Enhanced Detection of Community-Acquired Pneumonia Pathogens With the BioFire® Pneumonia FilmArray® Panel.

David Gilbert  
*Providence-Portland Medical Center, Portland, Oregon.*

James E Leggett  
*Providence Portland Medical Center, Portland, Oregon, USA.*

Lian Wang  
*Medical Data Research Center, Providence Health and Services, Portland, OR, USA*

Shirin Ferdosian  
*Department of Medical Education, Providence Portland Medical Center, Portland, Oregon.*

Gita D Gelfer  
*Department of Medical Education, Providence Portland Medical Center, Portland, Oregon.*

See next page for additional authors

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Original Article

Enhanced Detection of Community-Acquired Pneumonia Pathogens With the BioFire® Pneumonia FilmArray® Panel


Department of Medical Education, Providence Portland Medical Center, Portland, Oregon

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ABSTRACT

Background: Although most observational studies identify viral or bacterial pathogens in 50% or less of patients hospitalized with community-acquired pneumonia (CAP), we previously demonstrated that a multi-test bundle (MTB) detected a potential pathogen in 73% of patients. This study compares detection rates for potential pathogens with the MTB versus the BioFire® Pneumonia FilmArray® panel (BPFA) multiplex PCR platform and presents an approach for integrating BPFA results as a foundation for subsequent antibiotic stewardship (AS) activities.

Methods: Between January 2017 to March 2018, all patients admitted for CAP were enrolled. Patients were considered evaluable if all elements of the MTB and the BPFA were completed, and they met other a priori inclusion criteria. The primary endpoint was the percentage of potential pathogens detected using the MTB (8 viral and 6 bacterial targets) versus the BPFA (8 viral and 18 bacterial targets). Blood and sputum cultures were performed on all patients. Two or more procalcitonin (PCT) levels assisted clinical assessments as to whether detected bacteria were invading or colonizing.

Results: Of 585 enrolled patients, 274 were evaluable. A potential viral pathogen was detected in 40.5% with MTB versus 60.9% of patients with BPFA with an odds ratio (95% CI) of 9.00 (4.12 to 23.30) p < 0.01. A potential bacterial pathogen was identified in 66.4% with the MTB vs 75.5% with the BPFA odds ratio (95% CI) of 2.09 (1.24 to 3.59), p = 0.003). Low PCT levels helped identify detected bacteria as colonizers.

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Key words:
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SUMMARY

Compared to a standard multi-test diagnostic bundle, the sputum BioFire® Pneumonia FilmArray® multiplex platform, significantly increased the detection of potential viral and bacterial pathogens in hospitalized adult patients with community-acquired pneumonia.

1. INTRODUCTION

Pneumonia is the leading cause of death due to infection in the United States with an estimated 1.2 million annual hospitalizations (Self et al., 2017). Management of community-acquired pneumonia (CAP) entails empiric antibiotic therapy that targets the most likely bacterial pathogens. The American Thoracic Society-Infectious Diseases Society of America CAP guidelines reserve microbiologic testing for patients with severe CAP (Metlay et al., 2019). Further, “rapid, cost-effective, sensitive, and specific diagnostic tests to identify organisms causing CAP have potential to improve routine care by supporting the use of targeted therapy…” (Metlay et al., 2019)

A benchmark study reported that microbiologic testing identified the microbiologic etiology in only 853 of 2259 (38%) hospitalized CAP patients (Jain et al., 2015). To improve this diagnostic yield, we reported that a multi-test bundle (MTB) consisting of sputum and

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blood bacterial cultures, polymerase chain reaction (PCR) testing of nasopharyngeal swabs (NP) samples by the multiplex Biofire® (Bio-fire® Co., Salt Lake City, Utah) Respiratory FilmArray® panel, anterior nasal swab PCR for *Staphylococcus aureus*, nasopharyngeal swab for *Streptococcus pneumoniae*, and urine antigen testing for *S. pneumoniae* and *Legionella pneumophila* detected a potential bacterial and/or viral pathogen in 73% of patients (Gilbert et al., 2016, Carvalho Mda et al., 2007). The feasibility of this approach was hampered by long turn-around times (TAT) for multiple MTB components, and the failure of the MTB to detect common potential bacterial pathogens, such as *Moraxella* and *Haemophilus species*.

