Use of the BIOFIRE® FILMARRAY® Pneumonia *plus* Panel to characterize the etiology of lower respiratory tract infections in Southern Province, Sri Lanka

Zachary A. Weishampel[1¶*], Ajith Nagahawatte[2,3,4¶], Gaya Wijayaratne[3,4], Subodha S. Wickramasinghe[3,4], Bradly Nicholson[4,5], Jack Anderson[1], Sky Vanderburg[6,7], C Lakmal Fonseka[4,8], Madureka Premamali[4], Robert J. Rolfe[1,2], U.H.B.Y. Dilshan[4], Ruvini Kurukulasooriya[4], Bhagya Piyasiri[9], Truls Østbye[1,2,4], Christopher W. Woods[1,2,4], Lana Abusalem[1], Armstrong Obale[1,4], L. Gayani Tillekeratne[1,2,4,8&*], Champica K. Bodinayake[2,4,8&]

[1] *Duke University, Durham, North Carolina, United States of America*

[2] *Duke Global Health Institute, Durham, North Carolina, United States of America*

[3] *Faculty of Medicine, Department of Microbiology, University of Ruhuna, Galle, Sri Lanka*

[4] *Ruhuna-Duke Centre for Infectious Diseases, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka*

[5] *Institute for Medical Research, Durham Veterans Affairs Medical Center, Durham, North Carolina, United States of America*

[6] *Pulmonary and Critical Care, Montage Health, Monterey, California, United States of America*

[7] *University of California San Francisco, San Francisco, California, United States of America*

[8] *Faculty of Medicine, Department of Medicine, University of Ruhuna, Galle, Sri Lanka*

[9] *National Hospital Galle, Galle, Sri Lanka*

* Corresponding authors

E-mail: zachary.weishampel@duke.edu (ZAW)

E-mail: gayani.tillekeratne@duke.edu (LGT)

¶ Co-primary authors

& Co-senior authors

Keywords: BIOFIRE® FILMARRAY® Pneumonia *plus* Panel; Lower Respiratory Tract Infections; Gram-Negative Bacteria; Antimicrobial Resistance; Southern Province, Sri Lanka

Word Count: 4,413

Abstract

BIOFIRE® FILMARRAY® Pneumonia *plus* Panel, an assay with bacterial, viral, and antibacterial resistance gene targets, was applied to sputum samples to evaluate the etiology of community-acquired lower respiratory tract infections (LRTIs) among hospitalized patients in southern Sri Lanka from 2019 to 2021. Sputum was tested by culture and the panel. Among 267 patients sampled, the most commonly detected bacteria were the *Klebsiella pneumoniae* group, *Staphylococcus aureus*, and the *Acinetobacter calcoaceticus-baumannii* complex. The most commonly detected viruses were human rhinovirus/enterovirus and influenza A. In total, 79.0% of patients had at least one gram-negative bacterium and 52.1% had at least one gram-positive bacterium. Of the patients sampled, 40.0% had at least one carbapenem resistance gene target, 21.0% had an extended-spectrum beta-lactamase related gene, and 15.7% had a methicillin resistance gene. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* were the most likely to be detected in the highest genomic concentration bin reported by the panel. Only 5.6% of patients had matching panel and culture results. Our findings suggest that patients with LRTI in southern Sri Lanka have a high prevalence of gram-negative bacteria and antibacterial resistance gene targets in their sputum. However, it remains difficult to differentiate colonizers versus the causes of infection.

Introduction

Lower respiratory tract infections (LRTIs), including tracheitis, bronchitis, bronchiolitis, and pneumonia, are leading causes of morbidity, disability, and death worldwide. They particularly impact areas associated with a low sociodemographic status; low- or middle-income countries (LMICs) experience the highest disability-adjusted life years due to LRTIs [1; 2]. This high burden of morbidity and mortality warrants improved access to diagnostic tools, as well as accurate, reliable measurements of the incidence, etiology, and contributing risk factors of LRTIs.

Determining the etiology of LRTIs is challenging for multiple reasons. The low sensitivity of currently available tests like sputum and blood cultures has caused many bacterial pathogens associated with LRTIs to be missed [3]. Additionally, it remains difficult to identify pathogens causing LRTI based on testing of upper respiratory tract samples such as nasal or nasopharyngeal samples [4]. Finally, even if an organism is detected, it is challenging to differentiate colonization (*i.e.*, the presence of bacteria or viruses not causing disease) from infection [5].

As such, there have been various studies investigating the clinical utility of broad or multiplexed nucleic acid amplification testing panels for detecting both viruses and bacteria as well as their role in polymicrobial or multidrug-resistant infections [6-11]. These panels have attracted increasing attention due to the rapidity of results compared to results from cultures and the ability to detect a variety of viral and bacterial pathogens through a single test [12-15].

