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A multiplex polymerase chain reaction assay for antibiotic stewardship in suspected pneumonia

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A B S T R A C T

Background: Multiplexed molecular rapid diagnostic tests (RDTs) may allow for rapid and accurate diagnosis of the microbial etiology of pneumonia. However, little data are available on multiplexed RDTs in pneumonia and their impact on clinical practice.

Methods: This retrospective study analyzed 659 hospitalized patients for microbiological diagnosis of suspected pneumonia.

Results: The overall sensitivity of the Unyvero LRT Panel was 85.7% (95% CI 82.3–88.7) and the overall specificity was 98.4% (95% CI 98.2–98.7) with a negative predictive value of 97.9% (95% CI 97.6–98.1). The LRT Panel result predicted no change in antibiotics in 12.4% of cases but antibiotic de-escalation in 65.9% (405/615) of patients, of whom 278/405 (69%) had unnecessary MRSA coverage and 259/405 (64%) had unnecessary P. aeruginosa coverage.

Interpretation: In hospitalized adults with suspected pneumonia, use of an RDT on respiratory samples can allow for early adjustment of initial antibiotics, most commonly de-escalation.

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1. Background

Pneumonia remains a leading cause of hospital admissions and is associated with substantial morbidity, mortality, healthcare costs, and days of work lost. (Yu et al., 2012) Community-acquired pneumonia (CAP) is estimated to cause ~1.5 million hospitalizations and ~100,000 deaths in the US each year, (Ramirez et al., 2017) equating to an aggregate cost of nearly $9.5 billion for 960,000 hospital stays. (Thomas et al., 2012; Tong et al., 2018) Hospital-acquired pneumonia (HAP), including ventilator-associated pneumonia (VAP), is the most common healthcare-associated infection. (Magill et al., 2018) Despite clinical advances, the burden of pneumonia on the healthcare system continues to increase, with hospitalization rates for pneumonia increasing by 35% due to Klebsiella spp., 23% due to Pseudomonas spp., and 23% due to Staphylococcus aureus between 2001 and 2011. (Wuerth et al., 2016) An aging population, (Kline and Bowdish, 2016) poor sensitivity diagnostic tools, (Ewig et al., 1996; Bandyopadhyay et al., 2000; Jain et al., 2015) prolonged courses of empirical antibiotics, (Fooled et al., 2018) and the emergence of antimicrobial resistance (Rarrasa-Villar et al., 2017) are all contributing to the persistent and increasing burden of pneumonia.

A hospitalized patient with suspected pneumonia is generally treated empirically. Empirical treatment recommendations in CAP, HAP, and VAP guidelines are based on risk factors for the specific pathogens seen in each form of pneumonia and are consistently associated with overtreatment. (Attridge et al., 2011; Kett et al., 2011; Ekren et al., 2018) Empirical treatment of CAP includes coverage of atypical but uncommon organisms, (Arnold et al., 2007) and empirical antibiotic decisions are even more difficult for HAP/VAP due to other common...
antibiotic-resistant pathogens. Moreover, empirical treatment of both CAP and HAP/VAP focuses on MRSA and Pseudomonas coverage. (Kalil et al., 2016) Subsequent tailoring of antibiotics is recommended to treat the specific etiology depending on the microbiological diagnosis; (Pickens and Wunderink, 2019) however, the turnaround time for a final respiratory culture result can be upwards of 72 hours, during which time patients continue to receive empirical antibiotics. In addition, respiratory cultures are often negative due to their relatively poor sensitivity, particularly when obtained after initiation of antibiotic therapy, and can miss important pathogens when concealed by the growth of multiple organisms in culture. (Ewig et al., 1996; Bandyopadhyay et al., 2000; Jain et al., 2015)

These limitations of respiratory microbiological culture may fail to identify the causative microorganism, which is associated with longer lengths of hospital stay, higher morbidity and mortality, higher hospital costs, nephrotoxicity, and nosocomial infections. (Falcone et al., 2012; Jensen et al., 2012; Vincent and Manges, 2015) Guideline-driven empirical therapy for pneumonia with broad-spectrum antibiotics and the lag time to definitive microbiological diagnosis mean that (i) de-escalation cannot be performed quickly, making the use of inappropriate antibiotics more likely; and (ii) the prolonged use of broad-spectrum antibiotics promotes the development of antimicrobial resistance. (Fowler et al., 2007; Fair and Tor, 2014; Karam et al., 2016) Therefore, an urgent need exists for the rapid detection of the causative pathogen in pneumonia in order to tailor antibiotics and encourage appropriate antibiotic stewardship.