The Biofire® Pneumonia FilmArray® (BPFA) panel probes sputum samples for 8 respiratory viruses and 18 bacteria with a 1 to 2-hour TAT. However, the performance of BPFA as compared with multi-element testing, such as MTB, has neither been evaluated in large studies, nor integrated into clinical practice to identify invasive vs colonizing pathogens. Herein, we compare the detection of potential viral and bacterial pathogens with MTB as compared with BPFA. We hypothesized that the BPFA would detect more potential viral and bacterial pathogens than MTB. We also present our approach to integrating BPFA results into clinical practice by eliminating unnecessary tests and serving as the basis of antibiotic stewardship (AS) activities.

2. MATERIALS AND METHODS

2.1. Study Patients

The study was registered with clinicaltrials.gov (NC02580384) as a prospective, non-blinded comparison of pathogen detection with patients as their own controls was conducted at Providence Portland Medical Center, a 480-bed community teaching hospital in Portland, Oregon between January 2017 and March 2018 and approved by the Privacy Board and the Institutional Board (IRB) that waived informed consent. Subjects were identified from adults (over the age of 18) presenting to the Emergency Department with respiratory symptoms meeting inclusion criteria as to whether detected pathogens were colonizing or invasive.

2.2. Specimen collection

Emergency Department (ED) nurses collected two blood culture samples and expectorated or hypertonic saline-induced sputum (or endotracheal aspirate) for BPFA; in addition, sputum was submitted for culture. For MTB, nasopharyngeal swab samples were submitted for the BioFire® Respiratory FilmArray® panel and lab-generated PCR for *S. pneumoniae* (Carvalho Mda et al., 2007); anterior nasal swab for *S. aureus* PCR (BD Max SR®), and urine for antigen detection (Binaxnow, Abbott Labs, Abbott Park, IL) of *S. pneumoniae* and *L. pneumophila* (Table 1). Blood was collected for PCT testing (Used Vidas B.R.A.

2.3. Data collection

The laboratory entered MTB results into the electronic medical record (EMR), and study investigators re-entered EMR data in the FileMaker® database. Being investigational, the BPFA results were entered by investigators into the study database. Three authors (DNG, JEL, SF) verified the accuracy of all transcribed data.

2.4. Study objectives

The primary objective was to determine if BPFA improved detection rates for potential viral and bacterial pathogens as compared with MTB. We also present an approach to integrating BPFA results into clinical practice by eliminating unnecessary tests and serving as the basis of antibiotic stewardship (AS) activities.

2.5. MTB and BPFA Test Components

Table 1 lists the components of the MTB and BPFA entered into comparative analyses. See Table 2 for components of the Biofire® Respiratory and Pneumonia FilmArray® panels used in MTB and BPFA.

2.6. Clinical Categorization and Adjudication

The FileMaker® database calculated a pneumonia severity index (PSI) for each patient (Fine et al., 2020). To assess the clinical importance of detected pathogens, patients were divided into four categories (Table 3) based on their clinical and laboratory presentation, PCT test results, and likelihood based on clinical judgment (adjudication) as to whether detected pathogens were colonizing or invasive.

In adults with CAP, detection of a potential viral respiratory pathogen is accepted as compatible with the etiology of the patient’s invasive infection as the frequency of asymptomatic colonization is very low. In a prospective controlled detection trial in adult CAP patients, detection of influenza, hMPV, and RSV “probably indicate an etiologic role.” Detection of other respiratory viruses, especially in children, requires further study (Self et al., 2016).

The biomarker procalcitonin (PCT) was used to assist in the clinical decision as to whether a detected virus and/or bacteria was a commensal. PCT serum levels do not increase with pure viral infection but do increase with invasive bacterial infection or the combination of viral and bacterial disease (Gilbert, 2020, Gilbert, 2017).

