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The potential of molecular diagnostics and serum procalcitonin levels to change the antibiotic management of community-acquired pneumonia

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ABSTRACT

Two diagnostic bundles were compared in 127 evaluable patients admitted with community-acquired pneumonia (CAP). Diagnostic modalities in all patients included cultures of sputum (if obtainable) and blood, urine for detection of the antigens of *Streptococcus pneumoniae* and *Legionella pneumophila*, and nasal swabs for PCR probes for *S. pneumoniae* and *Staphylococcus aureus*. At least one procalcitonin level was measured in all patients. For virus detection, patients were randomized to either a 5-virus, lab-generated PCR panel or the broader and faster FilmArray PCR panel.

Overall, an etiologic diagnosis was established in 71% of the patients. A respiratory virus was detected in 39%. The potential for improved antibiotic stewardship was evident in 25 patients with only detectable respiratory virus and normal levels of PCT.

1. Introduction

Community-acquired pneumonia (CAP) is a common and potentially lethal infectious disease that requires concomitant attempts to determine a microbial etiology and the prompt initiation of broad spectrum empiric antibacterials (Mandell et al., 2007).

Our study was designed to: optimize the rapid detection of pathogenic bacteria and/or viruses; use normal serum procalcitonin (PCT) levels to exclude the presence of invasive bacteria; provide the microbiologic and PCT data to clinicians within 48 hours or less of admission; and determine if physician providers would respond to the data provided by switching from empiric to either no therapy (non-influenza viral illness) or a directed specific antimicrobial regimen.

The protocol described herein is the same used during January to March, 2014 (Gelfer et al., 2015), enrolling an additional 127 patients during the 2014-2015 winter months.

2. Materials and methods

2.1. Study conduct and design

2.1.1. Study conduct

This study was conducted as a non-blinded cluster randomization trial at a 480 bed community-teaching hospital in Portland Oregon (Providence Portland Medical Center-PPMC). The project was approved by both the Institutional Review Board (IRB) and the Privacy Board of PPMC. Only de-identified chart data was collected; the IRB indicated no need for informed consent.

Prior to study initiation, the investigators reviewed the study protocol with Emergency Department nurses, physicians, hospitalists, residents, and clerks.

A diagnosis of CAP requiring admission made by ED physicians prompted enrollment in the study. The ED physician ordered protocol-mandated diagnostic "bundles" which were initiated by ED nurses, who also ordered empiric antibiotic therapy. ED unit clerks notified investigators of a new patient. The protocol neither dictated nor suggested antibiotic management to either the ED or inpatient physicians.

Providers learned of test results via the electronic medical record (EMR), with two exceptions. Providers were notified immediately of positive blood cultures or identification of influenza.

2.1.2. Study design

A common core of diagnostic tests was applied to all patients in the study: i.e., two blood cultures, sputum culture and sensitivity, serum PCT level, urine antigen testing for *Legionella pneumophila,* serogroup 1 and *Streptococcus pneumoniae*, nasal swabs for PCR detection of the lyt gene of *S. pneumoniae* and *Staphylococcus aureus. S. aureus* PCR (BD Max Staph SR) was purchased from Becton-Dickinson.

PCT levels were determined using an immunoassay (bioMerieux) performed on a Vidas system. The protocol called for only one baseline PCT serum level; providers ordered additional PCT levels at their discretion. PCT results included an interpretative algorithm modeled after a widely-used used European format (Schuetz et al., 2012, 2013). Values below 0.1 ng/mL were interpreted as "bacterial etiology very unlikely"; values >0.25–0.5 ng/mL as "bacterial etiology likely"; and values >0.5 ng/mL as "bacterial etiology very likely". The algorithm suggests

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a repeat PCT level in 4-6 hours in those patients with levels ≤ 0.25 ng/mL and possible evolving bacterial infection.