One such test, the BIOFIRE® FILMARRAY® Pneumonia *plus* (PN*plus*) Panel (bioMérieux USA, Salt Lake City, Utah) is a multiplex polymerase chain reaction (PCR) assay that simultaneously detects 34 targets, including 9 viruses, 18 bacteria, and 7 genetic markers of antibacterial resistance, through the utilization of sputum, tracheal aspirate, or bronchoalveolar lavage samples [16]. The test is run for approximately one hour on the FILMARRAY® platform. Prior studies have shown the high performance capabilities and

accuracy of the PN*plus* Panel for bronchoalveolar lavage fluid and sputum samples [17-19]. Compared to the more conventional culture method, the PN*plus* Panel has the potential to improve the sensitivity of detection and speed of characterization in diagnosing the etiology of LRTI [20]. Furthermore, as genetic markers of antibacterial resistance are included in the panel, the PN*plus* Panel and other PCR assay panels can be used to tailor therapies based on antibacterial resistance [21].

However, additional studies are needed to evaluate the feasibility and performance of the PN*plus* Panel, specifically in LMICs where rates of antibacterial resistance are high [22-25]. In this study, we applied the PN*plus* Panel to characterize the etiology of community-acquired LRTIs in southern Sri Lanka, including the prevalence of bacterial, viral, and antimicrobial resistance gene targets from sputum samples. Since certain chest radiographic findings are more likely associated with bacterial infection versus viral infection-- opacity/consolidation versus interstitial patterns, respectively-- we assessed for associations between microbial targets and radiographic findings on chest imaging. We also determined associations between specific bacterial targets and genomic density, as these associations may shed light on organisms causing infection versus colonization. Finally, we compared the PN*plus* Panel results with sputum culture results to identify test performance.

Materials and Methods

Study cohort

We enrolled consecutive children and adults meeting a case definition for LRTI who were hospitalized at National Hospital Galle (previously known as Teaching Hospital Karapitiya), a public, 1800-bed tertiary care hospital located in Southern Province, Sri Lanka, between November 2019 and January 2021. As described previously, to be eligible, patients in the study needed to be ≥1 year of age, admitted <48 hours prior to enrollment, and have had an LRTI present for <14 days [26]. LRTI was determined using age-related criteria. For patients five years old or older, LRTI was defined as having at least one symptom consistent with acute infection (reported fevers, reported chills, documented fever, documented hypothermia, leukocytosis, leukopenia, or new altered mental status) and at least one symptom

consistent with acute respiratory illness (new cough, sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure). In addition, patients needed to have a chest radiograph performed by the time of enrollment, although the result of the radiograph was not considered. For patients younger than five years of age, LRTI was defined as having at least one symptom consistent with acute infection (lower chest wall indrawing, tachypnea, oxygen saturation <90%, central cyanosis, severe respiratory distress, inability to drink or breastfeed, vomiting, altered consciousness, or convulsions) and at least one symptom consistent with acute respiratory illness (cough or difficulty breathing). Patients younger than five years of age were not required to have a chest radiograph performed in order to be eligible. Patients were excluded if they were unable or unwilling to give consent for participation, unwilling to provide biological samples, hospitalized within 28 days prior to their current hospitalization, known to be or suspected to be infected at any other anatomic site requiring antibacterial therapy, or deemed to have a condition that precluded participation out of concern for safety as determined by the study physicians or other clinical providers.

Data collection

Following written informed consent, research staff completed a standardized questionnaire detailing the patient's demographic information and clinical history. Patients were followed longitudinally with repeated assessments throughout their hospitalization. Patients also had an additional visit approximately four weeks post-discharge. Microbiological results of sputum cultures obtained during clinical care, radiographic data, treatments, and clinical outcomes were obtained from the medical records. Each patient provided one sputum sample at enrollment for research testing.

Laboratory testing of sputum samples

One aliquot of sputum was immediately processed as a culture for research purposes using standard microbiological techniques according to the Clinical Laboratory Standards Institute to identify bacterial organisms [27]. The other aliquot of sputum was stored at -70°C and later tested with the PN*plus* Panel according to the manufacturer's instructions [16]. If a patient had a sputum culture performed during

routine care that was processed by the Clinical Microbiology Laboratory at the hospital, the result from this culture was also recorded.

The PN*plus* Panel detects the presence of 18 bacteria, 9 viruses, and 7 markers of antibacterial resistance. Of the 18 bacterial targets, 15 targets represent organisms present in typical bacterial pneumonia: *Klebsiella pneumoniae* group, *Acinetobacter calcoaceticus-baumannii* complex, *Pseudomonas aeruginosa*, *Enterobacter cloacae* complex, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella aerogenes*, *Serratia marcescens*, *Moraxella catarrhalis*, *Proteus* spp., *Klebsiella oxytoca*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Streptococcus pyogenes*. For only these 15 bacterial organisms, a semi-quantitative result is also reported via bins of 10^4, 10^5, 10^6, or $\geq 10^7$ copies/mL. The remaining three bacterial targets, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae,* represent organisms causing atypical bacterial pneumonia. Viral targets include those for adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, Middle East Respiratory Syndrome coronavirus, parainfluenza virus, and respiratory syncytial virus. Finally, the panel includes five carbapenem resistance-encoding genes (IMP, KPC, NDM, OXA-48-like, and VIM), one methicillin resistance-encoding target (*mecA/C* and MREJ), and one extended-spectrum beta-lactamase-encoding gene (CTX-M). The PN*plus* Panel reports all positive bacterial, viral, and antibacterial resistance gene targets within a sample run; however, antibacterial resistance genes are reported by the PN*plus* Panel only if commensurate bacterial targets are also detected as explained by the manufacturer [16].