**Table 1** Components of Multi-Test Bundle and BioFire® Pneumonia FilmArray® Testing

<table>
<thead>
<tr>
<th>Multi-Test Bundle</th>
<th>BioFire® Pneumonia FilmArray®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood cultures x 2</td>
<td>Blood cultures x 2</td>
</tr>
<tr>
<td>Sputum culture if sputum purulent</td>
<td>Sputum culture if sputum purulent</td>
</tr>
<tr>
<td>NP swab for Biofire® Respiratory FilmArray**</td>
<td>Sputum for Biofire® Pneumonia FilmArray**</td>
</tr>
<tr>
<td>Nasal swab for <em>Staphylococcus aureus</em> PCR (BD Max SR®)</td>
<td></td>
</tr>
<tr>
<td>NP swab for <em>Streptococcus pneumoniae</em> PCR</td>
<td></td>
</tr>
<tr>
<td>Urine for <em>Streptococcus pneumoniae</em> and <em>Legionella pneumophila</em> antigens (Binaxnow®)</td>
<td></td>
</tr>
</tbody>
</table>

NP = nasopharyngeal, PCR = polymerase chain reaction  
* sputum or sputum equivalent (ie, endotracheal aspirate, bronchoalveolar lavage)  
** Organism targets for BioFire® FilmArray® panels listed in Table 2.

H.M.S instrument and PCT reagents from Biomerieux, Durham, NC) with PCT retesting 4-6 hours later. A rapid response laboratory adjacent to the ED received all samples.
The BPFA panel includes genomic semi-quantitation of 15 of 18 bacterial targets with the premise that higher bacterial “loads” would be consistent with invasive infection. However, concordance between genomic semi-quantitation and sputum culture CFU/ml is reported between 40 and 54% and the degree of concordance varied by organism (Biofire 2020, Lee et al., 2019, Murphy et al., 2020). For these reasons, we elected to not use the BPFA semi-quantitation result in the adjudication of positive pathogen detection.

### 2.7. Cost Considerations

We determined the costs of BPFA versus all elements in the MTB based on 2017-2018 cost data.

### Table 2

Comparison of organism targets in the Biofire® Respiratory FilmArray and the Biofire Pneumonia FilmArray® Panels. The Respiratory Panel requires a nasopharyngeal swab and the Pneumonia Panel requires sputum.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Respiratory Panel</th>
<th>Pneumonia Panel</th>
<th>Atypical Bacteria</th>
<th>Respiratory Panel</th>
<th>Pneumonia Panel</th>
<th>Bacteria</th>
<th>Respiratory Panel</th>
<th>Pneumonia Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>X</td>
<td>X</td>
<td>Bordetella pertussis</td>
<td>X</td>
<td>O</td>
<td>Acinetobacter calcoaceticus-bau-</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>X</td>
<td>X</td>
<td>Chlamydia pneumonia</td>
<td>X</td>
<td>X</td>
<td>Enterobacter cloacae complex</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>X</td>
<td>X</td>
<td>Legionella pneumophila</td>
<td>O</td>
<td>X</td>
<td>Escherichia coli</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Human rhinovirus/enterovirus</td>
<td>X</td>
<td>X</td>
<td>Mycoplasma pneumonia</td>
<td>X</td>
<td>X</td>
<td>Haemophilus influenza</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Influenza A</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Klebsiella aerogenes</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Influenza B</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Klebsiella oxytoca</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Klebsiella pneumonia group</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Respiratory syncyntial virus</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Moraxella catarrhalis</td>
<td>O</td>
<td>X</td>
</tr>
</tbody>
</table>
| **Includes 4 strains of coronavirus, but not SARS-CoV-2**

### Table 3

Clinical Classification of Patients Based on Integration of Test Results with Clinical Judgment

**Uninfected: No evidence of CAP**
- Post-admission clinical, laboratory, and imaging studies document an alternative non-infectious diagnosis: e.g., congestive heart failure.
- Pure Bacterial Pneumonia
- Proven: A compatible clinical syndrome (fever, cough, new pulmonary infiltrates, elevated white blood count) with detection of only bacterial pathogen(s) by one or more of: Biofire® Respiratory FilmArray® NP S. pneumoniae PCR, nasal swab for S. aureus PCR, urine antigens, sputum and/or blood culture, or BPFA.
- Presumptive: Clinical CAP syndrome with compatible chest radiographic abnormalities without detection of a potential bacterial pathogen by any of the same tests listed in the last paragraph. A PCT level ≥0.25 ng/ml with no other explanation was interpreted as presumptive evidence of invasion by an undetected bacterial pathogen.
- Pure Viral Pneumonia
- Presumptive: High probability of viral pneumonia based on compatible clinical presentation, detection of a virus by the Biofire® Respiratory FilmArray® or BPFA absent detected bacterial pathogen by FilmArray®, and serum PCT level <0.25 ng/ml.
- Viral and Bacterial Co-Infection
- Presumptive: Detection of a virus by either the Biofire® Respiratory FilmArray® or BPFA. In addition, detection of a bacterial pathogen in sputum and/or blood cultures, urine antigen tests, the Biofire® Respiratory FilmArray®, the BPFA, the nasal swab for S. aureus and/or the NP PCR for S. pneumoniae.
- Pneumonia of Unclear Etiology
- Clinical criteria for CAP but no bacterial or viral pathogen detected by any test and no non-infectious disease diagnosed.

