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FULL PAPER

Evaluation of the Pneumonia Panel on laboratory samples from an Italian pediatric center: results from a monocentric study

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SUMMARY

Lower respiratory tract infections (LRTI) are still burdened by considerable morbidity and mortality. Rapid and appropriate treatment imply knowledge of the underlying causative pathogen; while it is tempting to offer broad spectrum antibiotics, Antimicrobial Stewardship Practices invite a judicious use of the latter, especially when bacteria are not the cause. However, the epidemiology shifts to multidrug resistant (MDR) pathogens that require optimization of molecules in order to provide optimal treatment.

Novel methods requiring direct sample result testing such as the Biofire Pneumonia (PN) panel have recently been made available on the market. Syndromic testing may hence provide support in the diagnosis of LRTI. There is paucity of data concerning experiences in high MDR settings, and even less concerning the performance of these panels in pediatric settings with moderate MDR prevalence.

Our study highlights the optimal sensitivity and importance of support from such methods in settings burdened by MDR presence and where fast and appropriate therapy is mandatory.

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INTRODUCTION

Lower respiratory tract infections (pneumonia, bronchiolitis, lung abscesses and empyema) still represent the fifth cause of mortality in the world, with over two and half million deaths yearly (Eshwara *et al*., 2020; Roth *et al*., 2018). Incidence is highest in children below the age of four and subjects aged >60 years. Moreover, infant mortality and adult hospitalizations for pneumonia show continuous increase in both low- and middle-income countries (Sattar, 2020).

Etiological causes of lower respiratory tract infections may be either bacterial and/or viral or fungal with overlapping clinical presentations; as a result, it is not possible to discern an etiological agent with the exception of laboratory-derived information concerning identification (tests of microbiology, inflammatory markers, hematological values) (Cals *et al*., 2007; Huang *et al*., 2018).

Moreover, identifying the pathogen in the etiology of the disease is crucial in order to provide adequate clinical and therapeutic treatment of the patient (Samuel *et al*., 2018; Kalil *et al*., 2016; Garau *et al*., 2008).

Key words:
Film Array, Pneumonia panel, standard-of-care (SOC).

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Early appropriate treatment impacts mortality in the critically ill; while immediate treatment is imperative in sepsis, there is, however, time for diagnostics in severe infections (Nauclér *et al*., 2021). Hence, identification may be feasible even in severe infections such as pneumonia.

The main bacteria causing typical pneumonia, both community and hospital, are *S. pneumoniae, S. aureus, P. aeruginosa, K. pneumoniae, H. influenzae,* and *M. catarrhalis*, while atypical pneumonia is caused mainly by *L. pneumophila, C. pneumoniae,* and *M. pneumoniae.*

The incidence of *S. Pneumonia* sustained pneumonia has indeed decreased over the years, with the introduction of vaccine campaigns involving children in the early stages of life. Conversely, the epidemiology of lower respiratory tract infections has now shifted towards a growing prevalence of Gram-negative bacteria, which unfortunately often exhibits intrinsic resistance or multi-resistance to commonly used antibiotics, making therapeutic choices challenging (Amati *et al*., 2020; Assafa *et al*., 2022).

In children, pneumonia or bronchiolitis of viral origin is the most common, and the main pathogens are Respiratory Syncytial Virus (RSV), Rhinovirus (HRV), Adenovirus (ADV), Influenza Virus A and B, Parainfluenza Virus (hPIV), and Coronavirus (hCOV). In these cases, once the etiological agent has been identified, antiviral therapy may be started immediately.

However, the diagnosis of lower respiratory tract infection of bacterial origin can be complex. This is due to a combination of time-consuming techniques, as germ growth requires extensive waiting, as well as skill required to distinguish between infection and colonization of the germ. Additionally, patients with symptoms are often already undergoing treatment with at least two or three antibiotics before the availability of the definitive laboratory report. The latter often implies both a more difficult identification of the pathogen, due to the stunted and reduced growth in the sample, and inappropriate use of antibiotics (Torres *et al*., 2016).

Current advances in the field of microbiology and virology include novel methods for quick detection, enabling quicker definition of the etiological agent and leading to more rapid, targeted, and appropriate therapy.

In particular, molecular biology-based methods can within an hour identify origin the underlying pathogen involved in the infective process (bacterial and/or viral) but also distinguish between colonization and infection, ensuring a highly reliable result and anticipating by 2-3 days definitive results obtained by means of traditional cultivation methods.