Rapid diagnostic tests (RDTs) that reduce diagnostic turnaround times from days to hours can address some of the aforementioned clinical challenges. The 2019 Infectious Diseases Society of America CAP guidelines acknowledged the need for rapid, cost-effective, accurate tests to improve directed antibiotic therapy. (Metlay et al., 2019) This crisis has led to a recommendation that antimicrobial prescriptions in high-income countries should be made only when supported by rapid diagnostic evidence, where such tests are available. (O’Neill, 2018) However, the majority of current RDTs for pneumonia only detect a single organism, (Torres et al., 2016) and experiences with high-sensitivity RDTs developed for malaria and tuberculosis in resource-poor settings have given rise to concerns that false positive results may result in overtreatment. (Ranadive et al., 2017; Houben et al., 2018; Weinrib and Caprado, 2019) However, data on the clinical application and impact of a new generation of highly multiplexed RDTs, which detect multiple organisms in a single respiratory sample by molecular testing, are lacking.

The Unyvero Lower Respiratory Tract (LRT) Panel is an RDT that uses multiplex polymerase chain reaction (PCR) to identify 20 causative agents of severe lower respiratory tract infections (LRITs) and ten antibiotic resistance determinants in clinical specimens. Targets comprise two Gram-positive bacteria (Staphylococcus aureus and Streptococcus pneumoniae), 14 Gram-negative bacteria (including Pseudomonas aeruginosa), three atypical pneumonia species (Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae), and one fungus (Pneumocystis jiroveci). The test uses an automated sample-to-answer platform with minimal hands-on time and takes approximately five hours, so it has the potential for use as a point-of-care test.

Using patients enrolled at two of the clinical sites for NCT01922024, a large non-interventional multicenter study that determined the operating characteristics of the Unyvero LRT Panel, we predicted the impact of the Unyvero LRT Panel RDT results on adjustment of empiric antibiotic regimens in hospitalized patients with suspected pneumonia.

2. Methods

2.1. Study population, inclusion criteria, and ethical approval

This retrospective study analyzed subjects enrolled in NCT01922024 at Northwestern Memorial Hospital (NMH) in Chicago, IL and Beaumont Health (BH); comprising three hospitals in Royal Oak, Troy, and Grosse Pointe in Michigan. NCT01922024 was a large non-interventional multicenter study conducted in 2015–2016 to determine the operating characteristics of the Unyvero LRT Panel. Any study subject enrolled in NCT01922024 with an available study ID linked to the electronic medical records (EMR) of NMH or BH was considered for inclusion. Inclusion criteria were hospitalization patients ≥18 years old; with a suspicion of a LRTI; and with an available surplus of >1 ml endotracheal aspirate (ETA) or bronchoalveolar lavage (BAL) fluid. Exclusion criteria were known infection with HIV, HBV, or tuberculosis. The Northwestern University and Beaumont Health Institutional Review Boards approved retrospective chart review of clinical parameters and outcomes prior to local study site closure (IRB reference number (s) STU00260608 and IORG0000367, FWA 00002516. The date of hospital admission to discharge; date and time of antibiotic administration; culture results from blood, urine, and sites other than respiratory; other microbiologic tests; and discharge disposition were retrieved from the EMR.

2.2. Unyvero LRT panel

The Unyvero LRT Panel is an RDT specifically designed for the detection of LRITs. Specimens were processed with the Unyvero LRT Panel assay as per the manufacturer’s instructions and as described previously. (Ozongwu et al., 2017; Gadsby et al., 2019) Organisms detected in the assay are shown in Table 1.

2.3. Definition of concordance and discordance of RDT and culture results

Results of the LRT Panel were compared to the final culture results in order to more accurately mimic clinical practice. A sample was defined as concordant if it was: (i) LRT Panel negative, culture negative; (ii) LRT Panel positive, culture positive; (iii) LRT Panel negative, culture positive; (iv) LRT Panel positive, culture negative.