**2.8. Statistics**

The performance between MTB and the BPFA was compared based on the number of pathogen species (bacterial and/or viral) detected in each patient by the two methods, using the paired t-test and Wilcoxon signed rank test. The same analysis was repeated for the number of bacterial species detected and for the number of viral species detected.

McNemar test assessed the difference in the proportions of patients detected with pathogen(s) (combined bacterial plus viral, bacterial, viral, and selected individual specific pathogen species) between MTB and BPFA.

The detection of individual specific pathogen species by the BPFA method was evaluated and presented as positive percent agreement (PPA) and negative percent agreement (NPA) against the MTB method; p-value <0.05 considered statistically significant. The R statistical program (www.r-project.org, R Foundation for Statistical Computing, Vienna, Austria) performed all analyses.

### 3. RESULTS

Of 585 enrolled patients, 274 (47.2%) were evaluable and 311 were excluded (Figure 1). Non-evaluability most commonly resulted from a final diagnosis other than CAP [159 of 311 (51.1%)], not obtaining a sputum or equivalent for BPFA [139 of 311 (44.7%)], and/or non-infectious disease diagnosed.
The BPFA outperformed the MTB in identifying all patients with *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus agalactiae*, and *Enterobacteriaceae*, p ≤0.001 (Figure 2). Detection rates for *S. pneumoniae* varied widely in the 274 patients between individual elements of MTB (Figure 3). The BPFA detected *S. pneumoniae* more often (in 78 patients, 28.5%), than any individual component in MTB but not with the composite MTB (Figure 3).

*S. aureus* was detected by nasal swab PCR in 94 of 274 (34.3%) patients vs. 71 of 274 (25.9%) by BPFA, p=0.002. Six patients (all detected by BPFA probe of sputum) had *S. aureus* bacteremia, three methicillin-sensitive *S. aureus* (MSSA) and three methicillin-resistant *S. aureus* (MRSA).

Only one patient had a positive urine antigen test for *Legionella pneumophila*.

The BPFA detected significantly more patients with potential viral pathogens (coronavirus, hMPV, influenza, parainfluenza, and rhinovirus) than the BioFire® Respiratory FilmArray® component of MTB (Figure 4). Influenza virus was detected in 61 (22.3%) with BPFA as compared to 39 (14.2%) with MTB, p <0.001. The number of patients with one or more detectable viruses increased from 41% with MTB to 61% with BPFA, p<0.001.

### 3.3. Positive Blood Cultures

Of 274 CAP patients, 22 (8%) had positive blood cultures deemed pathogens rather than contaminants. The same organism was detected in the sputum culture in 9 of the 22 (41%) patients. On the other hand, *S. aureus* by sputum BPFA in 17 of the 22 (77%) patients.

*S. pneumoniae* was identified in the blood of 10 patients, *S. aureus* in 6, *S. pyogenes* in 2, and *Enterobacter cloacae, E. coli, S. agalactiae*, and *Aerococcus viridans* each in one patient. A concomitant viral pathogen was detected by BPFA in 10 of the 22 patients with positive blood cultures.

### 3.4. Multiple Pathogen Detection

The superiority of the BPFA as compared with MTB in detecting co-infections is reported in Table 6. In one extreme example, BPFA detected 1 virus (RSV) and 5 bacterial species (*E.coli*, *P. aeruginosa*, *K. pneumoniae*, *S. marcescens*, and MRSA) while the MTB detected no virus and 3 bacteria (*P. aeruginosa*, *E. coli*, and MSSA).

