary outcome, enabling a robust and precise comparison of median times between the two cohorts. A multivariate model was also constructed, adjusting for potential confounding factors, including immunosuppression, the number of ventilator days at the time of empirical antibiotic prescription, the day of the week, whether the prescription occurred during the weekend and the presence of holidays within the subsequent 48-72 h.

For the analysis of the total duration of antibiotic therapy with vancomycin, meropenem and colistin, mixed-effects linear regression models were similarly employed.

The diagnostic performance of the PnP was assessed by calculating its sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for detecting mecA-mediated methicillin resistance and Extended-Spectrum Beta-Lactamases (ESBL) compared to standard cultures. These calculations were based on the prevalence rates observed for methicillin-resistant Staphylococcus aureus (10%) and ESBL-producing organisms (23%) in the studied population.

Ethical Aspects

The study was approved by the Ethics Committee of Hospital Italiano de Buenos Aires, Argentina. It was conducted in strict compliance with both national and international regulations, including the Declaration of Helsinki by the World Medical Association and the International Council for Harmonisation (ICH) E6 Good Clinical Practice Guidelines. All study data were handled with the utmost confidentiality, ensuring anonymity and de-identification, with access strictly limited to authorized personnel for research purposes. This process adhered to the National Law for the Protection of Personal Data 25.326 (Habeas Data Law), which governs data privacy and security in Argentina.

The research team declares no conflicts of interest related to the objectives of this study. Furthermore, no external funding or equivalent financial support was received for the study. The planning, execution, analysis and reporting of the study and its findings were conducted independently.

Fundings

The study was supported by 90 FilmArray PnPs provided by Biomerieux Laboratory, which had no role in study design or execution. Other expenses were covered by the hospital's Infectious Diseases.

Results

Baseline Characteristics

A total of 170 suspected VAP events were evaluated, with 90 episodes during the pre-intervention period and 80 during the intervention period. After exclusions (55 and 27 events, respectively), 88 VAP events were included in the final analysis, of which 60.2% (53 events) belonged to the intervention group (Figure 1). These events were distributed among 26 ICU patients in the pre-intervention period and 46 ICU patients in the intervention period.

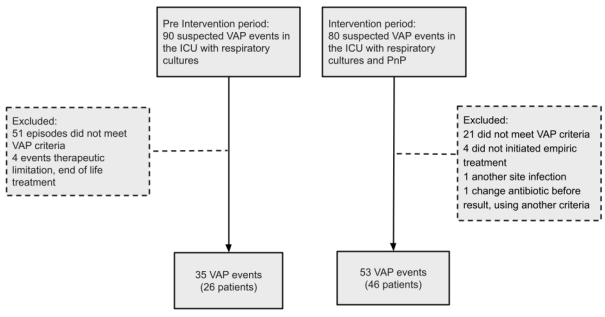

Fig. 1: Flowchart of included ventilator associated pneumonia events and patients

There were some significant differences between the two cohorts. Antimicrobial treatment for VAP was

DOI: 10.3844/ajidsp.2025.15.25

initiated more frequently during weekends in the pre-intervention group (52%, 14 patients, p = 0.026). Additionally, carbapenems were prescribed significantly more often in the pre-intervention period (96%, 26 patients, p = 0.010). Conversely, a higher proportion of patients in the intervention group received empirical treatment aligned with hospital guidelines (87%, 40 patients, p = 0.013), with colistin being more frequently used as part of the regimen (87%, 40 patients, p = 0.003) (Table 1).

Table 1: Patient characteristics; IQR Interquartile range, p Statistical significance value