In addition to the common bundle, patients were cluster-randomized in one week blocks to undergo additional diagnostic testing with either the PPMC laboratory-generated respiratory pathogen PCR panel (Standard) or a commercial multiplex PCR panel (FilmArray), from Biofire (Salt Lake City, UT).The Standard panel probes for influenza A and B, adenovirus, human metapneumovirus, respiratory syncytial virus, and rhinovirus. Specimens were run daily at least 6 days per week; results were available within 12-48 hours. On alternate weeks, nasaopharyngeal (NP) swabs were processed with FilmArray, that probes for five types of influenza, four types of parainfluenza, rhinovirus/enterovirus, adenovirus, human metapneumovirus, four types of coronavirus, respiratory syncytial virus, *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* and *Bordetella pertussis*.

2.1.3. Data collection

The authors extracted data from the patients' EMR, using an assigned study number and database file (Filemaker, Pro 13). Data extraction began at enrollment, continued periodically during hospitalization, and was completed post-discharge. All data entry was verified by two or three of the authors.

Infectious diseases pharmacists entered data referable to use of antibacterial and/or anti-influenza therapy. Using a standardized list of the purchase expense of individual antibiotics, one investigator (DNG) determined the days of, and expense of, antimicrobial therapy. On any given day, empiric therapy with 3 different antibiotics, regardless of the number of doses, was defined as 3 days of therapy (DOT). The length, or number of days, of therapy (LOT), regardless of the number of drugs administered each day, was also calculated. Results were normalized to 1000 hospital patient-days.

2.2. Inclusion and exclusion criteria

Inclusion required an ED diagnosis of CAP of sufficient severity to require hospitalization in a patient 18 years of age or older. Patients were excluded if it was not possible to obtain a NP swab or if antibiotics were withheld and comfort care initiated. Post-enrollment, patients were excluded if two sites of infection were present: e.g., CAP plus a non-CAP infection, if patients were placed on comfort care with discontinuation of anti-infectives, or if there was a failure to collect the protocol-mandated diagnostic tests. Patients unable to provide an acceptable sputum for culture were not excluded.

2.3. Final clinical categorization

The final database for each enrolled patient was reviewed by two of the investigators (JL and DNG) for the purpose of final categorization as per the definitions below. In the event of disagreement, adjudication was by a third investigator (GG). The criteria for the assigned final clinical diagnosis were:

2.3.1. Uninfected; no evidence of CAP

Post-admission clinical, laboratory and imaging studies document an alternative non-infectious diagnosis: e.g., congestive heart failure.

2.3.2. Bacterial pneumonia

Proven: Pulmonary infiltrates and a bacterial pathogen in sputum, blood, or pleural fluid; a positive *S. pneumoniae* NP swab PCR and/or *S. pneumoniae* urine antigen was accepted as bacterial pneumonia.

Presumptive: Multifocal pulmonary infiltrates and detection of *S. pneumoniae* or S. *aureus* by PCR of a nasal swab in patients in whom it was not possible to obtain sputum or a bronchoalveolar lavage specimen. Elevation of the serum procalcitonin was used as evidence of bacterial invasion as opposed to asymptomatic colonization.

In the presence of clinical pneumonia, a serum procalcitonin level of ≥0.25 ng/mL was accepted as presumptive evidence of bacterial pneumonia in the absence of detection of a bacterial pathogen; e.g., the patient with documented aspiration.

2.3.3. Viral pneumonia

Presumptive: Identification of the presence of adenovirus, coronavirus, human metapneumovirus, influenza, parainfluenza, respiratory syncytial virus, or rhinovirus by one of the PCR probes and a compatible clinical syndrome. In distinction to potential bacterial pathogens like *S. aureus* and *S. pneumoniae*, asymptomatic nasal colonization by respiratory viral pathogens is a rare occurrence.

2.3.4. Bacterial-viral co-infected

Presumptive: Respiratory virus detected and either serum PCT was above 0.5 ng/mL, and/or a bacterial pathogen found in a sputum culture, by urine antigen, or PCR. Bacterial and viral pathogens were identified as "potential" etiologic agents as no seroconversion studies were performed.

2.4. Determination of protocol adherence of patient data

Each patient file was reviewed by three investigators (GG, JL, DG). A patient was considered evaluable only if all protocol-required diagnostic studies were performed, except for sputum culture if no sputum could be obtained. Each patient file was reviewed to determine if the patient's pneumonia diagnosis was, in hindsight, correct. Of those patients with a clinical pneumonia syndrome, the investigators classified the etiology of the pneumonia in one of 4 ways: viral, bacterial, or a combination of viral and bacterial, or, when no pathogen was found, clinical pneumonia of unclear etiology. If a respiratory virus was detected, an associated bacterial infection was deemed present if a bacterial pathogen was identified by culture PCR or urine antigens, or if the serum PCT concentration was >0.5 ng/mL.

