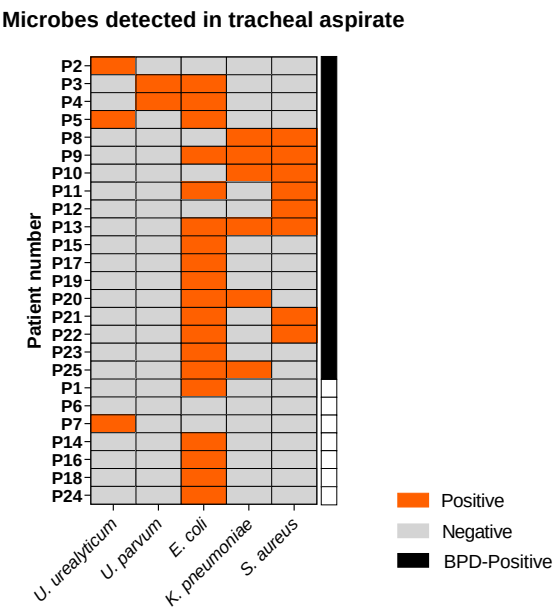


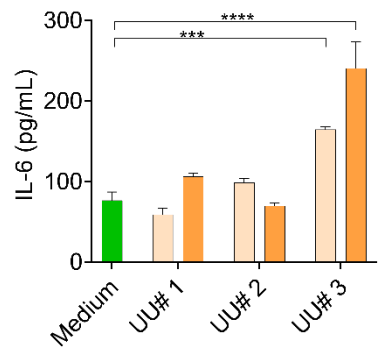
Supplemental Material

Supplemental Figure S1 Visualization of bacteria in neonatal tracheal aspirates



Supplemental Fig S1 Tracheal aspirates from 25 preterm neonates were analyzed for presence of *U. urealyticum*, *U. parvum*, *E. coli*, *K. pneumoniae*, *S. aureus* and GBS using PCR. The positive and negative results for each bacterium are indicated for each patient. One sample was ran out (P6) and could not be tested.

Supplemental Figure S2 The amounts of IL-6 induced by *U. urealyticum*



Supplemental Fig S2 The amounts of IL-6 induced by *U. urealyticum* determined by ELISA. Bars represent the mean $\pm$ SD of triplicate cultures. Representative results of three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$ (One-way ANOVA followed by Dunnett's multiple comparison test).

Supplemental Table S1 The primers used for the neonatal tracheal aspirate polymerase chain reaction assays.

Multiplex PCR				
Pathogen	Primer/probe	Sequence	Target gene	Reference
<i>Ureaplasma urealyticum</i>	Forward primer	5'-AAGGTCAAGGTATGGAAGATCCAA-3'	Urease gene	1
	Reverse primer	5'-TTCCTGTTGCCCTCAGTCT-3'		
	Probe:	5'-ACCACAAGCACCTGCTACGATTTGTTC-3'		
<i>U. parvum</i>	Forward primer	5'-AAGGTCAAGGTATGGAAGATCCAA-3'	Urease gene	1
	Reverse primer	5'-TTCCTGTTGCCCTCAGTCT-3'		
	Probe:	5'-TCCACAAGCTCCAGCAGCAATTG-3'		
<i>Escherichia coli</i>	Forward primer	5'-CATGCCGCGTGTATGAAGAA-3'	16S rDNA	2
	Reverse primer	5'-CGGGTAACGTCAATGAGCAAA-3'		
	Probe:	5'-TTCAGCGGGGAGGAAGGGAGTAAAGTTAATA-3'		
<i>Klebsiella pneumoniae</i>	Forward primer	5'-CGAAACCGCTCGTAAACACA-3'	gap A gene	3
	Reverse primer	5'-AGGAAGCGTTGGAAACGATG-3'		
	Probe:	5'-GTTCTGACTGGCCCGTCCAAAGA-3'		
<i>Streptococcus agalactiae</i>	Forward primer	5'-TTCACCAGCTGTATTAGAAGTACATGC-3'	cfb gene	4
	Reverse primer	5'-CCCTGAACATTATCTTTGATATTCTCA-3'		
	Probe:	5'-CAAGCCCAGCAAATGGCTCAAAAGCT-3'		
Singleplex PCR				
Pathogen	Primer/probe	Sequence		
<i>Staphylococcus aureus</i>	Forward primer	5'-CATCGGAAACATTGTGTTCTGTATG-3'	Sa442	5
	Reverse primer	5'-TTTGGCTGGAAAATATAACTCTCGTA-3'		
	Probe:	5'-AAGCCGTCTTGATAATCTTTAGTAGTACCGAAGCTGGT-3'		
All bacteria	Universal primers	Sequence		
	Forward primer	5'-AGAGTTTGATCCTGGCTCAG-3'	16S rRNA	6
	Reverse primer	5'-CTGCTGCCTCCCGTAG-3'		
Internal Control PhHV-1	Primer/probe	Sequence		
	Forward primer	5'- GGGCGAATCACAGATTGAATC-3'	gB gene	7
	Reverse primer	5'-GCGGTTCCAAACGTACCAA-3'		
	Probe	5'-TTTTTATGTGTCCGCCAC CATCTGGATC-3'		

References:

- 1 Yi, J., Yoon, B. H. & Kim, E. C. Detection and Biovar Discrimination of *Ureaplasma Urealyticum* by Real-Time Pcr. *Mol Cell Probes* **19**, 255-260 (2005).
- 2 Huijsdens, X. W. et al. Quantification of Bacteria Adherent to Gastrointestinal Mucosa by Real-Time Pcr. *J Clin Microbiol* **40**, 4423-4427 (2002).
- 3 Ahn, C., Yoon, S. S., Yong, T. S., Jeong, S. H. & Lee, K. The Resistance Mechanism and Clonal Distribution of Tigecycline-Nonsusceptible *Klebsiella Pneumoniae* Isolates in Korea. *Yonsei Med J* **57**, 641-646 (2016).
- 4 van den Brand, M. et al. Development of a Multiplex Real-Time Pcr Assay for the Rapid Diagnosis of Neonatal Late Onset Sepsis. *J Microbiol Methods* **106**, 8-15 (2014).
- 5 Peters, R. P. et al. Quantitative Detection of *Staphylococcus Aureus* and *Enterococcus Faecalis* DNA in Blood to Diagnose Bacteremia in Patients in the Intensive Care Unit. *J Clin Microbiol* **45**, 3641-3646 (2007).
- 6 Waldeisen, J. R., Wang, T., Mitra, D. & Lee, L. P. A Real-Time Pcr Antibigram for Drug-Resistant Sepsis. *PLoS One* **6**, e28528 (2011).
- 7 Nassuuna, J. et al. Helminth Driven Gut Inflammation and Microbial Translocation Associate with Altered Vaccine Responses in Rural Uganda. *NPJ Vaccines* **10**, 56 (2025).