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Guidance on Use of the Pneumonia Panel for Respiratory Infections

Although the number of pathogens that cause pneumonia is lengthy, establishing the microbiologic etiology of pneumonia is inherently difficult. A recent large multi-center study of community-acquired pneumonia (CAP) found that only 38% of 2259 CAP cases had a microbiologic diagnosis with 23% having viruses detected, 11% bacterial, and 3% had both viruses and bacteria detected.[1] Current tools to assist in pneumonia diagnosis include respiratory tract cultures (sputum, BAL, tracheal aspirate, mini-BAL), urine antigens (pneumococcal, *Legionella*), serology, and PCR for viral and certain bacterial pathogens. While these tools are useful, the study noted above used all these tools and was unable to document an etiology causing pneumonia in 62% of patients. Thus, more sensitive tools for detection of respiratory pathogens are still needed.

Nebraska Medicine has recently introduced a new FDA-approved multiplex PCR panel to assist in determination of the etiology of pneumonia, termed the Pneumonia Panel (PP). This test uses a nested multiplex PCR-approach to amplify nucleic acid targets directly from sputum or bronchoalveolar lavage (BAL) in patients with suspected pneumonia. The list of pathogens and resistance genes included in the panel is found in **Table 1**. Note that the bacterial targets are detected semi-quantitatively whereas the atypical pathogens and the viral targets are detected qualitatively.

Table 1: Pneumonia Panel Pathogen Targets and Associated Resistance Genes

Semi-quantitative Detection:	
Gram Positive Organisms: *Staphylococcus aureus* *Streptococcus pneumoniae* *Streptococcus agalactiae* *Streptococcus pyogenes*	Resistance Genes (*Staph aureus* only): *mecA/C* and MREJ
Gram Negative Organisms: *Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* complex *E. coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumonia* *Moraxella catarrhalis* *Proteus* spp. *Pseuodomonas aeruginosa* *Serratia marcescens*	Resistance Genes (All Gram Negatives): CTX-M IMP KPC NDM VIM OXA-48-like
Qualitative Detection:	
Atypical Pathogens: *Chlamydia pneumoniae* *Legionella pneumophila* *Myocplasma pneumoniae*	Viral Pathogens: Adenovirus Coronavirus Human Metapneumovirus Rhinovirus/Enterovirus Influenza A Influenza B Parainfluenza RSV

Pneumonia Diagnostic Issues:

The diagnosis of pneumonia is difficult as many other conditions can mimic pneumonia such as heart failure, pleural effusion, malignancy, etc. Thus, the Pneumonia Panel should only be ordered in patients with a clinical syndrome highly suggestive of pneumonia. It should not be used to decide if a patient has pneumonia. Patients can be colonized with organisms that are detected by the panel even when pneumonia is not present. Therefore a positive result on the pneumonia panel does not mean the patient has pneumonia or that antibiotics should be started. The decision to start antibiotics should be a clinical decision, while the panel should be used to refine and adjust the use of antibiotics, not to decide whether they are needed. Biomarkers such as procalcitonin can be helpful in determining if antibiotics are indicated (see guidance on ASP website).

Respiratory Tract Cultures:

All specimens submitted for Pneumonia Panel testing will simultaneously be cultured. If a sputum is obtained and judged to be of poor quality based on Gram stain (>25 squamous cells/hpf) it will be rejected and a new specimen should be obtained. Complete culture results are generally reported within 48-72 hours.

Criteria for Pneumonia Panel Use: This test should only be used in patients who have clear evidence of pneumonia (signs and symptoms + increased oxygen need + new or progressive radiographic infiltrate). Use should be restricted to situations where the result will change therapy. Recent CAP guidelines suggest that clinicians should not use diagnostic testing in patients with non-severe CAP being treated with typical therapy (see ASP pneumonia guidelines).[2] The Pneumonia Panel should be considered in the following situations:

1) Patients with severe CAP (admitted to ICU, respiratory failure, etc.).
2) CAP patients on expanded-spectrum therapy (vancomycin, cefepime, etc.).
3) Patients not responding to typical therapy.
4) Patients with hospital-acquired or ventilator-associated pneumonia.

Pneumonia panel ordering is restricted to the ICU or the Infectious Disease and Pulmonary team. If a sputum test was performed, the panel may be ordered on a subsequent BAL specimen, independent of the time between specimens' collection. Otherwise, the Pneumonia panel cannot be repeated within 10 days unless discussed with the microbiology director.