2.5. Statistics

For comparisons between the two diagnostic methods, *t* test or Wilcoxon test was performed for continuous variables, and chi-square test or Fisher's Exact test was performed for categorical variables. Kruskal-Wallis test or one-way ANOVA test was used for comparisons among the three distinct etiology groups (viral, bacterial, or a combination of viral and bacterial).

3. Results

From December 4, 2014, to March 6, 2015, the ED admitted 211 patients with a diagnosis of CAP (Fig. 1). Of the 99 patients randomized to the Standard group, 31 patients were non-evaluable, due to inadequate evidence of pneumonia in 26, incomplete diagnostics in 3, and transition to comfort care within a day in 2 patients. Inadequate evidence of pneumonia was attributable to patients with bronchitis or COPD exacerbation (8), sepsis from another source (7), CHF (5), cystic fibrosis (2), metastatic cancer (2), MAI (1) and chemical aspiration (1). Of the remaining 68 evaluable patients, 1 or more pathogens were identified in 47 (69%).

Of the 111 patients randomized to the FilmArray group, 52 patients were non-evaluable, due to inadequate evidence of pneumonia in 40, incomplete diagnostics in 3, and transition to comfort care in 9 patients within a day. Inadequate evidence of pneumonia was attributable to patients with bronchitis or COPD exacerbation (13), sepsis from another source (12), CHF (6), metastatic cancer (6), asthma, pulmonary embolism, or chemical aspiration (1 each). Of the remaining 59 evaluable patients, 1 or more pathogens were identified in 43 (73%).

Non-evaluable patients were otherwise similar to those evaluable with respect to demographics, comorbidities, and other features listed in Table 1.

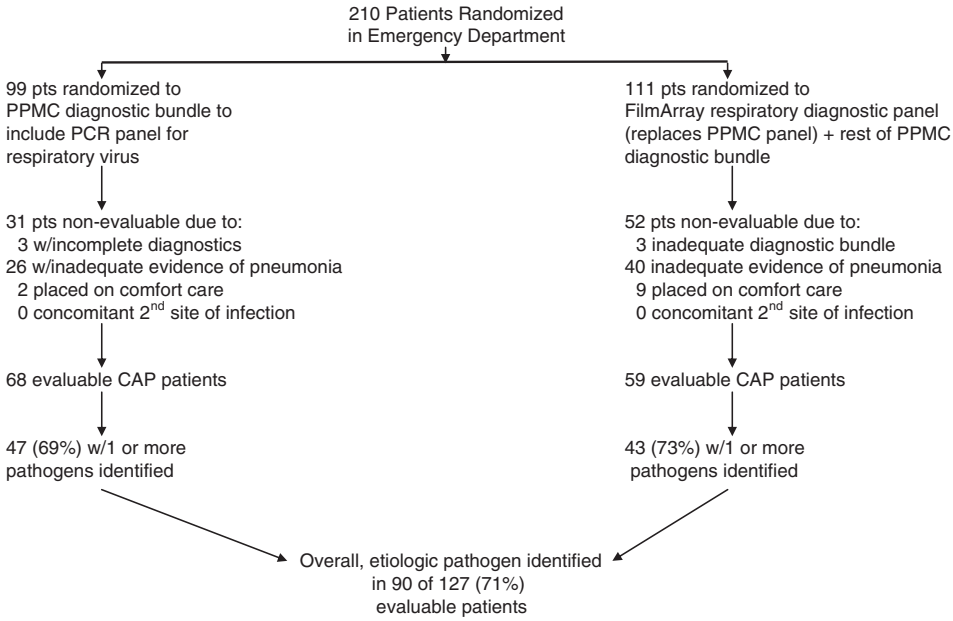

Fig. 1. Screening, eligibility, and enrollment of hospitalized adults with CAP.