In the Microbiology Analysis Laboratory of the Santobono Hospital in Naples (Azienda di Rilievo Nazionale Santobono-Pausilipon), we have implemented such systems for supporting the diagnosis of respiratory infections (BioFire FilmArray, BIOMERIEUX). Our current routine includes both the BioFire Respiratory Panel (for upper respiratory tract infections) and the BioFire Pneumonia Panel (for lower respiratory tract infections). Specifically, the BioFire Film Array Pneumonia Panel enables rapid identification of pathogens along with a precise semi-quantitative count of genomic material of germs, in order to distinguish between colonization and infection of the lower respiratory tract, which is crucial for clinicians in order to determine the most appropriate treatment choice, either by starting, stopping or expanding the spectrum of antimicrobials to ensure the most appropriate and timely treatment. The aim of the current study was to assess correspondence between results obtained by means of the BioFire Film Array Pneumonia (PN) panel and compare them with standard of care (SOC) methods involving standard culture laboratory techniques, in samples from a pediatrics Center.

MATERIALS AND METHODS

115 samples from the Intensive Care Unit or other Units with critical care patients (28/115 from Emergency and Pediatrics) of the Santobono Hospital of the "Santobono-Pausilipon" Hospital in Naples, from January 2021 to September 2022, were retrospectively assessed. 87 BAL and 28 sputum samples were collected.

For each sample, two sterile samples were provided, one for molecular investigation and one for culture examination.

Molecular diagnostics

Pneumonia PN panel BioFire Diagnostics, LCC (PN). The BioFire Film Array Pneumonia Panel (PN panel, BioFire Diagnostics, LLC) is based on a nested PCR and on bronchoalveolar lavage (BAL or mini-BAL), endotracheal aspirate, and sputum samples, and requires 75 minutes. Targets include the following pathogens: 15 bacteria (*A. baumannnii*, *E. cloacae complex, E. coli, H. influenzae, K. aerogenes, K. oxytoca, K. pneumoniae, M. catarrhalis, Proteusspp., P. aeruginosa, S. marcescens, S. aureus , S. agalactiae, S. pneumoniae, S. pyogenes*), 3 atypical bacteria (*C. pneumoniae, M. pneumoniae* and *L. pneumophila*), 9 viruses (Adenovirus, Coronavirus, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A virus, Influenza B virus, Parainfluenza virus, Respiratory syncytial virus, Middle East respiratory coronavirus syndrome) and 7 bacterial resistance genes (mecA/mecC and MREJ, CTX-M, KPC, NDM, OXA-48-like, VIM, IMP).

Results for bacterial pathogens (the 13 common ones) are semi-quantitative (no. of genomic copies/mL of sample), while atypical bacteria, viruses and resistance genes are detected qualitatively.

Sterile whole samples without addition of enrichment broth initially underwent PN panel testing in accordance with the manufacturer's instructions. Briefly, 200 µL of sample (BAL, mini-BAL or sputum), were directly pipetted into the injection tube provided in the kit. After reconstitution of the reaction wells with the hydration vial of the kit, the sample was applied in the cartridge and the run started, using the dedicated software (FilmArray). The panel is equipped with all necessary reagents for lysis, nucleic acid extraction, reverse transcription, two PCR amplifications and detection of 33 target sequences, as well as with two internal controls, the RNA process control (to monitor each stage of the process) and the control for the second PCR.

Viruses and "atypical" bacteria are reported with a qualitative result, while for the other bacteria, in addition to the qualitative result, the semi-quantitative count expressed in "bin" (no. copies/mL), from 10^4 to 10^7 no. copies/mL is also indicated. The panel detects germs starting at $10^{3.5}$ copies/mL; for lower values, the negative result is reported as "not detected."

The method also detects the possible presence of the main bacterial resistance genes: mecA/mecC and MREJ, CTX-M, KPC, NDM, OXA-48-like, VIM, IMP. Results are readily available in 75 minutes.

Standard Culture Methods

Each sample was submitted in parallel to traditional culture testing, for both identification and suscepti-

bility. Each BAL/mini-BAL or sputum sample was plated on blood agar, Mannitol agar, MacConkey agar, Sabourad chromogenic agar, and chocolate agar with bacitracin, and incubated for 24 h at 37°C in a thermostat, with addition of 5% CO_2 where necessary. Samples were also plated in order to define the bacterial count, expressed as "colony forming units" (CFU/mL), ranging from 10^3 to 10^6 copies.
The identification and susceptibility of isolated pathogens were detected by means of automated Vitek2 system (Biomerieux), using specific cards, while results were interpreted in accordance with the Guidelines of The European Committee on Antimicrobial Susceptibility Testing - EUCAST.
NG-Test CARBA-5 BIOTECH were used to assess Carbapenem resistance by means of SOC, though these methods were not employed to confirm Biofire PN panel data (conventional phenotypic testing was used to confirm resistance patterns). Resistances were confirmed on isolated strains by means of CARBA-5 BIOTECH test, while ESBLs were confirmed by SOC using standard phenotypic techniques by VITEK.

