Evaluation of a Multiplex Molecular Assay for the Detection of Respiratory Pathogens from Tracheal Aspirates and Sputa

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Introduction

- Pneumonia is the leading cause of infection-related mortality, and empiric antimicrobial treatment is typically broad.
- Rapid molecular diagnostics have been shown to help guide appropriate therapy and permit early discontinuation of unnecessary antimicrobial treatment.
- Rapidly ruling out the most common bacterial etiologies of pneumonia can assist in the early differential diagnosis for pneumonia.
- The Curetis Unyvero LRT panel is a molecular multiplex test that detects 19 of the most common etiologies, along with 10 associated resistance markers, of bacterial pneumonia from lower respiratory tract specimens. (Table 1)

Table 1: Targets detected by the Unyvero LRT panel

Gram negative organisms	Gram positive organisms
Acinetobacter spp.	*Staphylococcus aureus*
Citrobacter freundii	*Streptococcus pneumoniae*
Enterobacter cloacae complex	**Atypical organisms**
Escherichia coli	*Chlamydia pneumoniae*
Haemophilus influenzae	*Mycoplasma pneumoniae*
Klebsiella oxytoca	*Legionella pneumophila*
Klebsiella pneumoniae	**Resistance markers**
Klebsiella variicola	*mecA*
Moraxella catarrhalis	*tem*
Morganella morganii	*ctx-m*
Proteus spp.	*kpc*
Pseudomonas aeruginosa	*ndm*
Serratia marcescens	*vim*
Stenotrophomonas maltophilia	*oxa 23 / 24 / 48 / 58 (reported individually)*

Methods

- Remnant specimen from tracheal aspirates and sputa processed for routine culture were frozen and archived.
- 184 specimens yielding cultures with a distribution of no growth, normal flora, and positive culture results were tested using the Unyvero LRT panel.
- Specimens were transferred to the sample tube by one of three methods depending on their viscosity: pipetting, a wide-bore specimen transfer tool attached to a syringe, or by swabbing the specimen and eluting the swab in 300 µL saline.
- Organisms detected by the multiplex assay were compared to those reported from routine culture.
- Results were sorted into 3 categories:
  - **Match**: All organisms reported on culture were detected by the LRT panel and vice versa.
  - **LRT(+)**: The LRT panel identified one or more pathogens that were not reported on culture.
  - **Culture(+)**:One or more organisms were reported on culture that the LRT panel did not detect.

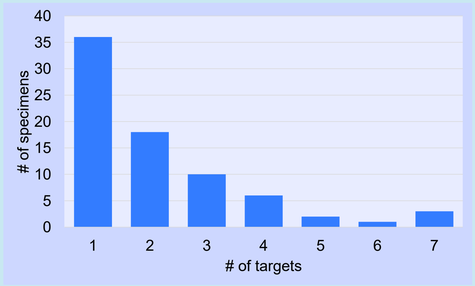

Fig. 2: Number of additional targets in LRT(+) specimens

Results

- Out of 184 specimens tested, 179 ultimately yielded valid results and were analyzed: 95 (53%) were categorized as Match, 73 (41%) as LRT(+), 8 (4%) as Culture(+), and 3 (2%) as both LRT(+) and Culture(+). (Fig. 1)
- 23 out of 28 specimens were repeated to resolve invalid results for one or more targets. Remnant specimen volume was QNS for 5 specimens. Invalid rates immediately improved upon transition of all viscous and semi-viscous specimens to the swab and elution method of sample preparation.

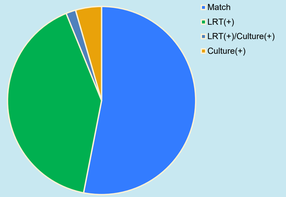

Fig. 1: Results of the Curetis Unyvero LRT panel compared to routine culture

- For **LRT(+)** results, a total of 163 additional targets were identified, on average 2.1 per specimen. (Fig. 2)
- The most common additional targets were *S. maltophilia* (32), *M. catarrhalis* (18), *H. influenzae* (17), *P. aeruginosa* (16) and *S. aureus* (15), of which 5 were also positive for the mecA gene. (Fig. 3)
- **Culture(+)** results included *S. aureus* (8), *mecA* (2), *S. marcescens* (1), and E. cloacae complex (1). (Table 3) In one specimen, the mecA gene was detected, whereas methicillin-susceptible S. aureus grew on culture.
- No atypical bacteria or carbapenemase genes were detected in our study cohort.

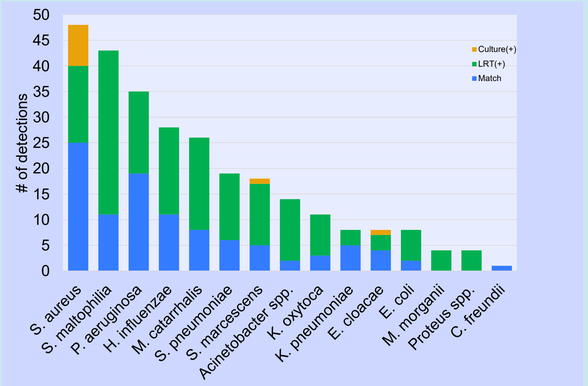

Fig 3: Distribution of organisms detected by culture only, the LRT panel only, or both

Table 3: Results for Culture(+) specimens

Culture result	Unyvero LRT result
1+ *S. aureus* 1+ NRF incl. mixed GNR	***Acinetobacter* spp.** ***S. maltophilia***
Few *S. aureus*	No organism detected
2+ *S. pneumoniae* **1+ *S. aureus*** Few NRF	***H. influenzae*** *S. pneumoniae*
2+ *S. aureus*	No organism detected
1+ S. aureus 1+ *S. agalactiae* 1+ NRF incl. *P. aeruginosa*	***M. catarrhalis*** *P. aeruginosa*
S. aureus **resistant to oxacillin**	no *mecA* detected
S. aureus **resistant to oxacillin**	no *mecA* detected
2+ *E. cloacae* **2+ *S. marcescens***	*E. cloacae* complex
3+ K. pneumoniae **3+ *E. cloacae* complex** **2+ *S. aureus***	K. pneumoniae
1+ *S. aureus* Few NRF	No organism detected

Discussion

- The LRT panel rapidly detected a large number of bacteria that were either not isolated or reported as normal flora during routine culture. These bacteria included likely pathogens and/or common colonizers. The clinical significance of these organisms is under further review (see below).
- Early rule-out of MRSA and *P. aeruginosa* could lead to faster de-escalation of empiric antibiotic treatment; 31 additional isolates of these pathogens were detected by the LRT panel, 8 isolates of *S. aureus* were not detected by the LRT panel.
- Semi-quantitation of growth from respiratory cultures often determines which organisms are reported and can play a role in assessing colonization. Therefore rapid reporting of molecular multiplex test results from non-sterile sites should be carefully considered and guided by antimicrobial stewardship principles.
- An IRB approved study reviewing patient charts to evaluate the potential clinical implications of these results is currently underway.

Conclusions

- The Curetis Unyvero LRT panel is an easy-to-use molecular multiplex panel that allows for rapid detection of common bacterial causes of pneumonia, including atypical etiologies.
- This technology offers the opportunity to explore the impact of rapid reporting of results on the therapeutic management of patients with suspect lower respiratory tract infection and the benefit to early and specific antimicrobial stewardship intervention.