Table 1

| Organism                                      | Resistance       | Gene  |
|-----------------------------------------------|-----------------|-------|
| Acinetobacter spp.                           | Carbapenem      | kpc   |
| Chlamydia pneumoniae                         |                 |       |
| Citrobacter freundii                         |                 |       |
| Enterobacter cloacae complex                  |                 | oxa-23|
| Escherichia coli                             |                 | oxa-24|
| Haemophilus influenzae                       |                 | oxa-48|
| Klebsiella oxytoca                           |                 | vim   |
| Klebsiella pneumoniae                        |                 |       |
| Klebsiella variicola                         |                 | 3rd generation cephalosporins |
| Legionella pneumophila                        |                 | ctx-M |
| Moraxella catarrhalis                        |                 |       |
| Morganella morganii                          |                 |       |
| Mycoplasma pneumoniae                        |                 |       |
| Pneumocystis jiroveci                        |                 |       |
| Proteus spp.                                 |                 | oxa-58|
| Pseudomonas aeruginosa                       |                 |       |
| Serratia marcescens                          |                 |       |
| Staphylococcus aureus                        |                 |       |
| Streptococcus pneumoniae                     |                 |       |

* Acinetobacter spp. detected by LRT panel: A. baumannii, A. calcoaceticus, A. haemolyticus, A. junii, A. lwofii, A. nosocomialis, A. parvus, A. pittii.  
* Enterobacter cloacae complex includes: E. asburiae, E. chengduensis, E. chumadensis, E. cloacae, E. hormaechei (incl. Ssp. xiangfengensis), E. iobei, E. ludwigi, E. roggenkampii, E. sichuanensis as well as E. bugandensis (not yet recognized as member of the E. cloacae complex).  
* Klebsiella pneumoniae includes two variants: K. pneumoniae (variant 1), and K. quasipneumoniae (variant 2).  
* Proteus spp. includes P. cibarius, P. hauseri, P. mirabilis, P. penneri, and P. vulgaris.
Panel positive, culture positive for the same organism; (iii) LRT Panel positive, culture positive for the same organism and a non-panel organism; or (iv) LRT panel negative, culture positive for a non-panel organism. A sample was defined as discordant if it was: (i) LRT Panel positive, culture negative; (ii) LRT Panel positive, culture positive for a different panel organism; or (iii) LRT Panel negative, culture positive for a panel organism. A sample was defined as both concordant and discordant if the LRT Panel and culture results had the same organism plus an additional panel organism reported by either assay.

2.4. Assessment of predicted changes to empirical antibiotic administration

Our assessment of the potential changes to empirical antibiotics based on LRT Panel results assumed that pneumonia was the most likely source of infection. Appropriate versus inappropriate antibiotic regimens were based on the published guidelines for definitive treatment of CAP and HAP (Fig. 1) and the organisms detected by the LRT Panel. (Kalil et al., 2016; Metlay et al., 2019) In this study, we did not use resistance markers to guide de-escalation of antibiotic therapy with the exception of mecA for S. aureus, since mecA is the central determinant of MRSA. (Spratt, 1994) However, we did use detection of a carbapenemase gene to suggest the need to escalate antibiotic therapy. If MRSA or P. aeruginosa were not detected by the panel, then anti-MRSA and/or anti-pseudomonal therapy were deemed unnecessary.

Predicted changes in therapy based on the LRT panel results were defined as: (1) No antibiotic change indicated, where an appropriate antibiotic regimen was used to treat the organism identified by the LRT Panel OR no pathogen was identified by LRT Panel and no anti-MRSA or anti-pseudomonal therapy was administered; (2) Favors de-escalation, where antibiotics could have been narrowed earlier based on the LRT Panel result; this category included cases where the LRT Panel result was negative but patients were on anti-MRSA and/or anti-pseudomonal therapy; (3) Favors expansion, where the antibiotic regimen used would not typically have adequately treated the pathogen identified by the LRT Panel; (4) De-escalation and expansion favored, where multiple antibiotics were used and one drug was too broad while the other was too narrow, e.g., LRT Panel reported P. aeruginosa but the empirical antibiotic regimen was vancomycin (too broad) and ceftriaxone (too narrow); (5) Initiate antibiotics, where antibiotics were not initiated at the time of testing but an organism was identified by the LRT Panel. Results from the LRT Panel were not available to the treating clinician during the study.

