

Use of a Multiplex PCR Pneumonia Panel for the Microbial Analysis of Lower Respiratory Tract Specimens from Children



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BACKGROUND

Prompt identification of pathogens is essential for optimal management of pneumonia patients. Culture requires 48-72h or longer to obtain results, during which patients receive empiric antimicrobial therapy and remain hospitalized. The Curetis Unyvero LRT Panel enables rapid and simultaneous detection of 19 bacterial pathogens and 10 antimicrobial resistance genes within 5h of specimen collection. Our prospective study aimed to evaluate the performance of the Unyvero LRT assay compared to culture of endotracheal aspirates (ETA) and bronchoalveolar lavage (BAL) specimens collected from children.

METHODS

Residual ETA (127) and BAL (62) specimens obtained for culture were included (n=189). Patients >18yo were excluded unless they were tested for continuity of care. Cultures were performed per routine practice. Aliquoted specimens were frozen within 24h of collection and thawed immediately before testing on the Unyvero system. Unyvero results were compared to initial culture results in 104 specimens, and in 85 specimens that had a follow-up culture inoculated simultaneous to Unyvero testing.

Figure 1: Unyvero LRT Panel

| | | |
|-------------------------------------|-------------------------------|----------------|
| <i>Acinetobacter</i> spp. | Carbapenem | <i>kpc</i> |
| <i>Chlamydia pneumoniae</i> | | <i>ndm</i> |
| <i>Citrobacter freundii</i> | | <i>oxa -23</i> |
| <i>Enterobacter cloacae</i> complex | | <i>oxa -24</i> |
| <i>Escherichia coli</i> | | <i>oxa -48</i> |
| <i>Haemophilus influenzae</i> | 3rd generation Cephalosporins | <i>oxa -58</i> |
| <i>Klebsiella oxytoca</i> | | <i>vim</i> |
| <i>Klebsiella pneumoniae</i> | | |
| <i>Klebsiella variicola</i> | | |
| <i>Legionella pneumophila</i> | Oxacillin/Cefoxitin | <i>ctx -M</i> |
| <i>Moraxella catarrhalis</i> | | |
| <i>Morganella morganii</i> | | |
| <i>Mycoplasma pneumoniae</i> | Penicillin | |
| <i>Proteus</i> spp. | | <i>mec A</i> |
| <i>Pseudomonas aeruginosa</i> | | |
| <i>Serratia marcescens</i> | | |
| <i>Staphylococcus aureus</i> | | |
| <i>Stenotrophomonas maltophilia</i> | | <i>tem</i> |
| <i>Streptococcus pneumoniae</i> | | |

RESULTS

133 specimens were positive for one or more pathogens on the Unyvero LRT panel. The positivity rate was higher for ETAs than BALs by both methods (Fig. 2) and our data indicated that Unyvero was more sensitive than culture for several key pathogens (Fig. 3&4). Culture missed one *Pseudomonas* and two *Haemophilus* in BALs. Additionally, Unyvero detected several pathogens in ETAs (*Acinetobacter*, *Pseudomonas*, *Haemophilus*, and *Serratia*) that were undetected by culture. Unyvero had no false negatives in BALs, and only two *S. aureus* falsely negative results were observed in ETAs. Culture missed one *S. pneumoniae* that was detected by Unyvero. Variability in semi-quantitative scores was observed between the initial and follow-up cultures. The overall sensitivity and specificity for Unyvero results were 88% and 95% compared to the initial cultures, and 98% and 98% compared to the follow-up cultures, respectively (Fig. 5).