Statistical analysis

Descriptive analyses were performed to determine the proportion of patients with community-acquired LRTIs who had bacterial, viral, and antimicrobial resistance gene targets detected by the PN*plus* Panel in their sputum samples. Then, heatmaps were created to evaluate co-detections of bacterial, viral, and antimicrobial resistance gene targets.

We explored the association between microbial targets and specific patterns on radiographic imaging, as bacterial infections are classically associated with consolidation or opacities on chest imaging, and viral infections are classically associated with interstitial patterns [28]. Fisher's exact test was used to identify the association between patients who had a positive bacterial, viral, and/or antibacterial resistance gene target result on the PN*plus* Panel and patients with opacities/consolidation on chest radiograph or with an interstitial pattern on chest radiograph during hospitalization. We also explored the association between specific bacterial targets and bacterial density, as higher densities may be indicative of an organism causing true infection, while lower densities may be indicative of an organism which is asymptomatically colonizing the patient. Fisher's exact test was used to evaluate the association between specific bacterial organisms and the bacterial genomic copies/mL bins. Principal component analysis and hierarchical clustering analysis were then performed on the bacterial genomic copies/mL bins.

Performance of the PN*plus* Panel at identifying an organism was compared with the results from clinical and research-based sputum cultures. Organisms on sputum cultures were classified based on standard procedures used by the two clinical and research laboratories in Sri Lanka and availability of reagents at the time of testing. In the comparison between the PN*plus* Panel and sputum culture results, a match was identified if the PN*plus* Panel detected any bacterium that fit the categories identified by the clinical or research culture results. Thus, identification of *E. cloacae* complex, *E. coli*, *K. aerogenes*, *K. oxytoca*, *K. pneumonia*e group, *P. spp.*, or *S. marcescens* by the PN*plus* Panel was considered a match with *Enterobacteriaceae* by sputum culture. A mismatch was defined as the PN*plus* Panel and clinical or research cultures detecting different bacteria, the clinical or research cultures detecting bacteria not detected from the PN*plus* Panel, or the PN*plus* Panel detecting bacteria not detected in the cultures.

Statistical analyses were conducted using GraphPad Prism Version 10 (Boston, MA) and R Statistical Software (R Core Team 2022). Heatmaps and bar-plots were created using Prism. Principal component analysis and hierarchical clustering analysis were performed with RStudio. Significance was determined at a p-value <0.05 for all analyses.

Ethical procedures

Ethical approval for this study was obtained from both the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka and from the Duke University Institutional Review Board, USA. For patients who were 18 years or older, written informed consent was obtained. For patients younger than 18 years of age, written informed consent was acquired from the parent or legal guardian. Written assent was also obtained from patients 12-17 years of age.

Results

Study cohort

A total of 267 patients were enrolled in the study; 253 patients (94.8%) were 18 years of age or older, 120 patients (44.9%) identified as female, and 173 patients (64.8%) were recorded to have a chronic co-morbidity. The most common co-morbidities included asthma, hypertension, and diabetes (**Table 1**). Additionally, 139 patients (52.1%) reported potentially using an antibiotic prior to hospitalization, as defined by patients responding with “yes” or “unsure” on the questionnaire. Of those who responded “yes” to antibiotic use prior to hospitalization, the most commonly used antibiotics were amoxicillin and clavulanic acid, azithromycin/erythromycin, and doxycycline/tetracycline.

B*IOFIRE® FILMARRAY® PNplus Panel*

Of the 267 sputum samples collected, the PN*plus* Panel detected the following: 211 (79.0%) with at least one gram-negative bacterium, 139 (52.1%) with at least one gram-positive bacterium, 111 (41.6%) with at least one virus, and 142 (53.2%) with at least one antibacterial resistance gene target (**Table 2**). Of the bacteria detected that are known to cause a typical pneumonia, the most prevalent were the *K. pneumoniae* group (41.9% of total patients), *S. aureus* (34.5%), the *A. calcoaceticus-baumannii* complex (32.6%), and *P. aeruginosa* (26.6%). Among the three species of bacteria representative of atypical pneumonia which are detectable by the panel, only *Mycoplasma pneumoniae* (1.1%) was detected in this sample set. Of the bacterial co-detections identified via the panel (*e.g.*, two bacteria detected in a single sample), the two most common co-detections were the *K. pneumoniae* group and the *E. cloacae*

complex, identified in 19.5% of patients, and the *K. pneumoniae* group and *A. calcoaceticus-baumannii* complex, identified in 19.1% of patients (**Figure 1A**). Two patients had eight gram-negative bacterial organisms detected each (**Supplementary Figure S1**).