Feature	Pre Intervention (26)	Intervention (46)	Statistical Significance
Female sex - % (no.)	28% (7)	37% (17)	P = 0.333
Age - median(IQR)	67 (41-76)	61 (47-73)	P = 0.519
Charlson - median (IQR)	3 (1-6)	3 (1-5)	P = 0.455
Immunosuppression - % (no.)	20% (5)	24% (11)	P = 0.591
Solid organ transplant	8% (2)	17,4% (8)	
Ventilator days - median (IQR)	8 (3-20)	8 (5-14)	P = 0.853
Type of sample - % (no.)			
Tracheal aspirate	64% (18)	61% (28)	P = 0.620
Bronchoalveolar lavage			
Bronchoalveolar lavage	36% (9)	39% (18)	
Day of the week - % (no.)			
Day of the week - % (no.)			
Monday	20% (8)	13% (7)	P = 0.198
Tuesday	8% (4)	8,7% (7)	
Wednesday	4% (1)	20% (10)	
Thursday	16% (5)	26% (12)	
Friday	20% (8)	20% (9)	
Saturday	8% (3)	4,3% (3)	
Sunday	24% (6)	9% (4)	
Weekends			
Holidays 48 h later	52% (14)	26% (12)	P = 0.026
Holidays 72 h later	16% (4)	6,5% (3)	P = 0.359
	20% (5)	10,9% (5)	
Antibiotic initiated - % (no.)	84% (23)	76% (35)	P = 0.353
Vancomycin	96% (26)	71,7% (33)	P = 0.010
Total Carbapenem	72% (20)	72% (33)	P = 0.829
Meropenem	56% (15)	87% (40)	P = 0.003
Colistin	8% (2)	9% (4)	P = 0.721
Piperacillin-tazobactam	0% (0)	7% (3)	
Cefepime	0% (0)	9% (4)	
Aztreonam	0% (0)	11% (5)	
Ceftazidime-avibactam	8% (3)	2% (1)	P = 0.147
Empirical adequate - % (no.)	60% (16)	87% (40)	P = 0.013
Antibiotic change - % (no.)	96,2% (25)	91,3% (42)	P = 0.847
Positive blood culture- % (no.) Negative	44% (12)	32,6% (15)	P = 0.312
	44% (12)	54% (25)	
Positive: Pneumonia	12% (3)	13% (6)	
Positive: Another source	4% (2)	2% (1)	
Positive contaminated	28% (7)	17% (8)	
Not performed	12% (3)	13% (6)	
Mortality 28 days - % (no.)	28% (7)	21,7% (10)	P = 0.619

Table 2: Bacterial isolates identified by conventional culture and pneumonia panel (≥10 4 copies/mL). In both periods.

Organisms	Pre-intervention Cohort		Intervention Cohort			
	Conventional Culture		Conventional Culture		PCR Pneumonia Panel	
	n = 56 organisms	%	n = 69 organisms	%	n = 104 organisms	%
Gram positive						
Staphylococcus aureus MS	14	25	9	13	14	13
Staphylococcus aureus MR	2	4	2	3	6	6
Streptococcus pneumoniae	1	2	0	0	3	3
Streptococcus agalactiae	0	0	0	0	1	1
Enterococcus faecalis	1	2	1	1	0	0
Gram negative coccobacillus						
Haemophilus influenzae	1	2	3	5	12	11
Moraxella catarrhalis	1	2	1	1	2	2
Fermenting Gram negative bacilli						
Escherichia coli	1	2	1	1	7	7
Klebsiella pneumoniae	5	9	12	17	15	14
Klebsiella oxytoca	0	0	0	0	2	2
Proteus mirabilis	0	0	2	3	5	5
Serratia marcescens	1	2	3	4	3	3
Enterobacter cloacae complex	7	13	1	1	3	3
Citrobacter freundii	1	2	2	3	Non detected	
Klebsiella aerogenes	0	0	3	4	5	5
Non fermenting Gram negative bacilli						
Pseudomonas aeruginosa	17	30	20	29	22	21
Acinetobacter baumanii	1	2	5	7	8	8
Stenotrophomona maltophilia	3	5	3	4	Non detected	
β lactamase						
ESBL			12*	27**	8***	20****
KPC	1	2	0	0	1	1
MBL	0	0	4	6	6	6
OXA	0	0	1	1	0	0

*MS Methicillin sensitive, MR Methicillin resistant, EPC Carbapenem resistant enterobacterales, MBL Metallo-beta lactamases, OXA Oxacillinases, NA Not applicable; * 3rd generation cephalosporin resistance detected, suspected ESBL mechanism; ** 12 suspected ESBL from 44 gram negative bacilli detected; *** CTX-M (ESBL mechanism) detected by PCR **** 8 CTX-M from 40 rapid diagnostic methods for pneumonia that detected at least one gram negative bacilli*

Regarding microbiological findings, most microorganisms were found less frequently in standard cultures compared to PnP, except for *Enterococcus spp*. The microorganisms detected included *Methicillin-Sensitive Staphylococcus Aureus (MSSA), MRSA, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus agalactiae, Klebsiella spp., Enterobacter cloacae complex, Proteus mirabilis, Escherichia coli, Moraxella catarrhalis, Pseudomonas spp. and Acinetobacter spp.*. Notably, the PnP identified a sample with a Metallo-Beta-Lactamase (MBL) resistance mechanism that was not detected by culture. However, standard cultures identified organisms outside the PnP's detection scope, such as *Citrobacter freundii* and *Stenotrophomonas maltophilia* (Table 2).