2.5. Statistical analysis

Results were analyzed using descriptive statistics. For sensitivity and specificity calculations, routine culture was considered the gold standard. All statistical analyses were performed in SPSS v21 (IBM Statistics Inc., Chicago, IL).

3. Results

3.1. Sample characteristics

Six-hundred and fifty-nine unique samples were enrolled in NCT01922024 at the two hospital systems. Study IDs were unavailable for 39 samples, and 5 samples had culture data but no antibiotic use data. Thus, 659 samples were available for determining the assay operating characteristics, 620 samples were available for concordance analysis, and 615 samples for antibiotic change analysis. Three-hundred and ninety-five samples were non-bronchoscopic or bronchoscopic BALs.

![Fig. 1. Selection of appropriate antibiotic regimen based on published guidelines.](https://example.com/fig1.png)
and 225 samples were ETAs obtained from patients in intensive care units and chronic ventilator units of the included hospitals.

3.2. Operating characteristics of the LRT panel and concordance between RDT and culture results

Compared to the bacterial culture standard, the sensitivity, specificity, positive predictive value, and negative predictive value of the Unyvero LRT Panel in this patient population are shown in Table 2. While the sensitivity varied for different organisms, the specificity was uniformly high (96.5–99.5%). The overall sensitivity of the Unyvero LRT Panel was 85.7% (95% CI 82.3–88.7) and the overall specificity was 98.4% (95% CI 98.2–98.7). Accordingly, the assay had a very high negative predictive value of 97.9% (95% CI 97.6–98.1). This compared favorably to a 91.4% sensitivity and 99.5% specificity for pathogen detection in the original nine-center study (ClinicalTrials.gov: NCT01922024), which determined operating characteristics using culture plus an independent PCR test as the gold standard for common pathogens, rather than culture alone.

Table 3 Concordance between RDT and culture results.

| LRT panel and culture agreement Total % | CONCORDANT | DISCORDANT | BOTH CONCORDANT and DISCORDANT |
|----------------------------------------|------------|------------|-------------------------------|
| Negative, Culture negative for panel organism | 203 (32.7) | 57 (9.2) | 17 (2.7) |
| Negative, Culture positive for panel organism | 211 (34.0) | 23 (3.7) | 7 (1.1) |
| Positive, Culture negative | 34 (5.5) | 25 (4.0) | 19 (3.0) |
| Positive, Culture positive for non-panel organism | 17 (2.7) | 3 (0.5) | 9 (1.5) |

The concordance between the Unyvero LRT Panel and culture results is reported in Table 3. According to our criteria, 75.0% of results were discordant, 9.2% were discordant, and 15.8% were both discordant and discordant. Of the discordant results, 4% each were due to either LRT Panel negative/culture positive discordance or vice versa, suggesting that the LRT panel and culture misclassified similar numbers of cases for the major organisms responsible for LRTIs.

3.3. Predicted changes to antibiotic therapy based on RDT testing

Having determined the organism and mecA status by the LRT Panel, we next predicted changes to antibiotic regimens based on the published guidelines for treatment of CAP and HAP (Fig. 1). (Kalil et al., 2016; Metlay et al., 2019) Reassured by the excellent negative predictive value of the LRT Panel, we determined that if MRSA or P. aeruginosa were not detected by the panel, then anti-MRSA coverage and 259/405 (64%) had unnecessary antibiotic expansion group, the most common organisms not initially covered by the LRT Panel was 85.7% (95% CI 82.3–88.7) and the overall sensitivity of the Unyvero LRT Panel was 85.7% (95% CI 82.3–88.7) and the overall speci

Table 2 The operating characteristics of the Unyvero LRT panel.