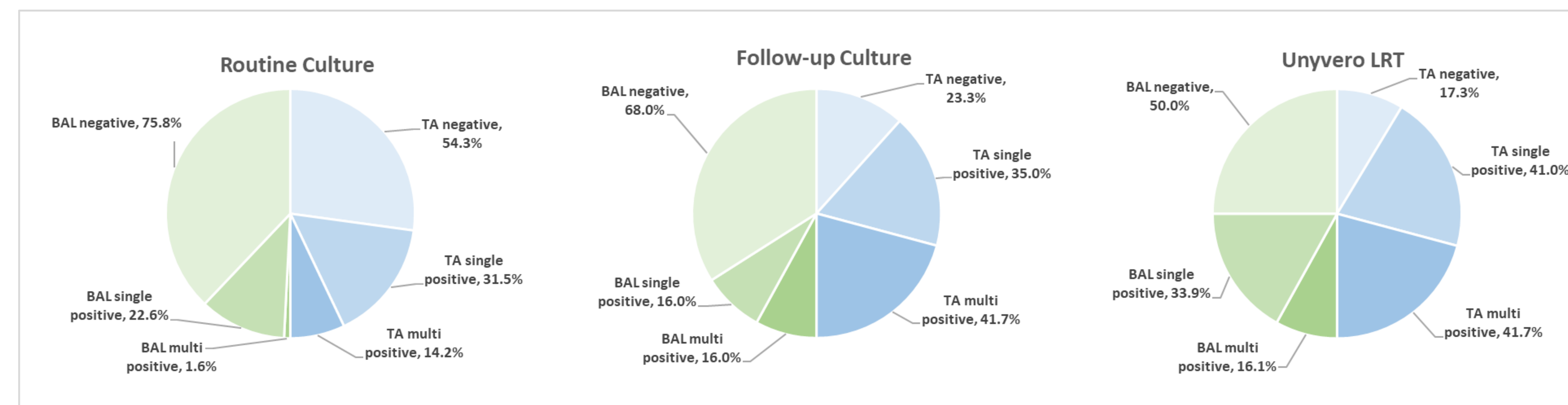


Figure 2: Detection of Potential Pathogens

Our results indicated that Unyvero LRT was more sensitive and had a higher detection rate of potential pathogens. The correlation was stronger between Unyvero LRT and follow-up culture than it was with initial culture. The highest rates for no potential pathogens detected were seen in the initial culture results for TA and BAL specimens.

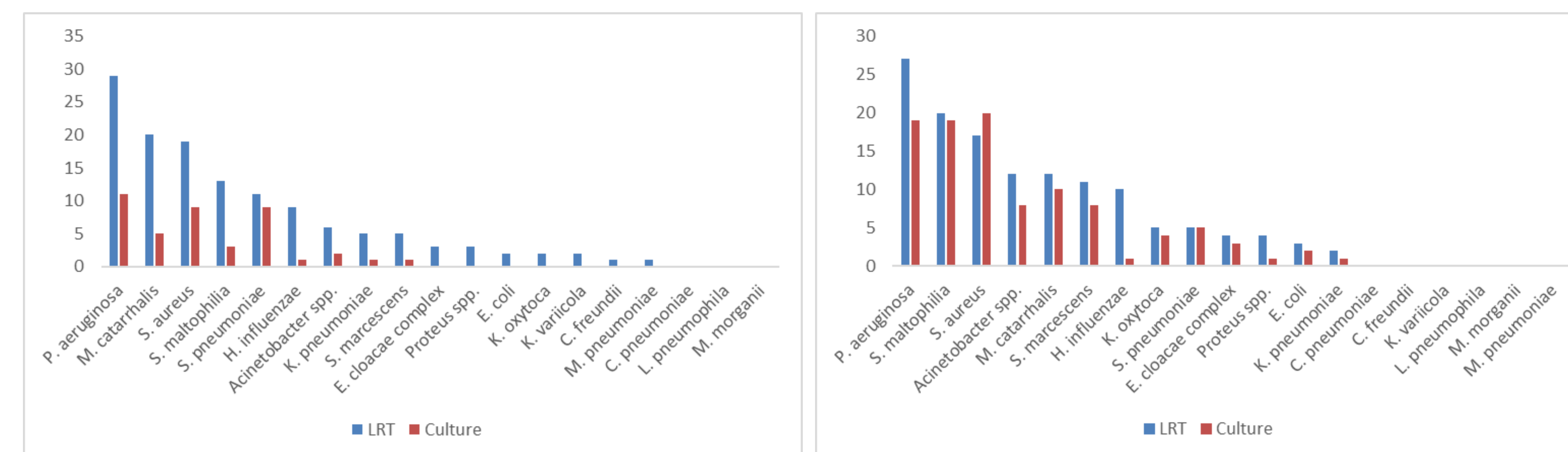


Figure 3: Unyvero results compared to initial culture results (n=104 TA and BAL specimens)