Primary Outcome

The time from antimicrobial prescription to antibiotic change was significantly reduced in the intervention group, with a median of 21 h (IQR 8-31) compared to 76 h (IQR 63-100) in the pre-intervention group (Figure 2). A linear mixed-effects regression model accounting for correlations between patients with multiple VAP events demonstrated a reduction of 55 h (95% CI-67,-42; p<0.001). After adjusting for variables such as immunosuppression, ventilator days, day of the week, weekend or holiday for the algorithm implementation, the adjusted coefficient was -58 h (95% CI-70,-46; p<0.001).

The median time from culture admission to antimicrobial modification also decreased substantially, from 67 h (IQR 47- 91) in the pre-intervention group to 9 h (IQR 5-20) in the intervention group (Fig. 3). Using a linear mixed-effects regression model, the reduction in time was consistent, with a coefficient of-55 h (95% CI-67,-42; p<0.001).

For each of the most frequently used antimicrobials (vancomycin, carbapenems and colistin), although reductions in the time to antibiotic modification were observed, they were not statistically significant. Vancomycin was prescribed in 70 VAP events, carbapenems in 71 events and colistin in 67 events as empiric treatments. The total duration of vancomycin use decreased from 77.1 h in the pre-intervention cohort to 42.9 h in the intervention cohort, with a time difference of -28 h (95% CI -64, 8; P = 0.129). For carbapenems, the duration dropped from 88.9-72.5 h, with a difference of -3.4 h (95% CI-51, 44; P = 0.888). Similarly, colistin use decreased from 74.4-45.5 h, with a time difference of -25 h (95% CI -56, 5; P = 0.104). Linear mixed-effects regression models were used for each antimicrobial, accounting for patients in the intervention period who experienced multiple events (9 for vancomycin, 12 for carbapenems and 9 for colistin). These models demonstrated the correlations and adjusted the estimates accordingly. Despite these reductions in use, the results indicate that the observed differences were not statistically significant for any of the three antimicrobials separately.

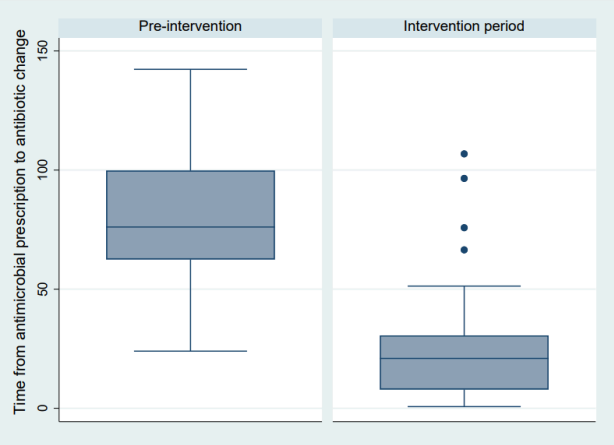

Fig. 2: Time difference from antimicrobial prescription to antibiotic change in both periods

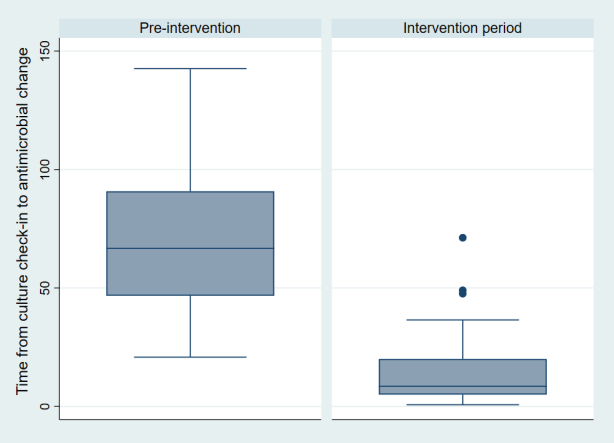

Fig. 3: Time difference from culture check-in to antimicrobial change in both periods

Secondary Outcomes

There was a 49% (26) complete agreement between the PCR panel results and culture findings. In 47% of cases, the agreement was partial, primarily because the PCR panel detected additional pathogens not identified by culture, particularly *Haemophilus influenzae*, *Streptococcus pneumoniae*, or *Moraxella catarrhalis*. In one sample *Stenotrophomonas maltophilia* was identified by culture but not by the PCR panel. Complete discordance occurred in only 4% (2) of cases, involving episodes with negative cultures where the PCR panel identified *Haemophilus influenzae* in one case and *methicillin-sensitive Staphylococcus aureus* in the other.