### 3.2. Comparison of Detection Rates for Specific Bacterial and Viral Pathogens

### 3.1. MTB and BPFA Pathogen Detection Rates

The proportion of all patients detected by MTB or BPFA with potential bacterial pathogens (± a viral pathogen) or potential viral pathogens (± a bacterial pathogen) are shown in Table 5 along with the aggregate proportion of patients detected with any potential pathogen (bacterial and/or viral). The BPFA panel as compared with MTB detected more patients with bacterial pathogens (75.5% vs 66.4%, p=0.003) and viral pathogens (60.9% vs 40.5%, p<0.001). Significantly more patients were detected by BPFA as having any potential pathogen (viral, bacterial or both) (90.6% vs 80.9%, p=0.001), Table 5.

### Table 5

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MTB</th>
<th>BPFA</th>
<th>Effect Measure</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any bacteria, patient No. (%)</td>
<td>182 (66.4%)</td>
<td>207 (75.5%)</td>
<td>OR (95% CI)= 2.09 (1.24,3.59)</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of bacterial species per patient</td>
<td>Mean (SD) 0.92 (0.82)</td>
<td>1.35 (1.19)</td>
<td>Diff (95% CI)=0.43 (0.30,0.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 1 (0.1)</td>
<td>1 (1.2)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any virus, patient No. (%)</td>
<td>111 (40.5%)</td>
<td>167 (60.9%)</td>
<td>OR (95% CI)=9.00 (4.12,23.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of virus species per patient</td>
<td>Mean (SD) 0.43 (0.55)</td>
<td>0.68 (0.61)</td>
<td>Diff (95% CI)=0.25 (0.18,0.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 0 (0.1)</td>
<td>1 (0.1)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Combined bacteria and virus species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any pathogen detected, patient No. (%)</td>
<td>224 (80.9%)</td>
<td>251 (90.6%)</td>
<td>OR (95% CI)=3.33 (1.71, 6.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of pathogen species per patient</td>
<td>Mean (SD) 1.35 (0.94)</td>
<td>2.03 (1.27)</td>
<td>Diff (95% CI)=0.68 (0.53,0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 1 (1.2)</td>
<td>2 (1.3)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MTB = Multi-test bundle; BPFA = BioFire® Pneumonia FilmArray®

* p-value was based on McNemar test for odds ratio, paired t-test for mean difference, and Wilcoxon signed rank test for distribution.
Figure 2. Comparison of MTB versus BPFA for detecting number of patients with potential bacterial pathogens, with or without detectable virus. Some patients were detected with more than one bacterial species.

Figure 3. Comparison of number of patients detected with Streptococcus pneumoniae by individual elements of the multi-test bundle vs the BioFire® Pneumonia FilmArray®. Some patients had more than one test element positive for S. pneumoniae. PCR denotes polymerase chain reaction.
3.5. Potential Role of Serum PCT Levels

Detected pathogens are either colonizing or invading and stimulating host innate immune inflammatory response. All 274 evaluable patients had a clinical syndrome consistent with pneumonia; one or more potential pathogens were detected in 261. As the clinical syndromes lack discriminating features, we assessed whether the biomarker PCT could help adjudicate whether the detected potential virus and/or bacteria were colonizing or invading.

PCT levels are a marker of activation of the host innate immune inflammatory response by invading bacterial pathogens but not by pathogenic respiratory viruses (Gilbert, 2020, Gilbert, 2017). PCT levels increase in the presence of concomitant invasive disease by a virus and/or bacteria.

We divided our patients into four categories depending on the pathogens detected (Figure 5). By convention, a serum PCT elevation of ≥0.25 ng/ml is selected as a clinically meaningful increase.

In 87 of 274 patients, only potential bacterial pathogens were detected by the MTB and/or BPFA of which 69 (79.3%) had PCT levels ≥0.25 ng/ml, a result consistent with invasive bacterial infection. In the remaining 18 (20.7%) patients, the PCT of <0.25 ng/ml was considered consistent with colonization by the detected bacteria.

For 143 patients, both viral and bacterial pathogens were detected by MTB and/or BPFA. Ninety-six (96) (67.1%) of these patients had PCT levels ≥0.25 ng/ml, which was interpreted as consistent with bacterial and viral co-infection. Forty-seven (47) patients (32.8%) had PCT values <0.25 ng/ml consistent with bacterial colonization and viral infection.