RESULTS

Results are reported in *Table 1*.
On the whole, 49/82 samples (65%) exhibited concordance with both methods for at least one pathogen; 12/82 samples (10.4%) yielded positive results on the PN panel while no pathogen was detected by standard culture (absence of growth or normal bacterial flora), 5 samples yielded negative results by both methods (concordance with PN panel and culture), and 4 samples had pathogens only detected by culture, as they were not included in the panel targets. Moreover, 4 samples yielded discordant results (no common pathogen between the two methods) (3.4%), and 12 samples were positive for at least one virus (Respiratory Syncytial Virus, rhinoviruses, adenoviruses, metapneumoviruses). Viral testing was performed only by means of PN panel, as no other method was available.
Standard Culture was not performed on 28 samples as they were patients who were either in the emergency room or in pediatrics and had another clinical course.
Concerning bacterial samples, sensitivity and specificity were 92.5% and 29.4%, respectively, while PPA and NPA were 80.3% and 55.6%, respectively. Discordance was mainly driven by pathogens not included on the panel or undetected by culture.

DISCUSSION

In patients with pneumonia, both the rapid identification of the pathogen, along with its susceptibility pattern, are associated with a strong reduction in mortality, ascribable to the establishment of a correct and appropriate therapy (Huang *et al*., 2018). Historically, the initial approach performed in clinical laboratories and mainstay for the diagnosis of lower respiratory tract infections is the standard culture method, along with a semi-quantitative colony count. However, these methods are hampered by lengthy procedures and time requirements, as results may be available only after at least approximately 48 hours.
The recent introduction and dissemination of molecular biology methods in laboratory practice, as well as in the field of respiratory infections, with faster turn-around times to identification, has made it possible to obtain important indications for a faster and more efficient therapeutic approach, reducing the duration of empirical antimicrobial therapy started upon admission.
In line with these observations, recent data reported that rapid identification of methicillin resistant *S. au-*

Table 1 - *Results from analysis of PN panel testing versus SOC.*

BiofirePN panel	*Overall perfomance of the BioFire Film Array Pneumonia plus panel*	*SoC + Bacteria*	*SoC- Bacteria*	*Virus only no SoC*	*Total*
Biofire +		49	12	12	73
Biofire -		4	5	0	9
Total		*53*	*17*	*12*	*82*
Overall concordance[a]	((5+49)/82)*100=65,9%				
Concordant positive[b]	(49/82)*100=59,8%				
Concordant negative[c]	(5/82)*100=6,1%				
Sensitivity	(49/53)+100=92,5%				
Specificity	(5/17)*100=29,4%				
PPA	49/(49+12)*100=80,3%				
NPA	5/(4+5)*100=55,6%				

[a]Overall concordance: samples fully concordant for analytes detected by PNplusPanel and SOC and for samples reported as negative by both methods.
[b]Concordant positive: PNplusPanel and SOC in agreement for all pathogens detected.
[c]Concordant negative for any pathogen: PNplusPanel and SOC both reported as negative for any pathogen.

reus (MRSA strains) has led to a reduction in the unnecessary use of anti-MRSA antibiotics from 80% to 60%, also contributing to a reduction in public health expenditure (Trevino *et al.*, 2017; Miller *et al.*, 2018). Timely recognition of resistance mechanisms also led to appropriate targeted therapy in case of gram-negative pathogens, reducing the use of inactive or toxic agents, with impact on length of stay but no difference in mortality.

In the present study, we aimed to compare the results obtained by means of the PN panel in our routine laboratory assessments with those obtained with the classical culture method, as unfortunately correspondence is not always immediate, due to various factors such as previous antibiotic therapy for at least 78 hours, fastidious bacteria with difficult growth, and the presence of normal bacterial flora (Buchan et al., 2020).

When all bacteria samples are considered, our data show an overall 65.9% concordance between SoC and PN panel samples concerning bacterial targets. Overall concordance is higher than in previously reported data from Ginocchio et al. amounting to 49.10% overall.