The sensitivity and specificity of the PnP were assessed in comparison to standard cultures for bacterial targets. For methicillin resistance in Staphylococcus aureus (detected through mecA), with a prevalence of 10%, the PnP demonstrated a sensitivity of 83.3% (95% CI: 35.9–99.6%) and a specificity of 100% (95% CI:

92.3–100%), with a negative predictive value of 98.2%. For Extended-Spectrum Beta-Lactamase (ESBL) detection, with a prevalence of 23%, the sensitivity was 62.5% (95% CI: 24.5–91.5%) and the specificity was 84.1% (95% CI: 69.9–93.4%), yielding a negative predictive value of 88.2%.

Compliance with the ID team therapeutic suggestions was evaluated in the intervention cohort. Among the 80 suspected VAP events during the intervention period, 53 (66.25%) met the inclusion criteria. In all 53 cases, the ICU team accepted the recommendations for antimicrobial treatment based on the PnP findings, achieving 100% adherence to the suggested modifications.

Mortality rates for all ICU patients, regardless of inclusion in the study, were similar between the pre-intervention and intervention periods, 12.33% and 12.02%, respectively (p = 0.25). Among the study participants, the 28 day mortality rates were 28% in the pre-intervention group and 21.7% in the intervention group (p = 0.619).

Discussion

The main finding of this study is that implementing a multimodal strategy -comprising a rapid diagnostic method, a treatment algorithm, education and enhanced multidisciplinary communication- can significantly reduce the duration of empirical broad-spectrum antibiotic use in VAP management. Moreover, these results were achieved without a significant difference in patient mortality between the two periods. Additionally, a high negative predictive value for MRSA was observed when comparing PnP to standard culture.

Although prior studies have evaluated the role of PnP in reducing the "time to change" antimicrobials in pneumonia cases, to our knowledge, none have demonstrated statistically significant reductions and some have estimated a possible significant impact but have not been able to demonstrate it in real life. For example, one study reported a time to discontinuation of anti-MRSA agents of 49.1 h in the non-PnP group compared to 41.8 h in the PnP group (P = 0.28). Similarly, the median time to discontinuation of antipseudomonal agents was 134.4 h in the non-PnP group versus 98.1 h in the PnP group (P = 0.47) (Miller *et al.*, 2023).

While previous studies have explored the potential of rapid diagnostic tests (PnP) to expedite antimicrobial adjustments in pneumonia, none have demonstrated statistically significant reductions in antimicrobial usage time. For instance, Miller *et al.* (2023) reported a mean time to discontinuation of anti-MRSA agents of 49.1 h in the non-PnP group versus 41.8 h in the PnP group (P = 0.28). Similarly, the median time to discontinuation of antipseudomonal agents was 134.4 h in the non-PnP group compared to 98.1 h in the PnP group (P = 0.47). Other studies have projected a potential impact, but have not validated these findings in clinical practice.

A key finding of this study was the observation of a significant improvement in the time to antibiotic modification, which can be attributed to several factors. Firstly, the study implemented a proposed diagnostic and treatment algorithm developed in collaboration with the microbiology and intensive care teams. The involvement of the treating physicians in the design of this proposal may have facilitated not only a 100% acceptance of the proposed treatment changes, but also an understanding of the need for rapid implementation to optimize outcomes. The same consideration applies to the microbiologists, who participated in this project from its very beginning. Secondly, the rapid diagnostic method itself offers the advantage of earlier results compared to traditional culture techniques. Finally, we believe that the establishment of effective communication between the teams was a crucial element that contributed to the significant difference in the time to antimicrobial change observed in this study, in contrast to previously published studies. It is essential to recognize the synergistic effect of the optimized communication framework and educational interventions. The integration of streamlined communication channels between microbiology, infectious diseases and intensive care teams, coupled with real-time follow-up via the online consulting group, played a pivotal role. The proposed method leads to improved outcomes by reducing patient exposure time to broad-spectrum antimicrobials, thereby mitigating selective pressure and the development of antimicrobial resistance. Furthermore, the involvement of the Infectious Diseases (ID) team in communicating results enhanced interpretation and may have reduced the risk of mismanagement, although these aspects were not formally measured in our study. Overall, these are all core elements of the antimicrobial stewardship programs, however implementation can be challenging.