The most prominent viruses detected with the PN*plus* Panel were human rhinovirus/enterovirus (19.5%) and the influenza A virus (10.9%) (**Table 1**). When assessing co-detections between bacteria and viruses, the most prominent co-detections were human rhinovirus/enterovirus with the *A. calcoaceticus-baumannii* complex (6.7%) or *S. aureus* (6.7%) (**Figure 1B**). A few viral co-detections were identified; two sputum samples had human rhinovirus/enterovirus and influenza A viruses detected and two samples had human rhinovirus/enterovirus and adenovirus detected. (**Figure 1C**).

For antibacterial resistance gene targets, the most commonly identified targets were the carbapenem resistance gene NDM (25.5%) and the extended-spectrum beta lactamase gene CTX-M (21.0%) (**Table 1**). In total, 96 patients (40.0%) were identified to have at least one carbapenem resistance-encoding gene. The most commonly co-detected antibacterial resistance genes were two carbapenem resistance-encoding genes, NDM and VIM (10.1%), and NDM with the extended-spectrum beta-lactamase CTX-M (9.7%) (**Figure 1D**). When comparing the co-detection of antibacterial resistance genes and bacteria, the most common co-detections were with the carbapenem resistance-encoding gene OXA-48-like and the *A. calcoaceticus-baumannii* complex, which was identified in 48 patients (18.0%) and the *K. pneumoniae* group with CTX-M or NDM, which were both detected in 48 patients (18.0%) (**Figure 1E**).

Radiographic findings

No significant association was identified between type of bacterial organism and the presence of an opacity/consolidation on chest radiograph or presence of an interstitial pattern on chest radiograph (**Table 2**). Similarly, there was no significant association between type of viral organism and the presence of an opacity/consolidation on chest radiograph or presence of an interstitial pattern on chest radiograph.

Bacterial density results

Among bacteria that can cause typical pneumonia, seven types of bacteria had a distribution of copy numbers (i.e., 10^4, 10^5, 10^6, or 10^7 copies/mL) that were statistically significantly different compared to other bacteria (**Figure 2A**). The three bacteria with the highest percentage of positive samples detected in the 10^7 copies/mL bin were *S. pneumoniae*, *H. influenzae*, and *P. aeruginosa*. The three bacteria with the highest percentage of positive samples in the 10^4 copies/mL bin were *K. aerogenes*, *E. cloacae* complex, and *A. calcoaceticus-baumannii* complex. Using the genomic concentration bins, the principal component analysis with hierarchical clustering identified four separate clusters (**Figure 2B**). One such cluster included *E. coli, S. pneumoniae*, *H. influenzae*, and *P. aeruginosa*. Others identified included the *K. pneumoniae* group and *S. aureus* cluster as well as the *E. cloacae* complex and *A. calcoaceticus-baumannii* complex cluster.

Sputum culture results

A total of 18 patients (6.7%) had positive clinical or research sputum cultures (**Figure 3A**). Of the positive sputum cultures, classifications included *Enterobacteriaceae*, *Pseudomonas* spp., *Moraxella* spp., or gram-negative bacteria. Of the research-based cultures, a total of 15 patients (5.6%) had positive sputum cultures. Four patients (1.5%) had positive clinical sputum cultures. In total, the sputum cultures and the PN*plus* Panel had 15 positive matches and 220 mismatches. The two most commonly matching bacteria identified by both methods were *P. aeruginosa* and the *K. pneumoniae* group (**Figure 3B**).

Discussion

Although pneumonia is the fourth leading cause of death in Sri Lanka, little is known about the microbial etiology [29]. With a previous study identifying that 86.8% of sampled patients with virus-associated LRTI were given antibiotic prescriptions in Southern Province, Sri Lanka, a better understanding of the etiologies of LRTI could improve both individual patient and community health [26]. In this study, we provide an initial characterization of microbial detections in patients hospitalized with community-acquired LRTIs at a large tertiary medical center in Southern Province, Sri Lanka through the use of the BIOFIRE®

FILMARRAY® PN*plus* Panel. We show the high prevalence of gram-negative bacteria such as *K. pneumoniae* group detected among patients with LRTIs and the high proportion of patients with LRTI who had carbapenem resistance gene targets detected in their sputum. As with sputum cultures, it remains difficult to differentiate between true infection and colonization with the use of PN*plus* Panel.

Using the PN*plus* Panel, we identified 211 patients (79.0%) with at least one gram-negative bacterium, 139 (52.1%) patients with at least one gram-positive bacterium, and 111 patients (41.6%) with at least one virus. To contrast these findings, a previous study that applied the panel to patients with LRTI in a low/middle-income setting in South Africa detected typical bacteria, both gram-negative and gram-positive, in only 55.4% of samples [25]. A study conducted in the U.S. identified bacteria in only 53.1% of samples [30]. When looking at specific bacteria detected, similar to the study conducted in South Africa, the gram-positive bacterium *S. aureus* and the gram-negative bacteria *K. pneumoniae* group and *E. cloacae* complex were three of the five most commonly detected bacteria [25]. However, in contrast to the study in South Africa and in those conducted in the U.S. and Europe, we found the gram-negative bacteria *A. calcoaceticus-baumannii* complex to be more prevalent and the gram-positive *S. pneumoniae* to be less present [18; 25; 30]. As for viruses, consistent with the previous studies, the most commonly detected virus was human rhinovirus/enterovirus, a common upper respiratory virus [18; 25; 30].