| Organism | TP | FN | FP | TN | Sensitivity | Specificity | PPV | NPV |
|----------|----|----|----|----|-------------|-------------|-----|-----|
| Acinetobacter spp. | 23 | 2 | 14 | 620 | 92.0 (74.0–100.0) | 97.8 (96.3–98.8) | 62.2 (49.1–73.6) | 97.6 (96.0–98.6) |
| Citrobacter freundii | 0 | 1 | 6 | 652 | 0 (0–97.5) | 99.0 (98.0–99.7) | N/A | N/A |
| Enterobacter cloacae complex | 29 | 6 | 3 | 621 | 82.9 (66.4–93.4) | 99.5 (98.6–99.9) | 90.6 (75.6–96.8) | 98.6 (97.4–99.4) |
| Escherichia coli | 38 | 3 | 15 | 603 | 92.7 (80.0–98.5) | 97.6 (96.0–98.6) | 71.7 (60.4–80.8) | 97.3 (95.7–98.4) |
| Haemophilus influenzae | 10 | 4 | 10 | 635 | 71.4 (41.9–91.6) | 98.3 (97.2–99.3) | 50.0 (33.2–66.8) | 97.9 (95.8–98.5) |
| Klebsiella oxytoca | 16 | 5 | 11 | 627 | 76.2 (52.8–91.8) | 98.3 (96.9–99.1) | 99.9 (97.6–99.8) | 97.6 (95.8–98.6) |
| Klebsiella pneumoniae | 35 | 12 | 10 | 602 | 74.5 (59.7–86.0) | 98.4 (97.0–99.2) | 77.8 (64.9–86.9) | 98.1 (95.9–98.8) |
| Moraxella catarrhalis | 10 | 0 | 4 | 645 | 100.0 (100.0–100.0) | 99.4 (98.5–99.8) | 200.0 (8.6–39.9) | 100 (100–100) |
| Moxarella morganii | 2 | 0 | 7 | 646 | 100% (100–100) | 98.9 (97.8–99.6) | 46.2 (29.1–64.2) | 100 (100–100) |
| Proteus spp. | 19 | 3 | 12 | 625 | 86.4 (65.1–97.1) | 98.1 (96.7–99.0) | 61.3 (46.5–74.0) | 97.7 (95.3–98.7) |
| Pseudomonas aeruginosa | 72 | 9 | 16 | 562 | 88.9 (80.9–94.8) | 97.2 (95.5–98.4) | 81.8 (73.6–88.0) | 98.4 (97.1–99.1) |
| Serratia marcescens | 14 | 4 | 4 | 637 | 77.8 (52.4–93.6) | 99.4 (98.4–99.8) | 77.8 (56.1–90.6) | 99.4 (95.8–99.7) |
| Staphylococcus aureus | 109 | 17 | 17 | 516 | 86.5 (79.3–91.9) | 96.8 (94.9–98.1) | 86.5 (80.0–91.1) | 96.8 (95.1–97.9) |
| Stenotrophomonas maltophilia | 34 | 1 | 22 | 602 | 97.1 (84.9–100.0) | 96.3 (94.7–97.8) | 40.7 (50.5–70.1) | 99.8 (98.9–100.0) |
| Streptococcus pneumoniae | 7 | 2 | 3 | 647 | 77.8 (40.9–102.2) | 99.5 (97.8–99.9) | 70.0 (41.7–88.4) | 99.7 (95.9–99.9) |

Abbreviations: TP, true positive; FN, false negative; FP, false positive; TN, true negative; PPV, positive predictive value; NPV, negative predictive value.
not necessitate antibiotic escalation, and a further 28% (22/79) were organisms that would have been covered even with empirical treatment that removed MRSA and Pseudomonas coverage.

4. Discussion

The laboratory diagnosis of LRTIs is predominantly based on microbiological cultures, introducing delays and the prolonged use of empirical, broad-spectrum antibiotic therapy in large numbers of patients. Antibiotic resistance is a severe and increasing problem worldwide, mandating the increased use of improved RDTs to reduce antibiotic administration and increase the use of specific antimicrobial therapies. (Aliberti et al., 2013; Cilloniz et al., 2016) Also, in consideration of the current global COVID-19 pandemic, concerns about secondary bacterial infections in hospitalized COVID-19 patients have given rise to the need for timely and appropriate diagnosis of pneumonia to address the over and under-treatment of patients, enabling healthcare providers to practice better antibiotic stewardship, and helping to limit resistance and the development of super-bugs. (Gerberding, 2020) Rapid multiplex RDTs may play a critical role in this crisis setting. Rapid molecular techniques such as the Unyvero LRT Panel are a promising tool to help guide appropriate therapy and de-escalation from broad-spectrum antibiotic therapy in patients with suspected pneumonia. (Torres et al., 2016; Sullivan and Dien Bard, 2019) However, like any new medical technology, their potential clinical impact requires testing in clinical practice, and data on multiplex assays in particular are lacking. Here we analyzed the potential impact on antibiotic use in patients with suspected pneumonia with implementation of the Unyvero LRT Panel using data from a non-interventional study that originally evaluated the operating characteristics of the assay.