Issues with Interpretation:

Respiratory Tract Colonization: The interpretation of results from this panel are complicated by a number of issues. First, various organisms may colonize the respiratory tract without causing infection. For example, *S. aureus* or *S. pneumoniae* often colonize the nasopharynx whereas *Pseudomonas aeruginosa* often colonizes the lower respiratory tract, particularly in the setting of structural lung disease. Sputum and BAL are therefore not expected to be sterile. The highly sensitive nature of nucleic acid testing means that the Pneumonia Panel may detect colonizing organisms that would not be detected in culture or that would be reported as "normal respiratory flora" due to their limited quantity. Treatment decisions should be based on the clinical likelihood of pneumonia, not necessarily on the detection of organisms.

Multiple Organism Detection: Patients may have more than one organism detected in their sputum or BAL. In the validation study of the pneumonia panel, 38% of 413 positive BAL specimens and 56% of 602 positive sputum specimens had >1 organism detected. Combinations of both viruses and bacteria and multiple bacteria were found. BAL specimens were more likely to be mono-microbial than sputum. When multiple organisms

were present, the most abundant organism detected by the Pneumonia Panel was usually the most prevalent organism detected by culture (concordance 79% BAL, 86% sputum). Most bacterial pulmonary infections are monomicrobial in nature and generally only the most common bacterial pathogen should be targeted for antibacterial therapy.

Positive Panel with a Negative Culture: Molecular tests are more sensitive than traditional culture methods for the detection of bacterial organisms and the panel may detect organisms at very low levels. In validation testing, the pneumonia panel found at least one organism in 49% of BAL specimens and 72% of sputum specimens. The sensitivity was determined using quantitative culture confirmed in culture negative specimens using additional molecular tests. The results of this analysis is included below in **Table 3**. Overall the panel is very sensitive for the detection of most respiratory tract pathogens. Organisms detected by the panel that were not found in quantitative culture were frequently present at levels below what quantitative culture could detect or were only detected using other molecular methods. This may be because the organisms were in very low levels or were non-viable due to antibiotic pre-treatment. This occurred most frequently with *S. aureus*, *H. influenzae*, and *P. aeruginosa*. The pneumonia panel will always be accompanied by a clinical culture to confirm the presence of bacterial pathogens and determine antimicrobial susceptibility. Interpretation of positive panel result with a negative culture requires clinical consideration. Organisms such as *S. aureus* and *P. aeruginosa* are relatively easy to detect using routine cultures, while other organisms such as *H. influenzae* and *S. pneumoniae* are more difficult to detect, particularly after antibiotics have been started. With this in mind if *S. aureus* (especially MRSA) and/or *P. aeruginosa* are detected by the panel but not confirmed by culture, therapy directed at these organisms can be de-escalated to typical community-acquired pneumonia coverage. This is in line with current HAP/VAP guidelines, which recommend that when organisms are not detected in culture, therapy should be withheld or discontinued.[3]

Bin Number Interpretation: Bacterial pathogens will be reported as either Not Detected or semi-quantitatively via a "Bin." These Bin's represent the relative abundance of nucleic acid in the specimen and are reported as copies/mL. The Bin numbers do not correlate with quantitative cultures and are usually higher than what would be detected on quantitative culture. Samples with Bin numbers of 10^4 or 10^5, particularly with *S. aureus, H. influenzae* and *Pseudomonas aeruginosa* may not be detected in culture. Thus, with certain pathogens, such as *S. aureus,* Bin numbers of 10^4 or 10^5 may reflect colonization particularly from an expectorated sputum sample. Viral and atypical pathogens and resistance gene markers are reported qualitatively as Detected or Not Detected.

<u>Antimicrobial Resistance Gene Markers:</u>

- *mecA* encodes for PBP2A and functions to mediate methicillin (oxacillin) resistance in *S. aureus*. A positive result for *mecA* suggests that MRSA is present. A negative result for *mecA* suggests that the *S. aureus* is susceptible to semi-synthetic penicillins, β-lactam/β-lactamase inhibitor combinations or cefazolin/ceftriaxone and typical CAP therapy can be continued.
- CTX-M ($bla_{CTX\text{-}M}$) encodes for the most common extended-spectrum β-lactamase (ESBL) enzyme found in gram negative *Enterobacteriaceae* such as *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. ESBLs hydrolyzes expanded spectrum cephalosporins (ceftriaxone, cefepime) and piperacillin/tazobactam. A positive results suggests that gram negative therapy should usually be escalated to a carbapenem. A negative result does not exclude the presence of other ESBLs
- IMP, KPC, NDM, OXA-48-like, and VIM are all carbapenemase gene markers in *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Carbapenemases hydrolyze all β-lactam antibiotics including the

carbapenems. Detection of these genes should result in immediate consultation with Infectious Disease. The absence of these markers does not always predict carbapenem susceptibility as other mechanisms can result in carbapenem resistance, particularly within *Pseudomonas aeruginosa*.