As for the antibiotic resistance genes, about 40% of patients had at least one carbapenem resistance gene target detected, 21% of patients had an extended spectrum beta-lactamase resistance gene target detected, and 15% of patients had a methicillin resistance gene target detected. Although previous studies have found similar percentages of patients to have methicillin resistance genes, we identified a noteworthy higher percentage of patients to have carbapenem resistance-encoding genes [18; 25]; one study conducted in South Africa found the carbapenem resistance-encoding gene NDM to be present in only 13.6% of sampled patients [31]. However, similar to our findings, a recent study conducted in India identified NDM to be present in 24.2% of patients with LRTIs [32]. With about 1.4 million deaths in South Asia in 2019 being associated with antimicrobial resistance, the high percentage of patients with

carbapenem-resistant isolates identified in this study raises serious concerns about the need to monitor the presence of emerging antibacterial resistance, need for novel antibacterials, and need for improved testing in resource-limited settings [33].

Additionally, we identified several bacteria with genomic concentration distributions that were significantly different, as defined via Fisher's exact test, from the general bacterial population. Previous papers have hypothesized that the genomic concentrations reported for typical bacteria could be used as a potential marker to differentiate between bacterial colonizers and sources of infection [18; 34]. Thus, using this hypothesis, our findings would suggest that the bacteria with the highest percentage of positive samples detected in the $\geq 10^7$ copies/mL bin (e.g., *S. pneumoniae*, *H. influenzae*, or *P. aeruginosa*) are more likely to be the true cause of infections while those with a higher percentage of positive samples detected in the 10^4 copies/mL bin (e.g., *K. aerogenes*, *E. cloacae* complex, and *A. calcoaceticus-baumannii* complex) are more likely to be colonizers. Although *S. pneumoniae* and *H. influenzae* are well known bacteria that cause community-acquired LRTIs, *P. aeruginosa* is less associated with community-acquired LRTIs [35].

In our comparison between the results of the PN*plus* Panel with sputum culture results, 83% of patients with positive sputum culture samples were found to have a matchable result on the PN*plus* Panel screening. This finding of a high positive percent agreement supports the previous findings that highlight the high capability of microbial detection through the PN*plus* Panel [18; 20; 25; 36]. However, the large number of patients who had a positive PN*plus* Panel result with negative culture samples also underlines the previously identified concern of the PN*plus* Panel being unable to differentiate bacterial colonizers compared to those causing true infection [18; 25; 37].

This study had several limitations. Despite sputum samples being a less invasive method to characterize the etiology of LRTI, bronchoalveolar lavage fluid would have provided a more accurate representation of the specific microbiome associated with LRTI by limiting bacteria originating from the upper respiratory tract [24]. Additionally, because the time of collection for sputum samples overlapped with the beginnings of the SARS-CoV-2 pandemic and the PN*plus* Panel was developed prior to the disease onset,

SARS-CoV-2-related LRTIs were unable to be detected. Finally, future studies should include a comparison among patients without LRTI to better differentiate between bacterial colonizers and those causing infection.

In conclusion, we identified a high proportion of patients with LRTI in southern Sri Lanka to have gram-negative bacteria and carbapenem resistance gene targets in their sputum samples tested with the BIOFIRE® FILMARRAY® PN*plus* Panel. Given the large number of patients with a positive panel finding and negative culture result, it remains challenging to use the results from the PN*plus* Panel to identify the microbial etiology of LRTI in this setting. However, the finding of significant numbers of gram-negative bacteria as well as carbapenem resistance-encoding genes in patients hospitalized with LRTI may be an indication of the etiology of community-acquired pneumonia in this setting.

Data availability statement

For researchers who meet criteria for access, data may be available upon request to the Ethics Review Committee, Faculty of Medicine, University of Ruhuna (contact via telephone: 0912234801/803 extension 161 or e-mail: ethics@med.ruh.ac.lk).

Acknowledgements

We would like to acknowledge and thank the patients who participated in the study. Furthermore, we would like to extend our gratitude to the scientists, physicians, collaborators, and research staff at the Ruhuna-Duke Centre for Infectious Diseases

Author contribution

Conceptualization, L.G.T. and C.K.B.; Data curation, J.A. and A.O.; formal analysis, Z.A.W., L.A., and A.O.; funding acquisition, C.W.W. and L.G.T.; investigation, C.W.W., L.G.T., A.N., G.W., S.S.W., B.N.,

S.V., T, R.K., T.O., and R.J.R.; methodology, C.W.W., L.G.T., A.N., G.W., S.S.W., B.N., S.V., T, R.K., and T.O.; project administration, C.W.W., L.G.T., A.N., G.W., S.S.W., B.N., S.V., T, R.K., T.O., M.P., and U.H.B.Y.D.; software, C.W.W., L.G.T., A.N., G.W., S.S.W., B.N., S.V., T, R.K., and T.O.; resources, , C.W.W., L.G.T., A.N., G.W., S.S.W., B.N., S.V., T, R.K., and T.O.; supervision, C.W.W., L.G.T., A.N., G.W., S.S.W., B.N., S.V., T, R.K., and T.O.; validation, L.A. and A.O.; visualization, Z.A.W. and L.A.; writing – original draft, Z.A.W. and L.A.; writing – review & editing, all authors.