3.1. Patient characteristics

The demographic and clinical features of the Standard and FilmArray evaluable patients are summarized in Table 1. The pneumonia severity index (PSI) range results were similar, placing the bulk of the patients in risk group III (scores 71-90) and hence justifying hospitalization. Comorbidities were evenly distributed.

3.2. Potential microbial etiology of the patients' CAP

Combining all the evaluable patients in the Standard and FilmArray groups, one or more potential pathogens were detected in 90 of 127 (71%) patients. In 40 of 127 (32%) only a potential bacterial pathogen was found; in 25 of 127 (20%) only a potential viral pathogen was detected; and in 24 of 127 (19%) both viral and bacterial pathogens were found. No statistical differences in diagnostic yield existed between Standard and FilmArray patients. In the remaining 37 patients (29%) with CAP, no potential pathogen was found.

Sputum for culture was only obtainable in 74 of the 127 (58%) evaluable patients. A candidate bacterial pathogen was found in only 28 of 127 (22%) patients, Table 2. *S. pneumoniae* was identified in only 5 sputa (3.9%). Both *H. influenzae* and *S.* aureus were cultured in 9 (7%) patients. Two patients were bacteremic (1.6%), both due to *S. pneumoniae; S. pneumoniae* antigen was detected in the urine of both patients. The urine antigen test for *S. pneumoniae* was positive in 16 of 127 (13.5%) patients. No patients had a positive urine antigen test for *L. pneumophila.*

The *S. pneumoniae* NP PCR was positive in 24 of 127 (18.9%) of patients, Table 2, but in only 10 of the 24 was the concomitant urine antigen test positive.

Table 1
Characteristics of evaluable patients.

	Diagnostic group		*P*-value
	Standard (n = 68)	FilmArray (n = 59)	
Demographics			
Age, mean ± SD	70.9 ± 17.9	69.9 ± 17.7	0.75
Male sex (%)	36 (52.9%)	26 (44.1%)	0.41
Weight (kg), mean ± SD	75.8 ± 23.3	76.7 ± 29.6	0.84
Clinical features			
Highest temperature (°C) in 1st 24 hr	37.9 ± 0.9	37.8 ± 0.9	0.84
WBC, total	16,661 ± 15.617	14,612 ± 6,992	0.33
Pneumonia severity index	78.4 ± 13.4	81.8 ± 11.4	0.12
Comorbidity and habits			
Alcoholism	3 (4.4%)	5 (8.5%)	0.47
Alcohol use, current	10 (14.7%)	11 (18.6%)	0.72
Congestive heart failure	12 (17.6%)	14 (23.7%)	0.53
COPD	25 (36.8%)	20 (33.9%)	0.88
Diabetes mellitus	21 (30.9%)	20 (33.9%)	0.86
HIV	3 (4.4%)	1 (1.7%)	0.62
Illicit drug use	4 (5.9%)	8 (13.6%)	0.24
Liver disease, chronic	6 (8.8%)	9 (15.3%)	0.4
Malignancy	8 (11.8%)	6 (10.2%)	0.9
Obstructive sleep apnea	6 (8.8%)	7 (11.9%)	0.9
Renal insufficiency	21 (30.5%)	19 (32.2%)	0.9
Tobacco use, current	16 (23.5%)	13 (22%)	0.9
Home medications			
Antibiotics	8 (11.8%)	6 (10.2%)	0.9
Glucocorticoids	9 (13.2%)	7 (11.9%)	0.9
Narcotics	28 (41.2%)	15 (25.4%)	0.09
PPI/H2 blocker	21 (30.9%)	26 (44.1%)	0.18

Table 2
Diagnostic yield for bacteria with selected test methods.

	Standard	FilmArray	Total	% Total
n = number evaluable patients	68	59	127	
Sputum				
No. pts w/sputum cultures	38	36	74	58%
# positive for potential pathogen	14	14	28	22%
# positive for *S. pneumoniae*	3	2	5	3.9%
# positive for *H. influenzae*	4	5	9	7%
# positive for *S. aureus*	4	5	9	7%
Blood				
No. pts w/blood cultures	68	59	127	
# positive	1	1	2	1.6%
Urine				
No. pts w/antigens done	68	59	127	
No. pts w/positive Legionella antigen	0	0	0	
No. pts w/positive *S. pneumoniae* antigen	6	10	16	12.5%
Nasal PCR				
# *S. pneumoniae* positive	13*	11*	24*	18.9%
# *S. aureus* positive	9†	11†	20†	16.0%

* Of the total of 24 patients with a positive NP swab PCR for *S. pneumoniae,* 10 had concomitant positive tests for *S. pneumonia* urine antigen.