There were no significant differences in the population type between the two periods. However, variations were noted in certain behaviors related to antimicrobial use. During the intervention period, there was increased adherence to the empirical treatment proposed by the institutional guideline, a reduction in carbapenem usage and an increase in colistin usage, aligning with the recommended regimens based on the unit's epidemiology. It is plausible that the educational intervention and the participation of ICU physicians influenced these changes.

When analyzing vancomycin, meropenem and colistin individually, we did not observe a statistically significant difference in the time to discontinuation. However, it could be argued that there was a clinically relevant difference for vancomycin and colistin, reflecting a one-day reduction in treatment duration with our multimodal strategy. The lack of statistical significance for these two antibiotics may be due to an

insufficient sample size to detect an effect. Nevertheless, another study, involving 1181 patients, has demonstrated that the clinical use of these types of panels can lead to faster de-escalation for gram-positive bacteria (Virk *et al.*, 2024). Previous studies have reported high sensitivity (ranging from 100% to 93.9%) and variable specificity (from 45.9-87.2%) (Murphy *et al.*, 2020; Kakati *et al.*, 2024). The high negative predictive value for MRSA found in our study allowed for early discontinuation of vancomycin treatment. In this research, a significant percentage of patients received empirical vancomycin (83% in the pre-PnP cohort and 69% in the PnP cohort), despite a relatively low prevalence of MRSA isolated in respiratory cultures (4.5 and 5.2%, respectively) and considering that vancomycin is not recommended in the empirical treatment of VAP. This strategy could help address this issue.

In contrast, the results for meropenem may be related to the lower sensitivity and negative predictive value of PnP for Extended-Spectrum Beta-Lactamase (ESBL) producers compared to standard cultures. In our study, ESBLs were observed in 27and 20% of isolates, as determined by culture results and PnP, respectively. Suspicion of ESBL-producing organisms by the ID team, based on previous epidemiological studies in the unit and the exclusive presence of the CTX-M gene in the PnP without other ESBL genes, likely contributed to limited meropenem de-escalation.

The main isolated organisms were Methicillin-Susceptible Staphylococcus Aureus (MSSA), Haemophilus influenzae, Klebsiella pneumoniae and Pseudomonas aeruginosa. The rapid method demonstrated greater sensitivity in identifying all these organisms, with a particularly noticeable difference for Haemophilus influenzae. This difference in the sensitivity of the rapid method allows for more accurate targeted treatment. This increased sensitivity of the rapid methods (PnP) represents an advantage, but at the same time, it could pose challenges for ASPs as polymicrobial cultures could lead to increased antimicrobial usage. The identification of more organisms could be due to the molecular detection of nonviable bacteria, low bacterial burdens, the presence of difficult-to-culture microorganisms, or the impact of prior antibiotic use (Larry *et al.*, 2023). It is also important to note that the semiquantitative results (copies/ml) produced by PnP are not directly comparable to Colony-Forming Units/ml (CFU) from cultures; the PnP values are, on average, approximately one log higher than the colony counts reported by cultures. Consequently, potential pathogens present at counts below 10^4 CFU/mL in cultures may not be routinely reported according to current cut-off points, resulting in lack of treatment.

The implementation of this multimodal strategy, in conjunction with rapid diagnostic methods, holds the potential to optimize the management of patients with Ventilator-Associated Pneumonia (VAP). This approach aims to expedite the transition to targeted antimicrobial therapy based on microbiological results, thereby mitigating the risk of antimicrobial resistance, *Clostridium difficile* infections and adverse effects associated with broad-spectrum antimicrobials employed in empirical therapy. The empirical treatment often involves multiple antimicrobials and consequently, may increase the risk of renal insufficiency. It is well-established that the development of antimicrobial-induced renal insufficiency is contingent not only upon the inherent nephrotoxic potential and dosage of the administered agents, but also on the duration of exposure. In our study, we demonstrated a reduction in vancomycin exposure time, from 77.1-42.9 h and a corresponding decrease in colistin exposure time, from 74.4-45.5 h. Notably, renal insufficiency associated with vancomycin and colistin has been documented to manifest as early as the fourth day of treatment, corresponding to three full days of exposure (Miller *et al.*, 2023;Kan *et al.*, 2022). Consequently, our findings suggest that this intervention may have prevented patients from reaching the established threshold for renal dysfunction associated with these antibiotics. Although this aspect was not measured in our study.