Financial support

This work was supported by the National Institutes of Health Fogarty Training Grant (L.G.T., grant number D43 TW009337); National Institutes of Allergy and Infectious Diseases (L.G.T., grant number K23AI125677); and the Duke Hubert-Yeargan Center for Global Health. The BioFire® FILMARRAY® Pneumonia *plus* Panel kits were provided by BioFire Diagnostics.

Conflict of Interest

The authors declare no conflict of interest.

References

Troeger C*, et al.* (2017) Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: A systematic analysis for the global burden of disease study 2015. *Lancet Infectious Diseases* **17:** 1133-1161.

Momtazmanesh S*, et al.* (2023) Global burden of chronic respiratory diseases and risk factors, 1990-2019: An update from the global burden of disease study 2019. *Eclinicalmedicine* **59**.

Buchan BW*, et al.* (2020) Practical comparison of the biofire filmarray pneumonia panel to routine diagnostic methods and potential impact on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. *Journal of Clinical Microbiology* **58**.

Loens K*, et al.* (2009) Optimal sampling sites and methods for detection of pathogens possibly causing community-acquired lower respiratory tract infections. *Journal of Clinical Microbiology* **47:** 21-31.

Torres A*, et al.* (2016) Laboratory diagnosis of pneumonia in the molecular age. *European Respiratory Journal* **48:** 1764-1778.

Faron ML, *et al.* (2017) Clinical evaluation of a semi-quantitative multiplex molecular assay for the identification of bacteria, viruses and fungi in lower respiratory specimens. *Clinical Virology Symposium*.

Gadsby NJ*, et al.* (2016) Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. *Clinical Infectious Diseases* **62:** 817-823.

Jitmuang A*, et al.* (2022) A multiplex pneumonia panel for diagnosis of hospital-acquired and ventilator-associated pneumonia in the era of emerging antimicrobial resistance. *Frontiers in Cellular and Infection Microbiology* **12**.

Poole S*, et al.* (2022) Molecular point-of-care testing for lower respiratory tract pathogens improves safe antibiotic de-escalation in patients with pneumonia in the icu: Results of a randomised controlled trial. *Journal of Infection* **85:** 625-633.

Semret M*, et al.* (2017) Multiplex respiratory virus testing for antimicrobial stewardship: A prospective assessment of antimicrobial use and clinical outcomes among hospitalized adults. *Journal of Infectious Diseases* **216:** 936-944.

Stafylaki D*, et al.* (2022) Impact of molecular syndromic diagnosis of severe pneumonia in the management of critically ill patients. *Microbiology Spectrum* **10**.

Esplund JN*, et al.* (2023) Clinical impact of a multiplex rapid diagnostic pneumonia panel in critically ill patients. *Antimicrob Steward Healthc Epidemiol* **3:** e5.

High J*, et al.* (2021) Inhale: The impact of using filmarray pneumonia panel molecular diagnostics for hospital-acquired and ventilator-associated pneumonia on antimicrobial stewardship and patient outcomes in uk critical care-study protocol for a multicentre randomised controlled trial. *Trials* **22**.

Kyriazopoulou E*, et al.* (2021) Biofire® filmarray® pneumonia panel for severe lower respiratory tract infections: Subgroup analysis of a randomized clinical trial. *Infectious Diseases and Therapy* **10:** 1437-1449.

Markussen DL*, et al.* (2024) Diagnostic stewardship in community-acquired pneumonia with syndromic molecular testing. *Jama Network Open* **7**.

BIOMÉRIEUX (August 2023) Biofire® filmarray® pneumonia panel plus. In.

Gastli N*, et al.* (2021) Multicentric evaluation of biofire filmarray pneumonia panel for rapid bacteriological documentation of pneumonia. *Clin Microbiol Infect* **27:** 1308-1314.

Ginocchio CC*, et al.* (2021) Multinational evaluation of the biofire® filmarray® pneumonia panel as compared to standard of care testing. *European Journal of Clinical Microbiology & Infectious Diseases* **40:** 1609-1622.

Yoo IY*, et al.* (2020) Evaluation of the biofire filmarray pneumonia panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. *Int J Infect Dis* **95:** 326-331.

Edin A, Eilers H and Allard A (2020) Evaluation of the biofire filmarray pneumonia panel plus for lower respiratory tract infections. *Infect Dis (Lond)* **52:** 479-488.

Luyt CE*, et al.* (2020) Usefulness of point-of-care multiplex pcr to rapidly identify pathogens responsible for ventilator-associated pneumonia and their resistance to antibiotics: An observational study. *Critical Care* **24**.

Chen CL*, et al.* (2024) Application of a multiplex molecular pneumonia panel and real-world impact on antimicrobial stewardship among patients with hospital-acquired and ventilatorassociated pneumonia in intensive care units. *Journal of Microbiology Immunology and Infection* **57:** 480-489.