† Of the total 20 patients with a positive nasal swab PCR for *S. aureus*, 9 had concomitant positive sputum cultures.

Table 3
Comparison of potential etiologic pathogens detected by PPMC standard diagnostic bundle or diagnostic bundle with FilmArray multiplex PCR substituted for PPMC viral PCR respiratory virus panel.

Pathogen identified	Standard (47 pts)	FilmArray (43 pts)
Patients with viral pathogen only: Subtotal	**13**	**12**
- Adenovirus	0	0
- Coronavirus	0	1
- Human metapneumovirus	1	1
- Influenza	11	5
- Parainfluenza	0	1
- Respiratory syncytial virus	0	4
- Rhinovirus	1	0
Patients with bacterial pathogen only: Subtotal	**20**	**21**
- *S. pneumoniae*	8	6
- *S. aureus* (MSSA + MRSA)	4	6
- *S. pneumoniae* + *S. aureus*	1	2
- *H. influenzae*	3	5
- Streptococcus species	1	1
- *Moraxella catarrhalis*	0	1
- *Enterobacteriaceae* species	3	0
Patients with viral and bacterial pathogens: Subtotal	**14**	**10**
- Virus + elevated procalcitonin serum concentration	2	1
- *S. pneumoniae* + adenovirus	0	0
- *S. pneumoniae* + coronavirus	0	0
- *S. pneumoniae* + hMPV*	0	0
- *S. pneumoniae* + influenza	3	2
- *S. pneumoniae* + parainfluenza	1	1
- *S. pneumoniae* + RSV*	2	2
- *S. pneumoniae* + rhinovirus	0	1
- *S. aureus* + hMPV*	1	2
- *S. aureus* + influenza	2	0
- Streptococcus species + influenza	1	0
- Mixed bacterial flora + influenza	2	1

* hMPV = human metapneumovirus; RSV = respiratory syncytial virus.

The *S. aureus* PCR was positive in 20 of 127 (16%) patients, in 9 of whom *S. aureus* also grew in sputum culture.

In sum, a potential bacterial pathogen was detected by culture of sputum and/or blood and/or urine antigen and/or PCRs for *S. pneumoniae* and *S. aureus* in 78 of 127 (64%) patients. Viral PCR panels detected a respiratory virus in 49 of the 127 38.6%) of the patients. In 24 patients, a virus was detected concomitant with a bacterial pathogen, Table 3. Adding the 25 patients with only a respiratory virus to the 65 with a bacterial pathogen detected alone or with a virus, one or more pathogens were identified in 90 of the 127 (71%) evaluable patients.

3.3. Comparison of standard versus FilmArray diagnostic bundles

A viral or bacterial pathogen was identified in 47 patients randomized to the Standard panel and 43 patients randomized to the FilmArray panel, Table 3. In 29% of the patients with a clinical syndrome of CAP, no pathogen was identified.

No significant differences were noted between the limited PPMC viral diagnostic panel and the expanded FilmArray panel in total number of viruses detected. The number of pathogens and their distribution between Standard and FilmArray patients is presented in Table 3. Influenza was the most common virus detected either alone in 16 patients or in combination with *S. pneumoniae* (5 patients) or *S. aureus* (3 patients). *S. pneumoniae* without a concomitant virus was found in 17 patients and combined with a respiratory virus in 12 patients.

3.4. Turnaround time

The turnaround time, to include processing, running, and result reporting for the diagnostic tests is summarized in Table 4. The FilmArray panel turnaround time was a mean of 2.1 hours as compared to the PPMC standard viral panel at a mean of 26.5 hours, $P < 0.001$. The urine antigen results were reported in roughly 7-8 hours and the nasal PCRs for *S. pneumoniae* and *S. aureus* in approximately 30 and 18 hours. Sputum and blood culture results required several days to complete.