For 31 patients, only a potential viral pathogen was detected. In 11 (35.5%) the PCT level was ≥0.25 ng/ml consistent with viral infection and a superimposed, but undetected, bacterial pathogen.

The range of PCT levels in the 13 patients with no detected pathogens may reflect the presence of undetected viral and/or bacterial pathogens.

3.6. Comparative Cost of MTB vs Test Bundle with BPFA

Our laboratory estimated the costs for MTB as compared with the projected cost of a new test bundle anchored by the BPFA, Table 7. Results indicate cost savings for the BPFA approach partially because three nasal swab PCRs and the two urine antigen tests would no longer be needed.

4. DISCUSSION

Our results demonstrate, in adult patients hospitalized for CAP, that the Biofire® Pneumonia FilmArray® (BPFA) detected more
patients with potential viral and bacterial pathogens (90.6%) than the MTB (80.9%), the latter having been our standard of practice (Gilbert et al., 2016).

BPFA sputum detection of 6 of the 7 respiratory viruses was statistically more frequent than testing for the same 7 viruses with the nasopharyngeal Biofire® respiratory FilmArray® panel which is part of the MTB.

The higher yield with the BPFA may reflect higher viral loads in the lower as compared with upper airways (Jeong et al., 2014, Wunderink, 2017). Alternatively, the higher yield may be due to differences in the viral primers used in the BPFA vs the primers in the Biofire respiratory FilmArray® (BioFire® FilmArray® User Instruction booklets).

For bacterial pathogens, BPFA detected more patients with 4 of the common pathogens that cause CAP. Notably, BPFA detected more patients with S. pneumoniae than individual urine antigen testing, nasal PCR, or sputum and blood cultures although statistically equivalent to the composite MTB results.

S. aureus was the only bacterium detected more frequently using the nasal swab PCR in the MTB vs the sputum BPFA, p=0.001. The difference may result from differences in the PCR primers used for the nasal swab PCR in the MTB as compared with BPFA primers. In addition, replication of S. aureus is enhanced at 35°C (anterior nares) vs 37°C in the lower airway source of sputum sent for BPFA (Matoos-Mora et al., 1988, Milne et al., 1987).

An emerging body of evidence supports the use of PCR for the detection of etiologic pathogens for CAP. Previous studies combined cultures of sputum and blood, urine antigen detection, and NP multiplex platforms that primarily focused on viral pathogens and a few atypical bacteria. The reported detection rates for pathogens have varied between 38-53% of patients (Sel et al., 2017, Jain et al., 2015, Stockmann et al., 2018, Musher et al., 2013, Musher et al., 2017).

First generation multiplex PCR respiratory panels only detected three atypical bacteria. Cultures and antigen detection are needed to detect common pneumonia pathogens: i.e., S. pneumoniae, H. influenzae, M. catarrhalis and others. We generated a MTB that included PCR probes for S. pneumoniae and S. aureus plus the Biofire® Respiratory FilmArray® panel which includes 7 viruses (Gilbert et al., 2016).

Traditional sputum cultures and antigen detection may miss bacterial pathogens obscured by in vitro overgrowth of normal oral flora and/or suppression of growth by empiric antibiotic therapy. Hence, we compared the present study to determine if the BPFA, with the 18-targeted bacterial targets, would increase detection as compared to our standard of care (SOC) MTB.

### 4.1. Study of BPFA vs SOC by Others

Other studies have compared pathogen detection by BPFA with standard of care (SOC) diagnostics. The SOC is not standardized from study to study but includes some combination of cultures of sputum and blood, urine antigens and nasopharyngeal swabs for multiplex PCR panels that most often focus on seasonal respiratory viruses.