El-Nawawy AA*, et al.* (2022) Comparison of a point-of-care filmarray test to standard-of-care microbiology test in diagnosis of healthcare associated infections in a tertiary care pediatric intensive care unit. *Antibiotics-Basel* **11**.

Hanson KE*, et al.* (2020) Molecular testing for acute respiratory tract infections: Clinical and diagnostic recommendations from the idsa's diagnostics committee. *Clinical Infectious Diseases* **71:** 2744-2751.

Van Der Westhuyzen M*, et al.* (2023) Utility of the biofire filmarray pneumonia panel assay for syndromic testing of lower respiratory tract infections in a low/middle-income setting. *Jac-Antimicrobial Resistance* **5**.

Medrano PG*, et al.* (2024) Prevalence and predictors of antibiotic prescription among patients hospitalized with viral lower respiratory tract infections in southern province, sri lanka. *Plos One* **19**.

(2008) Abbreviated identification of bacteria and yeast, 2nd edition. In: *CLSI M35.* Second ed. Wayne, PA: Clinical and Laboratory Standards Institute.

Franquet T (2018) Imaging of community-acquired pneumonia. *J Thorac Imaging* **33:** 282-294.

(2015) Annual health bulletin. Ministry of Health, Nutrition and Indigenous Medicine.

Webber DM*, et al.* (2020) Evaluation of the biofire filmarray pneumonia panel for detection of viral and bacterial pathogens in lower respiratory tract specimens in the setting of a tertiary care academic medical center. *J Clin Microbiol* **58**.

Mitton B, Rule R and Said M (2021) Laboratory evaluation of the biofire filmarray pneumonia plus panel compared to conventional methods for the identification of bacteria in lower respiratory tract specimens: A prospective cross-sectional study from south africa. *Diagn Microbiol Infect Dis* **99:** 115236.

Shamim U*, et al.* (2024) Functional metagenomics highlights varied infection states with dynamics of pathogens and antibiotic resistance in lower respiratory tract infections. *Heliyon* **10:** e38380.

Antimicrobial Resistance C (2022) Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* **399:** 629-655.

Gong JR*, et al.* (2024) Evaluation and clinical practice of pathogens and antimicrobial resistance genes of biofire filmarray pneumonia panel in lower respiratory tract infections. *Infection* **52:** 545-555.

Guthrie R (2001) Community-acquired lower respiratory tract infections - etiology and treatment. *Chest* **120:** 2021-2034.

Murphy CN*, et al.* (2020) Multicenter evaluation of the biofire filmarray pneumonia/pneumonia plus panel for detection and quantification of agents of lower respiratory tract infection. *Journal of Clinical Microbiology* **58**.

Table 1. Sociodemographic and clinical characteristics of patients with lower respiratory tract infections.

Characteristics	Patients (N = 267)	Percentage (%)
Age (years)		
0-17	14	5.2
≥ 18	253	94.8
Sex		
Female	120	44.9
Male	147	55.1
Co-morbidities		
Asthma	97	36.3
Hypertension	73	27.3
Diabetes	40	15.0
Ischemic heart disease	25	9.4
Chronic obstructive pulmonary disease	19	7.1
Bronchiectasis	12	4.5
Chronic kidney disease	8	3.0
History of pulmonary tuberculosis	8	3.0
Immunosuppressed in past 30 days	3	1.1
Symptoms		
Cough	227	85.0
Fever	174	65.2
Dyspnea	153	57.3
Antibiotic use prior to hospitalization		
Yes	32	12.0
Unsure	107	40.1
No	124	46.4
Chest radiograph during hospitalization		
Opacity/consolidation	37	13.9
Interstitial pattern	12	4.5
Antibiotic prescribed on discharge		
Yes	49	18.4
Unsure	151	56.6
No	63	23.6
Discharge status		
Alive	258	96.6
Transferred	8	3.0
Dead	1	0.4

Table 2. BIOFIRE® FILMARRAY® Pneumonia *plus* Panel results for patients with lower respiratory tract infections. P-values show the association between panel detections and opacity/consolidation or interstitial chest radiograph results.