This study holds significant value due to its successful execution in a Latin American setting, a region marked by high antimicrobial resistance rates, where the implementation of rapid diagnostic tools has the potential to curtail broad-spectrum antimicrobial usage (Antimicrobial Resistance Collaborators, 2023). Importantly, in resource-constrained environments, such as those prevalent in Latin America, this research provides evidence supporting the integration of rapid diagnostic methods into antimicrobial stewardship programs (ASPs) in this setting. These methods may also offer potential cost savings in Broad-Spectrum Antimicrobial consumption, although this was not quantified within our study. Consistent with Fabre *et al.* (2023) recommendations, the implementation of rapid diagnostic methods, while potentially representing an investment in resource-limited settings, when integrated within an ASP and further aligned with a diagnostic stewardship approach, can optimize not only antimicrobial use but also reduce the frequently excessive sampling in febrile patients within intensive care units. The practical application of this approach in our study demonstrated a notable improvement in patient selection for respiratory sampling procedures, promoting a more rigorous evaluation of respiratory infection foci. A probable, albeit unquantified, reduction in clinical sample requests was observed, attributed to the implementation of a collaborative assessment between the ID and intensive care teams for patients with suspected VAP, which conditioned sample collection on the clinical relevance of the anticipated results. Notably,

this study facilitated the definitive incorporation of these diagnostic tools into the clinical management of VAP patients at our institution.

Despite these promising findings, several limitations should be acknowledged. First, although the study achieved statistically significant results, the sample size of this pilot study remains relatively small, limiting the generalizability of the findings. Second, the study design —a retrospective control cohort and a prospective interventional, quasi-experimental cohort—is inherently limited by the inability to control for all potential confounding factors. For example, the clinical rationale underlying diagnostic and therapeutic decisions was not fully captured, which could influence outcomes. Third, one of the major limitations of this study is the selection of study periods. Due to the pandemic and changes in patient management protocols, it is important to acknowledge that this could introduce confounding variables. To mitigate this effect, a multivariate model was also constructed. Furthermore, polymicrobial infections, which may prolong culture result times, were not specifically measured, potentially affecting the interpretation of results. Finally, the study period coincided with the COVID-19 pandemic, which likely disrupted standard patient populations and care processes.

Conclusion

The principal finding of this study is that the implementation of a multimodal intervention—including rapid diagnostic techniques, a treatment protocol, educational initiatives and enhanced interdisciplinary communication—leads to a significant reduction in the duration of empirical broad-spectrum antibiotic administration for VAP. Implementing this multimodal strategy may facilitate improved VAP patient care, optimizing treatment with rapid targeted treatment, with the narrowest effective spectrum and lessening the potential for adverse effects from multiple antibiotics and the development of antimicrobial resistance. Rapid diagnostic methods such as PnP and standard approaches like routine cultures can complement their respective strengths in sensitivity and specificity. However, to maximize the potential of PnP, it should be integrated into a comprehensive framework that prioritizes structured and effective communication. These tools can provide valuable support for multidisciplinary teams in their efforts to deliver the best available care while facilitating antimicrobial de-escalation for ICU patients undergoing treatment for VAP.

Acknowledgement

This study was made possible thanks to the contribution of 90 panels of Filmarray pneumonia panels from the Biomerieux Laboratory to the Hospital Italiano de Buenos Aires.

Funding Information

The authors of this study did not receive any funding for this manuscript or any of the activities related to this study. The Biomerieux Laboratory was not involved in the design or any stage of the study development.

Author Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Conflict of Interest

Potential conflicts of interest: M.I.S reports having received a research grant previously from BioMérieux for a Diagnostic Stewardship and Antimicrobial Stewardship program, not related to this study.