Positive samples according to the PN panel and negative by standard culture (10.4% PN+/SOC-), may be explained as mentioned above, as nucleic acids may also persist beyond live cells, implying greater sensitivity of the PN panel as opposed to culture methods. Indeed, in these cases, bacteria detected by the panel, *S. pneumoniae, M. catarrhalis, P. aeruginosa,* or *S. aureus* yielded 10^4 copies/mL, indicating difficult-to-grow or low-quantity germs. Conversely, the 4 samples showing positive growth by culture but negative by PN panel (PN-/SOC+), are due to isolation of germs not included in the panel (*S. maltophilia, M. morganii, C. albicans, C. tropicalis*).

Indeed, the panel settings enable detection as low as $10^{3.5}$copies/mL, in accordance with current guidelines to reduce the risk of considering clinically insignificant loads for BAL samples (7, 13) (Miller et al. 2018; Kalil et al., 2016). Additionally, the PN panel overestimates 1 log for sputum and 1.5 log for BAL, even though the agreement between copies/mL and CFU/Ml is >90% for batches of 10^6 CFU/mL and above.

Knowledge of the cut offs (10^4 CFU/ml for BAL and 10^5-10^7 CFU/mL) for the tracheal aspirate and sputum for both the cultures and the molecular biology (15, 16) (Leber, 2016; GBD, 2013) allows discrimination between normal colonization and infection, supporting clinicians as to whether to stop or de-escalate or escalate antibiotics. In our study, we observed semiquantitative count values agreement for bacterial loads between 10^5-10^7 CFU/mL, while loads of 10^4 CFU/mL were not always accompanied by growth of pathogens by Standard culture.

In most samples (62.5%), the same predominant pathogen was identified by both molecular and culture methods.

Finally, this method also enables the identification of viruses only responsible for lower respiratory tract infections (*Respiratory Syncytial Virus, Rhinovirus, Adenovirus, Metapneumovirus, Influenza and Parainfluenza viruses*), causing bronchiolitis and severe dyspnea, especially in newborns (12 samples).

Furthermore, the panel also allows identification of the main bacterial resistances. In our case it allowed for rapid detection of 6 CTX-M strains (2 *P. aeruginosa* and 4 *Klebsiella spp*. ESBL beta-lactamase producers) and 6 VIM carbapenemase producer strains (Verona Integron-Mediated Metallo-β-lactamase) (2 *K. pneumoniae* and 4 *P. aeruginosa*), providing clinicians with very important orientation in the choice of the antibiotic, as well as in the hospital management of the patient.

Indeed, the presence of genetic resistance determinants prompted step-up treatments which may not be considered standard of care, as few antibiotics are currently active against beta-metallo enzymes such as VIMs. Though time to treatment was not considered here, it is tempting to speculate that shorter time to results was achieved in a highly endemic resistance setting, such as in our center.

Finally, in our series, the PN panel also allowed identification of *Legionella pneumophila*, otherwise impossible to detect by means of standard culture method.

Being a retrospective analysis, it was no longer possible to perform additional molecular analyses on the samples; however, being a first level Laboratory, the use of molecular biology already guarantees considerable assistance in clinical practice. In addition, the right antibiotic therapy for lower respiratory tract infection, suggested by the result obtained with molecular biology, is always monitored by the dosage of Procalcitonin in serum.

The Laboratory receives samples from the Pediatric Hospital and Emergency Room; therefore, the molecular investigation is often requested upon entrance to the Emergency Room, as screening.

Clinical outcomes are missing due to inaccessibility of medical records.

The molecular test is certainly more expensive, but it is very important to give an initial response to clinicians in about 1.5 hours.

The important element is the eligibility of the sample and clinical request (the ratio). In these cases, the utility justifies the cost, above all for the correct choice of antibiotic and the problem of antibiotic resistance related to the incorrect use of antibiotics (also for empiric treatment when entering the Hospital).

All of the patients (or their authorized representatives) provided written informed consent.

CONCLUSION

This was a retrospective observational monocentric study, conducted by assessing samples over a two-year period. The data hereby presented is to our knowledge the first data concerning the use and comparison of PN panel results in a pediatric cohort at high risk of MDR infections.

Our results highlight the importance of incorporating this panel in clinical practice not only for departments such as intensive care, but also for emergency departments or Urgency pediatrics, for the rapid detection of etiological agents underlying causes of respiratory tract infection and for the establishment of targeted therapy, potentially reducing the unnecessary use of antibiotics and improving the quality of health.

However, a final diagnosis of lower respiratory tract infection must always involve interplay between the use of such methods and the clinical picture, the patient's history, the local epidemiology, and the appropriateness of the sample. Considering the increasing diffusion of multi-drug resistant strains, especially when gram negative bacteria are considered, such as carbapenemase-producing strains, it is advisable to implement these methods in routine practice in dedicated health care facilities.

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