Table 4
Turnaround time for diagnostic tests.

	Standard (n = 68)	FilmArray (n = 59)	*P*-value
Sputum culture and sensitivity (h)	55.2 ± 12.5	55.9 ± 18.5	0.85
Urine antigen:			
S. pneumoniae (h)	7.6 ± 5.3	7.5 ± 5.8	0.92
L. pneumoniae (h)	7.9 ± 6.1	7.3 ± 5.7	0.63
Blood culture (h)	130.3 ± 16.8	132.6 ± 14.9	0.41
Nasopharyngeal swab for:			
Respiratory virus PCR panels (h)	26.5 ± 15	2.1 ± 0.7	<0.001
S. pneumoniae PCR (h)	27.2 ± 20.3	32.2 ± 20.7	0.17
S. aureus PCR (h)	16.1 ± 8	17.3 ± 9	0.44

3.5. Serum procalcitonin (PCT) concentrations

The admission serum PCT concentrations are summarized in Fig. 2. The PCT levels are significantly lower in the patients infected with only a virus versus the patients infected with a bacteria or a combination of a virus and a bacteria, $P < 0.003$.

3.6. Influence of diagnostics on antibacterial therapy

All enrolled evaluable patients received their first doses of empiric antibiotic therapy within six hours of arrival and before leaving the ED. The LOT, DOT and cost of antibiotics and antivirals (for influenza) were calculated and normalized to 1000 patient-days, Table 5. Overall, the median cost of therapy was lower in FilmArray patients versus standard patients ($3037 vs $7952, $P = 0.02$). For each etiologic category, the cost was consistently lower for patients in the FilmArray group, but was only significant in patients with combined bacterial and viral pathogens ($P = 0.046$), the highest cost etiologic category. The LOT in virus-only patients was significantly lower than in patients with bacterial infection, $P =$

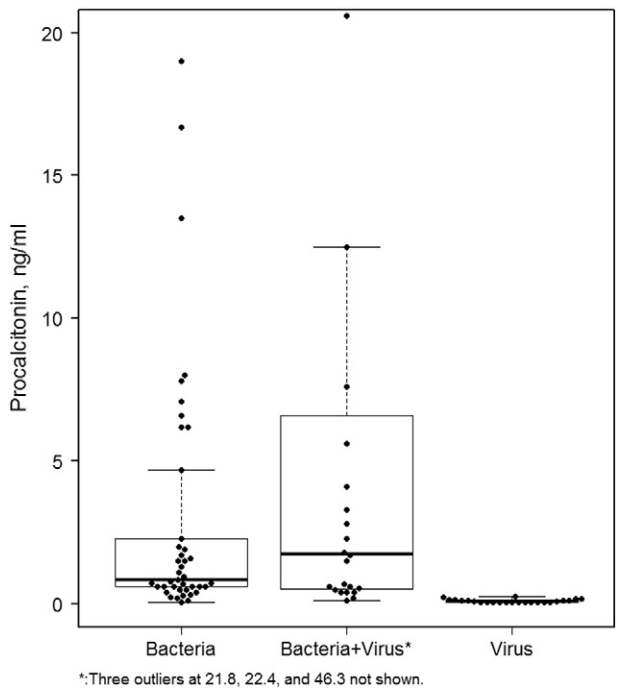

Fig. 2. Box plot of PCT values in patients with CAP caused by a virus, bacteria, or a combination of a virus and bacteria. The PCT values in patients with CAP due to bacteria alone or a virus plus bacteria are significantly higher than in patients with CAP caused only by a virus, $P = 0.003$.

Table 5
Influence of diagnostic results on antibacterial therapy (mean ± SD).