Table 2: Therapy Recommendations Based on Pneumonia Panel Results

Listed below are the potential results of the pneumonia panel and specific therapy recommendations based upon Nebraska Medicine-specific antibiogram data (respiratory tract-specific and overall antibiogram). Previous respiratory culture results and antibiotic allergies should be taken into account when making therapy decisions.

Pathogen Detected	Preferred Therapy	Comments
Staphylococcus aureus		
Negative *S. aureus*	Stop MRSA therapy if started	A negative pneumonia panel for *S. aureus* rules out MRSA pneumonia and anti-MRSA agents should be discontinued.
mecA/C negative = MSSA	Ampicillin/Sulbactam or Ceftriaxone	For sputum panel positive for MSSA continue treatment with typical CAP agents. • If only MSSA found on culture, narrow to cefazolin or oxacillin • If culture MSSA negative, continue typical CAP therapy
mecA/C positive = MRSA	Vancomycin or Linezolid De-escalation Options: *mecA/C* negative: Cefuroxime, Amoxicillin/clavulanate *mecA/C* positive: Linezolid, TMP/SMX	For sputum positive for MRSA, add vancomycin or linezolid and continue typical CAP agents: • If culture MRSA positive stop typical CAP agents • If culture MRSA negative stop MRSA agent at 72 hours unless clinical syndrome highly suggestive of MRSA
Streptococcus pneumoniae		
No concern for CNS infection	Penicillin or Ampicillin	In severe CAP or pneumococcal bacteremia use combination therapy with azithromycin
Concern for CNS Infection	Ceftriaxone PLUS Vancomycin De-escalation Options (non-CNS): Amoxicillin, Cefuroxime	Continue vancomycin until susceptibility results are reported
Streptococcus pyogenes **(Group A Strep) and** ***Streptococcus agalactiae*** **(Group B Strep)**	Penicillin or Ampicillin or Cefazolin De-escalation Options: Amoxicillin, Cephalexin	-hemolytic strep are uniformly susceptible to penicillin

***Acinetobacter calcoaceticus-baumannii* complex**	Meropenem +/- amikacin De-escalation Options: Levofloxacin 79-94% Minocycline 82-85%	**No β-lactam with >90% activity** Percent Susceptible: Meropenem 83-94% Cefepime 76-79% Consider addition of amikacin in severely ill or non-responding (cefepime +amikacin, meropenem + amikacin active 98%)
Enterobacter (Klebsiella) aerogenes	Cefepime De-escalation Options: Levofloxacin 92-100% TMP/SMX 96-97%	Percent Susceptible: Cefepime: 99-100% Ertapenem: 92-94% Pip/Tazo: 82-92% Ceftriaxone: 70-74%
Enterobacter cloacae	Cefepime De-escalation Options: Levofloxacin 100% TMP/SMX 92-94%	Percent Susceptible: Cefepime: 94-95% Meropenem: 98-100% Pip/tazo: 75-82% Ceftriaxone: 59-67%
E. coli *CTX-M* = Possible Extended-Spectrum Beta-Lactamse (ESBL)	CTX-M Negative: Cetriaxone or Pip/tazo CTX-M Positive: Ertapenem or Meropenem* De-escalation Options: CTX-M Negative: Cefdinir CTX-M Positive: Use culture data, limited oral options	Percent Susceptible: Ceftriaxone: 75-91% Cefepime: 77-92% Pip/tazo: 66-88% Ertapenem: 91-100% Meropenem: 95-100% Levofloxacin: 63-77% TMP/SMX: 64-73% Ampicillin/sulbactam: 40-56%
Haemophilus influenzae	Ampicillin/sulbactam or Ceftriaxone De-escalation Options: Amoxicillin/clavulanate Cefdinir/Cefuroxime	
Klebsiella oxytoca	Ertapenem or Meropenem* De-escalation Options: Levofloxacin 87-98% TMP/SMX 93-94% Mino/Doxycycline 91-100%	Percent Susceptible: Ertapenem: 99% Cefepime: 83-92% Pip/tazo: 81-89%
Klebsiella pneumoniae	Ceftriaxone De-escalation Options: Amoxicillin/clavulanate 83-91% Cefdinir 93-97% TMP/SMX 87-81% Levofloxacin 97-98%	Percent Susceptible: Ceftriaxone: 93-97% Ampicillin/sulbactam: 83-91% Cefepime: 93% Pip/tazo: 91-97% Ertapenem: 100%
Moraxella catarrhalis	Ampicillin/sulbactam or Ceftriaxone De-escalation Options: Amoxicillin/clavulanate Cefdinir/Cefuroxime	