In representative studies, the BPFA panel detected a viral and/or bacterial potential pathogen in 47.4 to 72% of the CAP patients (Lee et al., 2019, Murphy et al., 2020, Webber et al., 2020, Table 7

<table>
<thead>
<tr>
<th>Test</th>
<th>MTB, $</th>
<th>BPFA, $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine antigen</td>
<td>No need for urine antigens</td>
<td></td>
</tr>
<tr>
<td>Legionella</td>
<td>30.00</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>19.00</td>
<td></td>
</tr>
<tr>
<td>Nasal swabs – PCRs</td>
<td>No need for nasal swabs</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>42.00</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>PCR “resp panel”</td>
<td>91.00</td>
<td></td>
</tr>
<tr>
<td>Biofire Pneumonia FilmArray</td>
<td>180.00</td>
<td></td>
</tr>
<tr>
<td>Sputum C&amp;S</td>
<td>8.23</td>
<td>8.23</td>
</tr>
<tr>
<td>Blood culture x 2</td>
<td>9.80</td>
<td>9.80</td>
</tr>
<tr>
<td>Tech x 1 hr @536.50/hr</td>
<td>36.50</td>
<td></td>
</tr>
<tr>
<td>Tech x 1/2 hr @36.50/hr</td>
<td>18.00</td>
<td></td>
</tr>
<tr>
<td>PCT @ $23 ea x 2</td>
<td>46.00</td>
<td>46.00</td>
</tr>
<tr>
<td>SUBTOTAL</td>
<td><strong>307.53</strong></td>
<td><strong>262.03</strong></td>
</tr>
<tr>
<td>Overhead @ 30%</td>
<td>92.00</td>
<td>79.00</td>
</tr>
<tr>
<td>TOTAL</td>
<td><strong>399.53</strong></td>
<td><strong>341.03</strong></td>
</tr>
</tbody>
</table>

$ = Dollars: represents estimated cost as per 2018

Figure 5. Box plot of distribution of serum procalcitonin levels in each of the 4 categories of pathogen detection. Each dot represents a single patient. The red line marks a serum procalcitonin level of 0.25 ng/ml.
Buchan et al., 2020). The number of patients varied from 595 to 1682. Patient population, comorbidities, severity of illness, season of the year, variations in PCR primers are a few of many pertinent variables.

Akin to our results, Webber et al detected potential bacterial pathogens in 60 patients by both SOC and BPFA plus an additional 92 detections of bacteria by the BPFA (Webber et al., 2020). Buchan et al reported similar results from 259 adult patients (Buchan et al., 2020).

Gadsby’s research group has developed a PCR pneumonia panel with probes for 13 bacteria and 7 viruses. In 323 adult CAP patients, a potential bacterial pathogen was identified in 81.1%. H. influenzae and S. pneumoniae were the most frequently identified bacteria (Gadsby et al., 2016).

The BPFA is not the only commercial rapid syndromic molecular test panel for pneumonia pathogen identification (Noviello and Huang, 2019, Poole and Clark, 2020). The Curetis Unyvero Hospitalized Pneumonia panel is FDA-approved and includes 17 bacterial, 3 atypical bacteria, 1 fungal, and 19 resistance genes but no viral targets. There are at least 4 other commercial entities developing pneumonia diagnostic panels (Poole and Clark, 2020).

4.2. Colonization versus Infection

Several studies report increased detection of potential viral and bacterial pathogens in lower respiratory secretions of CAP patients when SOC diagnostics are compared to use of the BPFA (Lee et al., 2019, Murphy et al., 2020, Webber et al., 2020, Poole and Clark, 2020). The positive and negative percent agreements concur with some variation as expected with variations in the SOC tests employed.

Rapid, sensitive, and specific identification of potential bacterial pathogens is a premise of improving the care of CAP patients. In theory, identification of a pathogen will allow switching from empiric antibiotic therapy to more focused specific therapy. Before switching, clinicians need to consider whether the detected bacteria are only colonizing and not a concern.

The BPFA platform provides a semi-quantitative number of bacterial genomes/ml present. Traditional indicators of bacterial invasion correlate with greater the numbers of bacteria, but patients with selected co-morbidities may be only colonized and yet have a high density of bacteria: e.g., cystic fibrosis, bronchietasis, colonized tracheostomy. Growth characteristics of organisms are another variable. Antibiotic therapy can influence bacterial density. Hence, we elected to not utilize the results of bacterial quantitation in our adjudication of whether the detected bacteria are colonizing or invading.