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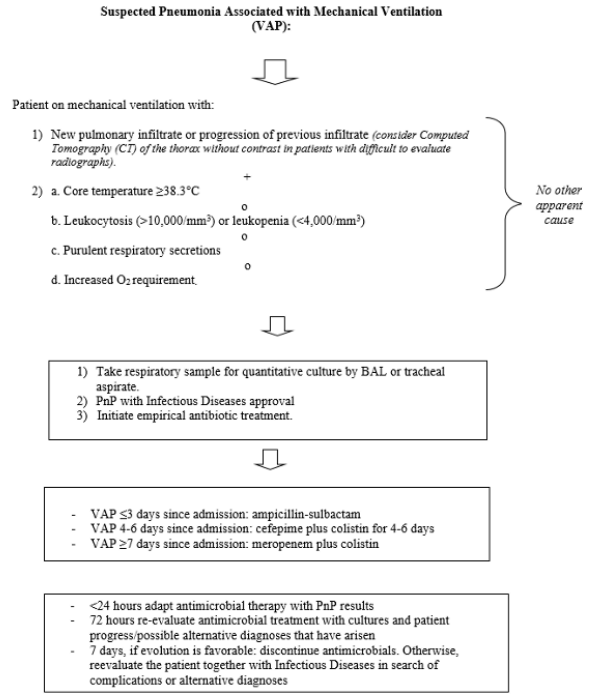
Appendices



Appendix 1: Algorithm for suspected pneumonia associated with mechanical ventilation (VAP)

Appendix 2: Follow-up spreadsheet for patients with suspected pneumonia

Patient and Study Information

- Patient Code
- Study Phase (Pre-intervention / Intervention Period)
- Reviewer
- Age
- Sex
- Charlson Comorbidity Index
- Day Mechanical Ventilation Started

Sample Collection

- Sample Type (e.g., AT, BAL)
- Sample Collection Date
- Sample Collection Time
- Sample Submitted to Lab: Date
- Sample Submitted to Lab: Time
- Day of the Week
- Was it a holiday? (Yes / No)
- Holiday in the Next 2 Days? (Yes / No)
- Holiday in the Next 3 Days? (Yes / No)

Patient Medical Background

- Immunosuppressed (Yes / No)
- Solid Organ Transplant (Yes / No)

Antimicrobial Therapy (ATM)

- ATM Start Date
- ATM Start Time
- Was ATM Changed? (Yes / No)
- ATM Change Date
- ATM Change Time
- ATM Scheme
- Protocol/Algorithm Followed? (Yes = 1, No = 0)
- Empirical TMJ Treatment According to Guidelines (Yes / No)

Antibiotics Administered

- Vancomycin (Yes / No)
  - Start Date / Time
  - Suspension Date / Time
- Meropenem (Yes / No)
  - Start Date / Time
  - Suspension Date / Time
- Colistin (Yes / No)
  - Start Date / Time
  - Suspension Date / Time
- Piperacillin-Tazobactam (Yes / No)
- Cefepime (Yes / No)
- Ceftazidime-Avibactam (Yes / No)
- Aztreonam (Yes / No)
- Other Antibiotic(s)
  - Antimicrobial 1: Start Date / Time, Suspension Date / Time
  - Antimicrobial 2: Start Date / Time, Suspension Date / Time
  - Antimicrobial 3: Start Date / Time, Suspension Date / Time
  - Antimicrobial 4: Start Date / Time, Suspension Date / Time

Microbiological Results

- MRSA Detected by PnP? (Yes / No)
- MRSA Detected by Culture? (Yes / No)
- ESBL Detected by PnP? (Yes / No)
- ESBL Detected by Culture? (Yes / No)
- Resistance Mechanism Identified
- PnP Results:
  - PnP 1 Result / Copy Count (ml)
  - PnP 2 Result / Copy Count (ml)
  - PnP 3 Result / Copy Count (ml)
  - PnP 4 Result / Copy Count (ml)
  - PnP 5 Result / Copy Count (ml)
- Culture Results:
  - Culture 1 Result / Count
  - Culture 2 Result / Count
  - Culture 3 Result / Count
  - Culture 4 Result / Count
- PnP and Culture Matching:
  - 1 = Yes
  - 2 = Partial
  - 3 = No
- Reason for Discrepancy

Clinical Decision Impact

- Was Treatment Modified Based on PnP? (Yes / No)
- Behavior Modification Due to PnP:
  - 0 = No Change
  - 1 = Escalated
  - 2 = De-escalated
- Behavior Modification Due to Culture
- Reason for Escalation
- Antibiotic Sensitivity Result

Clinical Outcomes

- Clinical Evolution on Day 14
- Total Days on Mechanical Ventilation
- 28-Day Mortality (Yes / No)
- Date of Death (if < 28 days)