Figure 4: Unyvero results compared to follow-up culture results (n=85 TA and BAL specimens)

Figure 5: Overall sensitivity and specificity for Unyvero results compared to initial and follow-up culture

| | initial routine culture vs. Unyvero LRT | | | | | | follow-up culture vs. Unyvero LRT | | | | | |
|---------------------------|---|------------|-------------|-----------|------------|------------|-----------------------------------|-----------|-------------|----------|------------|------------|
| | TP | FP | TN | FN | sens | spec | TP | FP | TN | FN | sens | spec |
| <i>Acinetobacter</i> spp. | 2 | 16 | 171 | 0 | 100% | 91% | 8 | 4 | 73 | 0 | 100% | 95% |
| <i>C. pneumoniae</i> | 0 | 0 | 189 | 0 | n/a | 100% | 0 | 0 | 85 | 0 | n/a | 100% |
| <i>C. freundii</i> | 0 | 1 | 188 | 0 | n/a | 99% | 0 | 0 | 85 | 0 | n/a | 100% |
| <i>E. cloacae</i> complex | 3 | 4 | 182 | 0 | 100% | 98% | 3 | 1 | 81 | 0 | 100% | 99% |
| <i>E. coli</i> | 0 | 5 | 184 | 0 | n/a | 97% | 2 | 1 | 82 | 0 | 100% | 99% |
| <i>H. influenzae</i> | 2 | 17 | 169 | 1 | 67% | 91% | 1 | 9 | 75 | 0 | 100% | 89% |
| <i>K. oxytoca</i> | 3 | 4 | 182 | 0 | 100% | 98% | 4 | 1 | 80 | 0 | 100% | 99% |
| <i>K. pneumoniae</i> | 2 | 5 | 178 | 4 | 33% | 97% | 1 | 1 | 83 | 0 | 100% | 99% |
| <i>K. variicola</i> | 0 | 2 | 187 | 0 | n/a | 99% | 0 | 0 | 85 | 0 | n/a | 100% |
| <i>L. pneumophila</i> | 0 | 0 | 189 | 0 | n/a | 100% | 0 | 0 | 85 | 0 | n/a | 100% |
| <i>M. catarrhalis</i> | 7 | 25 | 157 | 0 | 100% | 86% | 10 | 2 | 73 | 0 | 100% | 97% |
| <i>M. morganii</i> | 0 | 1 | 187 | 1 | 0% | 99% | 0 | 1 | 84 | 0 | n/a | 99% |
| <i>M. pneumoniae</i> | 0 | 1 | 188 | 0 | n/a | 99% | 0 | 0 | 85 | 0 | n/a | 100% |
| <i>Proteus</i> spp. | 1 | 6 | 182 | 0 | 100% | 97% | 1 | 3 | 81 | 0 | 100% | 96% |
| <i>P. aeruginosa</i> | 21 | 35 | 133 | 0 | 100% | 79% | 19 | 8 | 58 | 0 | 100% | 88% |
| <i>S. marcescens</i> | 4 | 12 | 172 | 1 | 80% | 93% | 6 | 4 | 75 | 0 | 100% | 95% |
| <i>S. aureus</i> | 15 | 21 | 151 | 2 | 88% | 88% | 17 | 0 | 66 | 2 | 89% | 100% |
| <i>S. maltophilia</i> | 10 | 23 | 156 | 0 | 100% | 87% | 19 | 1 | 65 | 0 | 100% | 98% |
| <i>S. pneumoniae</i> | 10 | 6 | 171 | 2 | 83% | 97% | 4 | 1 | 80 | 0 | 100% | 99% |
| Total | 80 | 184 | 3316 | 11 | 88% | 95% | 95 | 37 | 1481 | 2 | 98% | 98% |

CONCLUSION

Our findings indicate that the Unyvero LRT improves the detection of important and difficult to treat pathogens such as *Pseudomonas* and *Acinetobacter*. Culture reports of normal respiratory flora can be confusing to clinicians while Unyvero results are unbiased and straightforward. Such results enable clinicians to manage patients based on the specimen Gram stain and LRT results along with their clinical judgement. Five hour pathogen identification and antibiotic resistance detection enable early interventions and avoidance of ineffective antimicrobial therapy.

ACKNOWLEDGMENTS

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