Table 4

| Potential impact on therapy based on Unyvero LRT results alone | Total |
|---------------------------------------------------------------|-------|
| No antibiotic change indicated                                | 76 (12.4%) |
| Favors de-escalation (antibiotics could have been narrowed)   | 405 (65.9%) |
| Favors expansion (antibiotics could have been broadened)      | 67 (10.0%) |
| Favors both de-escalation and expansion of antibiotics         | 48 (7.8%) |
| Start antibiotics                                              | 19 (3.1%) |
| **Total Samples Available for Analysis**                       | 615 (100%) |

The overall sensitivity (85.7%) and specificity (98.4%) of the assay for organism detection in our mixed population of BALF and ETA samples were consistent with previous reports for this (88.8% and 94.9%) and another (90% and 97.4%) FDA-approved multiplex LRTI assay and comparable to the sensitivities and specificities reported for traditional ETA and BAL culture. (Baselski and Wunderink, 1994; Shin et al., 2021) A recent evaluation of the Unyvero LRT Panel in 175 BALF specimens reported a positive percentage agreement of 96.5% and negative percentage agreement of 99.6% with quantitative bacterial culture. (Collins et al., 2017) The laboratory diagnosis of LRTIs is predominantly based on microbiological cultures, introducing delays and the prolonged use of empirical, broad-spectrum antibiotic therapy in large numbers of patients. Antibiotic resistance is a severe and increasing problem worldwide, mandating the increased use of improved RDTs to reduce antibiotic administration and increase the use of specific antimicrobial therapies. (Aliberti et al., 2013; Cilloniz et al., 2016) Also, in consideration of the current global COVID-19 pandemic, concerns about secondary bacterial infections in hospitalized COVID-19 patients have given rise to the need for timely and appropriate diagnosis of pneumonia to address the over and under-treatment of patients, enabling healthcare providers to practice better antibiotic stewardship, and helping to limit resistance and the development of super-bugs. (Gerberding, 2020) Rapid multiplex RDTs may play a critical role in this crisis setting. Rapid molecular techniques such as the Unyvero LRT Panel are a promising tool to help guide appropriate therapy and de-escalation from broad-spectrum antibiotic therapy in patients with suspected pneumonia. (Torres et al., 2016; Sullivan and Dien Bard, 2019) However, like any new medical technology, their potential clinical impact requires testing in clinical practice, and data on multiplex assays in particular are lacking. Here we analyzed the potential impact on antibiotic use in patients with suspected pneumonia with implementation of the Unyvero LRT Panel using data from a non-interventional study that originally evaluated the operating characteristics of the assay.