We elected to use the serum biomarker procalcitonin (PCT) to assist in the assessment of whether detected bacteria were colonizing or invading. Elevations of serum PCT indicate activation of innate immunity (Gilbert, 2020, Gilbert, 2017). PCT gene activation does not occur with pure viral infection but promptly increases in response to invasion by bacteria to include dual infection with a virus (Branche et al., 2019, Schuetz et al., 2017, US Food and Drug Administration 2017). The other variables that influence PCT serum levels; e.g., impaired renal function, are known (Gilbert, 2020).

Jain and Pavia point out that the “holy grail” would apply molecular diagnostics to lung tissue (Jain and Pavia, 2016). In the absence of lung tissue, we integrated BPFA results with the clinical presentation and PCT test results to characterize with an a priori classification system the likelihood of CAP due to bacterial and/or viral pathogens (Table 3). This approach allows clinicians to focus the antimicrobial regimen and promote antibiotic stewardship.

Based on previous research, we selected PCT serum levels of ≥0.25 ng/ml as consistent with activation of an innate immune response by invasive bacteria (Branche et al., 2019, Schuetz et al., 2017). PCT levels of <0.25 ng/ml were interpreted as consistent with colonization (negative predictive value for invasive bacterial infection is >90%) (Self et al., 2017, Gilbert, 2017, Stockmann et al., 2018). The potential value of pathogen detection coupled to PCT levels is acknowledged in multiple publications (Branche et al., 2019, Schuetz et al., 2017, US Food and Drug Administration 2017, Jain and Pavia, 2016, Wunderink et al., 2020). A recent economic model supported use of PCT serum levels as a way to reduce hospital costs, decrease the risk of antibiotic resistance and Clostridium difficile infections, and enhance clinical decision-making beyond clinical judgment and heuristic approaches (Mewes et al., 2020).

4.3. Suggested Revised Multi-Test Bundle

The diagnostic yield of BPFA creates an opportunity to simplify diagnostic testing for CAP. The BPFA has PCR primers for all Legionella species causing disease in humans suggesting elimination of the urine antigen test for L. pneumophila. Also, the urine antigen test and NP PCR for S. pneumoniae may be eliminated because BPFA outperformed both. The low yield of blood cultures compared to BPFA suggests they can be reserved for ICU patients (Metlay et al., 2019). Of note, nasal swabs for S. aureus PCR may remain an optional test added to BPFA as nasal samples were 9% more sensitive than sputum samples submitted to BPFA. Sputum cultures remain necessary to provide antibiotic susceptibility testing of detected bacteria.

A new test bundle would include BPFA, sputum culture and sensitivity, blood cultures for the critically ill, and perhaps nasal S. aureus PCR. Two (2) PCT levels 4-6 hours apart for their high negative predictive value and to help discriminate between infection and colonization. This bundle replaces traditional testing, which may take 1-2 days to complete vs a bundle anchored by BPFA that takes less than 2 hours. Moreover, this proposed bundle decreases the cost of testing (Table 7) and, in theory, can reduce antibiotic consumption, and shorten length of hospitalization (Buchan et al., 2020).

4.4. Limitations

Patients inability to provide sputum and the difficulty of ED physicians in this single center study to make an accurate diagnosis of CAP excluded a majority of patients from the study. To avoid excluding patients from BPFA testing, studies are needed to assess the potential value of oropharyngeal swab specimens in lieu of sputum for testing, as has been investigated for children (Wang et al., 2019). Only 13 patients were excluded because of incomplete test results beyond failure to collect sputum. Second, despite the large panel size, a failure of the PCR panels to detect bacteria or virus does not exclude the presence of an undetected virus and/or bacteria. Third, without systematic antibody seroconversion testing, we could not provide definitive differentiation between invasive viral disease vs viral colonization. However, protracted viral colonization of adults is rare. Lastly, we did not document the influence of rapid pathogen detection on antibiotic therapy, antibiotic adverse events to include C. difficile infection, antibiotic resistance, or need for patient isolation.

5. Conclusion

For hospitalized adult patients with CAP, the BPFA multiplex platform detected significantly more common viral and bacterial potential pathogens as compared to a MTB. Serum PCT levels can help in the judgement as to whether detected bacteria are colonizing or causing infection.

Future studies are needed to critically assess the influence of enhanced pathogen detection on antibiotic usage, prevalence of antibiotic adverse effects and antibiotic resistance, and other facets of antimicrobial stewardship.

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REFERENCES