	Diagnostic method	No. of patients	Cost of therapy ($) per 1000 patient-days	LOT ǂ(days) per 1000 patient-days	DOT ǂper 1000 patient-days
Etiologic category					
Bacteria	FilmArray	21	9391 ± 12270	1274 ± 920	1549 ± 775
	Standard	20	9771 ± 9807	1491 ± 1463	2326 ± 2235
	Combined	41	9576 ± 10999	1380 ± 1205+	1928 ± 1682
Bacteria + virus	FilmArray	10	10482 ± 11682	1027 ± 740	1378 ± 658
	Standard	14	20562 ± 16383	1851 ± 20161	2574 ± 2100
	Combined	24	15362 ± 15194	1508 ± 1638+	2076 ± 1739
Virus	FilmArray	12	8392 ± 8327	841 ± 294	1388 ± 804
	Standard	13	10442 ± 6399	848 ± 219	3056 ± 4677
	Combined	25	9458 ± 7304	845 ± 252++	2256 ± 3458
No pathogen identified	FilmArray	16	5467 ± 7259	937 ± 179	1818 ± 1203
	Standard	21	9023 ± 10102	1586 ± 2058	1403 ± 449
	Combined	37	7485 ± 9047	1305 ± 1572	1583 ± 871
Pathogen					
Combined	FilmArray	59	8308 ± 10165*	1053 ± 657	1560 ± 895≠
	Standard	68	11890 ±11712**	1472 ± 1667	2232 ± 2574≠≠

ǂLOT = length of therapy; DOT = days of therapy
+vs++ $P = 0.04$.
* vs** $P = 0.02$.
≠vs≠≠ $P = 0.03$.

0.04. However, there was no difference in LOT between the FilmArray and Standard bundle patients with a viral infection.

The DOT was significantly lower in the FilmArray patients, $P = 0.03$. For each of the etiologic categories with an identified pathogen, DOT was consistently lower in the FilmArray group. However, the difference was only significant in the bacteria + virus patients, $P = 0.02$.

In 25 patients (13 standard and 12 FilmArray), the NP PCR detected a pathogenic virus without a concomitant bacterial pathogen, a clinical presentation consistent with a viral pneumonia, and a serum PCT level ≤0.1 ng/mL. Nonetheless, discontinuation of the empiric antibiotics within 48 hours of test results occurred in only 8 of the 25 (32%) patients. Despite the faster turn-around time for FilmArray patients, discontinuation of empiric antibiotics was almost identical (5 FilmArray patients and 3 standard patients).

4. Discussion

An etiologic pathogen was detected in 69% of the evaluable Standard bundle patients and 73% of the FilmArray bundle patients. Serum PCT levels separated patients with pure viral infections from patients with bacterial or mixed viral-bacterial pneumonia. The LOT was shorter for patients with pure viral infection, but the full potential for antibiotic de-escalation was not achieved.

In contrast, Musher, 2013 detected a bacterial pathogen in 28% and a viral pathogen in 30% of patients. A pathogen was found in only 24% of CAP patients (Restrepo et al., 2008) if only bacterial cultures of blood and sputum plus urine antigen testing for *L. pneumophila* was performed. Various studies, adding PCR probes for atypical respiratory pathogens and viruses plus serologies, have reported diagnostic yields of 38%, 53%, 67% and 89% in CAP patients, respectively (Falsey et al., 2013; Jain et al., 2015; Johansson et al., 2010; Shibi et al., 2010).

Gadsby et al., 2016, collected sputum cultures in 323 PSI class 4 or 5 CAP patients. Retrospective PCR probes of the sputa identified one or more bacterial pathogens in 87% of the patients, and respiratory virus in 30%. A mixture of bacterial and viral pathogens was found in 82% of the sputa containing a respiratory virus.

4.1. Colonization vs infection by S. pneumoniae and S. aureus

Depending on the detection method used, asymptomatic nasopharyngeal colonization of healthy adults by *S. pneumoniae* ranges from 8% to 22% (Janoff and Musher, 2015; van Deursen et al., 2016). Further, approximately 20% (range 12–30%) of otherwise healthy individuals are persistent nasal carriers of *S. aureus* (Que and Moreillon, 2015).

We accepted detection of *S. pneumoniae* by culture, urine antigen, or PCR as evidence of an etiologic pathogen, as opposed to colonization, if the patient had the clinical syndrome of CAP and an elevated serum level of procalcitonin. The same logic was applied to the detection of *S. aureus* by culture or nasal PCR. Only once was *S. aureus* interpreted as a solo pathogen; in 5 other patients *S. aureus* was detected along with either influenza or hMPV.