Table 6

| Organism                  | Number (%) |
|---------------------------|------------|
| Viridans Streptococcus*   | 15 (19.0%) |
| Corynebacterium*          | 13 (16.5%) |
| Enterobacter aerogenes    | 10 (12.6%) |
| Coagulase negative Staphylococcus* | 7 (8.9%) |
| Citrobacter koseri        | 5 (6.3%)  |
| Alcaligenes               | 4 (5.1%)  |
| Enterococcus spp.*        | 2 (2.5%)  |
| Prevotella*               | 1 (1.3%)  |
| Pantoea                   | 1 (1.3%)  |
| Enterobacter sakazakii    | 1 (1.3%)  |
| Group F Streptococcus*    | 1 (1.3%)  |
| Group C Streptococcus*    | 1 (1.3%)  |
| Moraxella/Phyrobacter     | 1 (1.3%)  |
| Pseudomonas putida        | 1 (1.3%)  |
| Pseudomonas fluorescen     | 1 (1.3%)  |
| Enterobacter gergoviae    | 1 (1.3%)  |
| Aeromonas                 | 1 (1.3%)  |
| Elekkenella corrodens     | 1 (1.3%)  |
| Providencia               | 1 (1.3%)  |
| Enterobacter amigenus     | 1 (1.3%)  |
| Streptococcus mitis*      | 1 (1.3%)  |
| Beta hemolytic Streptococcus* | 1 (1.3%) |
| Raoultella spp.           | 1 (1.3%)  |
| Aspergillus               | 1 (1.3%)  |
| Actinomyces*              | 1 (1.3%)  |
| Streptococcus agalactiae  | 1 (1.3%)  |
| Burkholderia cepacia      | 1 (1.3%)  |
| Lactobacillus spp.*       | 1 (1.3%)  |
| Citrobacter youngae       | 1 (1.3%)  |
| Enterococcus faecium      | 1 (1.3%)  |
| **Total non-panel organisms** | 79 |
recent report in the critical care setting that multiplex PCR for respiratory pathogens would alter the antibiotic choice earlier in >50% of cases. (Gadsby et al., 2019) Contrary to previous concerns that RDTs may lead to over-treatment, (Ranadive et al., 2017; Houben et al., 2018; Weinrib and Caparro, 2019) our data suggest that implementation of this RDT would de-escalate antibiotic use in the majority of cases. Even in cases where the LRT Panel result was negative for any organism, initial empirical antibiotic regimens usually still included anti-MRSA and anti-pseudomonal drugs. Conversely, in cases where the organism identified by the LRT panel was missed by culture, the patient was still usually covered by the empirical antibiotic regimen. In this scenario, LRT results may potentially prevent inappropriate de-escalation.

A recent study of the BioFire FilmArray Pneumonia panel concluded that the panel could also allow antibiotic adjustment in 71% of cases including discontinuation or de-escalation in 48% of patients. (Buchan et al., 2020) These findings, particularly the utility of a rapid diagnostic test for antibiotic de-escalation, were similar to the findings in our study of the LRT Panel. The two panels have important differences. The LRT Panel includes important etiologic agents of pneumonia like Stenotrophomonas maltophilia, Pneumocystis jirovecii, Klebsiella variicola, Morganella morganii and Citrobacter freundii; these organisms are not in the FilmArray Pneumonia panel. However, the FilmArray Pneumonia panel includes Klebsiella aerogenes, Streptococcus agalactiae and Streptococcus pyogenes, and common respiratory viruses not on the LRT panel. Another difference between these panels is that the LRT Panel includes ten resistance genes while the FilmArray Pneumonia Panel includes only six. The FilmArray Pneumonia Panel provides semi-quantitative results using different bin categories corresponding to 10^4, 10^5, 10^6, or ≥10^7 copies/mL while the LRT Panel does not. Correlations to quantitative culture results reported as CFU/mL are however difficult, and agreement rates may be variable between organisms and in different specimen types; and concordance may be low, especially at lower CFU/mL densities. (Poole and Clark, 2020) The role of reporting semi-quantitative PCR results has yet to be defined in clinical practice.

Our evaluations regarding antibiotic escalation and de-escalation were based on the assumption that pneumonia was the most likely source of infection, as the study had enrolled only patients with a suspected LRTIs. In practice, in cases where another positive culture or extra-pulmonary infection is documented, the LRT Panel result should be used in conjunction with the other culture results to make decisions on treatment. If pneumonia is the only suspected site of infection, treatment could be narrowed and/or combination therapy discontinued, with important antibiotic stewardship implications. However, in patients presenting with sepsis or septic shock of unclear etiology, antibiotic decisions should not be based solely on an RDT.

This study has a number of limitations. We constructed a hypothetical estimate of the utility of the test, assuming 100% compliance with the assay and its results, which is unlikely to be achievable in practice. The decision to alter antimicrobial therapy was based on clinical guidelines, so mainly exploited results from two of the 20 organism targets. The decision to alter antimicrobial therapy was based on clinical guide- lines of the utility of the test, assuming 100% compliance with the antimicrobial stewardship program. rapid tests that identify organisms in respiratory samples can optimize antimicrobial utilization and patient outcomes.

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