***Proteus* spp**	Ceftriaxone <u>De-escalation Options:</u> Amoxicillin/clavulanate 87-90% Cefdinir/Cefuroxime 96-100%	<u>Percent Susceptible:</u> Ceftriaxone: 94-100% Pip/tazo: 95-100%
Pseudomonas aeruginosa	Piperacillin/tazobactam <u>De-escalation Options:</u> Levofloxacin 64-73%	**No β-lactam with >90% activity** <u>Percent Susceptible:</u> Pip/tazo: 87-89% Meropenem 82-83% Cefepime 83-85% Ceftazidime 88-90% Consider tobramycin addition in severely ill or non-responding patients (P/T + tobra 99%, cefepime + tobra 98%)
Serratia marcescens	Cefepime <u>De-escalation Option:</u> Levofloxacin 96-98% TMP/SMP 98-100%	<u>Percent Susceptible:</u> Cefepime: 98-100% Ertapenem: 98% Pip/tazo: 69-78%
Gram Negative Resistance Genes: CTX-M IMP, KPC, NDM, OXA-48-like, VIM	Consider carbapenem use (ertapenem or meropenem) **Consult ID**	Place in contact isolation Genetic markers of resistance in GNR do not consistently equate to phenotypic resistance nor does their absence guarantee susceptibility to an agent. If a resistance gene is detected but cultures do not corroborate, consider de-escalation to agent active against pathogen detected by culture
Chlamydia pneumonia *Mycoplasma pneumoniae*	Azithromycin 500mg once, then 250mg X 4 days Doxycycline X 7 days	
Legionella pneumophila	Levofloxacin 750mg daily X 7 days Azithromycin 500mg daily X 7 days	Dual therapy is not recommended
Influenza A Influenza B	Oseltamivir 75mg BID X 5 days	Start within 48 hours of symptom onset if possible. If hospitalized, severe, immunocompromised, or evidence of pneumonia treatment recommended Evaluate for bacterial coinfection using pneumonia panel and procalcitonin Place in droplet/contact isolation
Adenovirus Coronavirus Human Metapneumovirus Rhinovirus/Enterovirus Parainfluenza RSV	Symptomatic therapy	Evaluate for bacterial coinfection using pneumonia panel and procalcitonin Place in droplet/contact isolation

Table 3: Performance of Pneumonia Panel Compared to Reference Culture or Molecular Testing[4]