Screening target	All patients (N = 267)	Opacity or consolidation on chest radiograph (n = 37)	P-value	Interstitial chest radiograph (n = 12)	P-value
Gram-negative bacteria					
Patients with ≥ 1 detection	211 (79.0%)	32 (86.5%)	0.13	10 (83.3%)	>0.99
Acinetobacter calcoaceticus-baumannii complex	87 (32.6%)	15 (40.5%)	0.36	5 (41.7%)	0.54
Enterobacter cloacae complex	68 (25.5%)	12 (32.4%)	0.43	4 (33.3%)	0.51
Escherichia coli	43 (16.1%)	7 (18.9%)	0.64	2 (16.7%)	>0.99
Haemophilus influenzae	65 (24.3%)	12 (32.4%)	0.31	3 (25.0%)	>0.99
Klebsiella aerogenes	18 (6.7%)	4 (10.8%)	0.32	1 (8.3%)	0.58
Klebsiella oxytoca	4 (1.5%)	0 (0%)	>0.99	0 (0%)	>0.99
Klebsiella pneumoniae group	112 (41.9%)	16 (43.2%)	>0.99	5 (41.7%)	>0.99
Moraxella catarrhalis	13 (4.9%)	3 (8.1%)	0.42	1 (8.3%)	0.47
Proteus spp.	5 (1.9%)	1 (2.7%)	0.54	0 (0%)	>0.99
Pseudomonas aeruginosa	71 (26.6%)	15 (40.5%)	0.08	4 (33.3%)	0.74
Serratia marcescens	16 (6.0%)	3 (8.1%)	0.71	0 (0%)	>0.99
Gram-positive bacteria					
Patients with ≥ 1 detection	139 (52.1%)	21 (56.8%)	0.86	4 (33.3%)	0.24
Staphylococcus aureus	92 (34.5%)	12 (32.4%)	0.86	1 (8.3%)	0.067
Streptococcus agalactiae	23 (8.6%)	2 (5.4%)	0.75	1 (8.3%)	>0.99
Streptococcus pneumoniae	51 (19.1%)	11 (29.7%)	0.13	2 (16.7%)	>0.99
Streptococcus pyogenes	11 (4.1%)	0 (0%)	0.37	0 (0%)	>0.99
Atypical bacteria					
Mycoplasma pneumoniae	3 (1.1%)	1 (2.7%)	0.41	1 (8.3%)	0.16
Viruses					
Patients with ≥ 1 detection	111 (41.6%)	16 (43.2%)	0.86	6 (50.0%)	0.57
Adenovirus	11 (4.1%)	1 (2.7%)	>0.99	0 (0%)	>0.99

Coronavirus	15 (5.6%)	4 (10.8%)	0.27	2 (16.7%)	0.16
Human metapneumovirus	2 (0.7%)	1 (2.7%)	0.32	0 (0%)	>0.99
Human rhinovirus/enterovirus	52 (19.5%)	5 (13.5%)	0.50	4 (33.3%)	0.27
Influenza A virus	29 (10.9%)	5 (13.5%)	0.58	0 (0%)	0.62
Influenza B virus	7 (2.6%)	1 (2.7%)	>0.99	0 (0%)	>0.99
Parainfluenza virus	3 (1.1%)	1 (2.7%)	0.41	0 (0%)	>0.99
Respiratory syncytial virus	2 (0.7%)	1 (2.7%)	0.32	0 (0%)	>0.99
Antibiotic Resistant Genes					
Patients with ≥ 1 detection	142 (53.2%)	23 (62.2%)	0.30	5 (41.7%)	0.56
CTX-M	56 (21.0%)	8 (21.6%)	>0.99	2 (16.7%)	>0.99
IMP	27 (10.1%)	3 (8.1%)	>0.99	2 (16.7%)	0.36
KPC	3 (1.1%)	0 (0%)	>0.99	0 (0%)	>0.99
mecA/C and MREJ	42 (15.7%)	3 (8.1%)	0.32	1 (8.3%)	0.70
NDM	68 (25.5%)	12 (32.4%)	0.43	0 (0%)	**0.043**
OXA-48-like	17 (6.4%)	5 (13.5%)	0.16	0 (0%)	>0.99
VIM	42 (15.7%)	10 (27.0%)	0.10	1 (8.3%)	0.70

Figure 1. Detected intersection of bacteria, viruses, or antibiotic resistance-encoding genes identified with the PN*plus* panel from sputum samples. Color ruler ranges between white (low value) and dark blue (high value) and represents the number of patients with specific co-detection. Crossed out boxes represent repeated comparisons or comparisons not potentially reported by PN*plus* panel. N = 267. (A) Intersection among all identified bacteria. (B) Intersection among all identified bacteria and viruses. (C) Intersection among all identified viruses. (D) Intersection among all identified antibiotic resistant genes. (E) Intersection among all identified antibiotic resistant genes and bacteria.

Figure 2. Genomic concentration of typical bacteria identified by the PN*plus* Panel from sputum samples. (A) Distribution of typical bacteria detected from patients based on reported genomic concentration bins (Copies/mL). Color ruler ranges between white (0 samples detected) and dark blue (60 samples detected). N = 267. Fisher's exact test applied to identify distinctness of bacterial bin distribution. * = P-value < 0.05. ** = P-value < 0.01. **** = P-value < 0.0001. (B) Principal component analysis with hierarchical clustering of the PN*plus* Panel results for typical bacteria based on genomic concentration bins.

Figure 3. Comparison of clinical and laboratory culture results with the PN*plus* Panel results. (A) Bar-plot demonstrating the comparison between bacterial culture from clinical and laboratory results with the results from the PN*plus* Panel. A match was defined as the PN*plus* Panel detecting a bacterium that was also detected in the clinical cultures or laboratory culture. A mismatch was defined as when the PN*plus* Panel and cultures detected different bacteria, when the PN*plus* Panel detected bacteria and cultures did not, or when the cultures detected bacteria and the PN*plus* Panel did not. (B) Bar-plot depicting the bacteria detected via the PN*plus* Panel that were also detected via culture samples.