4.2. Detection of S. pneumoniae by NP PCR

Spik et al., 2013 increased detection of *S. pneumoniae* from 6.7% by sputum culture to 22.8% by PCR of sputum. Albrich et al., 2014 studied 222 South African HIV-positive adults with CAP. *S. pneumoniae* was cultured from sputum in 46% whereas the PCR was positive in either the NP aspirate or sputum in 67.1%

4.3. Low yield of blood cultures

Only 2 of our 127 (1.6%) evaluable patients had positive blood cultures which is lower than the 20 to 25% rate reported by Said et al. (2013), but similar to the 0.8% and 1.9% reported by Falsey et al. (2013) and Musher et al. (2013). In children, 118 blood cultures would need to be collected to identify one bacteremic patient. If blood cultures were limited to PSI class 4 or 5 patients and the immunocompromised, only 42 cultures would be needed to detect one positive (Andrews et al., 2015).

4.4. Standard bundle vs FilmArray

As summarized in Table 3, the standard viral PCR panel detected as many virus-infected patients as the larger (more viruses plus 3 bacteria) FilmArray panel. We suspect the lower yield occurred due to the low incidence of coronavirus, parainfluenza, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* infections at the time of the study.

4.5. Time to results and serum PCT level

Our results demonstrate the ability to distinguish viral from bacterial infection within 2-8 hours of hospital admission, Table 4. It is thus possible to reconsider the need for respiratory isolation and antimicrobial therapy before the second scheduled dose of empiric antibacterials.

4.6. Influence of rapid viral diagnostics and serum PCT levels on de-escalation

The absence of an elevated PCT level in a patient with a clinical syndrome compatible with a viral illness strongly suggests the absence of an active bacterial infection, and no benefit to empiric antibiotic therapy (Becker et al., 2008; Branche et al., 2015; Falsey et al., 2013; Gelfer et al., 2015; Gilbert, 2011, 2015; Schuetz et al., 2012, 2013).

Despite the presence of only a respiratory viral pathogen and normal PCT levels, empiric antibiotics were discontinued within 48 hours in only 8 of 25 (32%) patients in this study, and 2 of 11 in that of Oosterheert et al. Branche et al., 2015 evaluated 151 patients hospitalized with lower, non-pneumonic respiratory tract infections, of whom 126 had a serum PCT level of <0.25 ng/mL and 42% or 50%(?) had a viral pathogen detected. The result was a shorter duration of antibiotic therapy, $P = 0.004$ and fewer patients discharged with a prescription for antibiotics, $P = 0.002$.

4.7. Limitations

The study of only 127 patients is a major limitation, but replicates our previous pilot study of 59 evaluable patients (Gelfer et al., 2015).

We recognize the need to compare pathogen detection by PCR with detection in a matched control group. Asymptomatic carriage of *S. pneumoniae* and some respiratory viruses (e.g., rhinovirus) are much higher in children than adults (Self et al., 2016). Hence, detection in adults with an appropriate clinical syndrome supports the pathogenic role of detected potential pathogens. Studies either suggest or refute a correlation of pathogen density ("load") with invasive disease (Collins et al., 2016). Seroconversion is often the "gold standard" but not helpful during the acute illness (Albrich et al., 2014).

5. Conclusion

Our study results, and the work of others, support routine expansion of rapid diagnostic test bundles to include a rapid multiplex PCR platform for respiratory viruses to determine the etiology of CAP. The fast turnaround time of the FilmArray offers quick assistance to antibiotic stewardship activities. In addition, our data support the value of anterior nasal swabs for *S. aureus* PCR and NP swab for *S. pneumoniae* PCR.

Testing urine for the antigens of *S. pneumoniae* and *L. pneumophila*, serogroup 1 may ultimately prove duplicative to the next generation PCR platforms and/or next generation gene sequencing.

Sputum cultures are needed for in vitro antimicrobial susceptibility testing. The challenge is the collection of a suitable specimen before, or concomitant with, initiation of antibiotic therapy.

Due to the low yield of blood cultures, it seems reasonable to limit blood cultures to patients with the highest PSI scores (Class 4 and 5).

Low PCT levels support the absence of invasive bacterial disease. A low PCT level supports an interpretation of colonization when *S. pneumoniae, H. influenzae, S. aureus* or other potential bacterial pathogens are identified.

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