		Sensitivity		Specificity	
Organism	**Source**	**TP/(TP+FN)**	**% (95% CI)**	**TN(TN+FP)**	**% (95% IC)**
A. calcoaceticus-baumannii cmplx	BAL	0/0	-	839/846	99.2% (98.3-99.6%)
	Sputum	10/11	90.9% (62.3-98.4%)	807/825	97.8% (96.6-98.6%)
E. aerogenes	BAL	6/7	85.7% (48.7-97.4%)	832/839	99.2% (98.3-99.6%)
	Sputum	3/4	75% (30.1-95.4%)	823/832	98.9% (98.0-99.4%)
E. cloacae cmplx	BAL	11/12	91.7% (64.6-98.5%)	11/12	98.6% (97.5-99.2%)
	Sputum	822/834	91.7% (64.6-98.5%)	803/824	97.5% (96.1-98.3%)
E. coli	BAL	12/12	100% (75.8-100%)	826/834	95.8% (79.8-99.3%)
	Sputum	23/24	99.0% (98.1-99.5%)	878/812	96.9%(95.5-97.9%)
H. influenza	BAL	10/10	100% (72.2-100%)	764/836	91.4% (89.3-93.1%)
	Sputum	16/18	88.9% (67.2-96.9%)	727/818	88.9% (86.5-90.9%)
K. oxytoca	BAL	2/2	100% (34.2-100%)	835/844	98.9% (98.0-99.4%)
	Sputum	9/9	100% (70.1-100%)	817/827	98.8% (97.8-99.3%)
K. pneumoniae grp	BAL	15/15	100% (79.6-100%)	819/831	98.6% (97.5-99.2%)
	Sputum	21/23	91.3% (73.2-97.6%)	769/813	94.6% (92.8-95.9%)
M. catarrhalis	BAL	0/0	-	817/846	96.6% (95.1-97.6%)
	Sputum	5/5	100% (56.6-100%)	761/831	91.6% (89.5-93.3%)
Proteus spp.	BAL	5/5	100%(56.6-100%)	837/841	99.5% (98.8-99.8%)
	Sputum	15/15	100%(79.6-100%)	813/821	99% (98.1-99.5%)
P. aeruginosa	BAL	36/36	100% (90.4-100%)	103/106	95.3% (93.6-96.6%)
	Sputum	103/106	97.2% (92.0-99.0%)	673/730	92.2%(90-93.9%)
S. marcescens	BAL	6/6	100% (61.0-100%)	834/840	99.3% (98.5-99.7%)
	Sputum	26/27	96.3% (81.7-99.3%)	782/809	96.7% (95.2-97.7%)
S. aureus	BAL	46/47	97.9% (88.9-99.6%)	729/799	91.2% (89.1-93.0%)
	Sputum	111/112	99.1% (95.1-99.8%)	631/724	87.2% (84.5-89.4%)
S. agalactiae	BAL	1/1	-	821/845	97.2% (95.8-98.1%)
	Sputum	9/9	100% (70.1-100%)	793/827	95.9% (94.3-97.0%)
S. pneumoniae	BAL	5/5	100% (56.6-100%)	817/841	97.1 (95.8-98.1%)
	Sputum	16/16	100% (80.6-100%)	785/820	95.7% (94.1-96.9%)
S. pyogenes	BAL	2/2	100% (34.2-100%)	838/844	99.3 (98.5-99.7%)
	Sputum	6/6	100% (61.0-100%)	825/830	99.4% (98.6-997%)
Viruses					
Adenovirus	BAL	8/8	100% (67.6-100%)	837/837	100% (99.5-100%)
	Sputum	13/17	76.5% (52.7-90.4%)	815/817	99.8% (99.1-99.9%)
Coronavirus	BAL	18/21	85.7% (65.4-95.0%)	810/823	98.4% (97.3-99.1%)
	Sputum	28/32	87.5% (71.9-95.0%)	796/802	99.3% (98.4-99.7%)
Human Metapneumovirus	BAL	8/8	100% (67.6-100%)	836/837	99.9% (99.3-100%)
	Sputum	20/21	95.2% (77.3-99.2%)	812/813	99.9% (99.3-100%)

Rhino/Enterovirus	BAL	52/54	96.3% (87.5-99.0%)	771/782	98.6% (97.5-99.2%)
	Sputum	96/96	100% (96.2-100%)	717/730	98.2% (97.0-99.0%)
Influenza A	BAL	10/10	100% (72.2-100%)	830/833	99.6% (98.9-99.9%)
	Sputum	13/13	100% (77.2-100%)	819/822	99.6% (98.9-99.9%)
Influenza B	BAL	5/6	83.3% (43.6-97%)	837/838	99.9% (99.3-100%)
	Sputum	12/12	100% (75.8-100%)	921/923	99.8% (99.1-99.9%)
MERS-CoV	BAL	0/0	-	846/846	100% (99.5-100%)
	Sputum	0/0	-	836/836	100% (99.5-100%)
Parainfluenza	BAL	16/18	88.9% (67.2-96.9%)	824/826	99.8% (99.1-99.9%)
	Sputum	28/29	96.6% (82.8-99.4%)	804/806	99.8% (99.1-99.9%)
RSV	BAL	3/3	100% (43.9-100%)	841/841	100% (99.5-100%)
	Sputum	43/43	100% (91.8-100%)	787/791	99.5% (98.7-99.8%)
Atypical Bacteria					
C. pneumoniae	BAL	0/0	-	844/845	99.9% (99.3-100%)
	Sputum	0/0	-	835/835	100% (99.5-100%)
L. pneumophila	BAL	2/2	100% (34.2-100%)	833/833	100% (99.5-100%)
	Sputum	0/1	-	826/826	100% (99.5-100%)
M. pneumoniae	BAL	3/3	100% (43.9-100%)	841/842	99.9% (99.3-100%)
	Sputum	7/8	87.5% (52.9-97.8%)	827/827	100% (99.5-100%)

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