Presentation Type:
Poster Presentation
Subject Category: Surveillance
Surveillance of Gram-Negative Bacteria in Pediatric Patients Requiring Mechanical Ventilation Using Whole-Genome Sequencing‡
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Background: Hospital-acquired infections remain a significant challenge for healthcare systems despite well-established infection prevention (IP) strategies, with ventilator-associated infections (VAI) being particularly challenging. This is especially true for pediatric patients, who often require mechanical ventilation for extended periods, increasing the risk of infection. Gram-negative bacteria, primarily Pseudomonas aeruginosa, are the leading causes of VAI. Traditional IP strategies are often reactive and may miss potential transmissions within the hospital, particularly for pathogens that are endemic to the hospital environment. Whole-genome sequencing (WGS) and genomic epidemiology have demonstrated value in identifying such events in adult hospitals, but their application in pediatric facilities remains limited. Here, we conduct a retrospective analysis to determine how this technology could be utilized in a pediatric facility. **Methods:** We conducted a retrospective genomic surveillance study of Gram-negative isolates collected between 2020 and 2024 at a pediatric tertiary hospital. Briefly, clones were selected by colony morphology, cultured, and DNA was extracted. DNA libraries were prepared and sequenced on the Illumina NextSeq 1000 platform. Phylogenetic analyses were performed using core-genome alignment pipelines. Likely transmission events were defined as bacterial clones differing by fewer than 10 single-nucleotide polymorphisms, based on previously described literature. Genetically related clones were evaluated with patient location and temporal hospital metadata to assess epidemiologic linkage. **Results:** A total of 687 clones from roughly 500 clinical samples were sequenced. We identified over 50 groups of genetically related isolates from multiple Gram-negative species. P. aeruginosa accounted for most of the genetically related groups, with over 40 discovered. Additional pathogens suspected to have genetic relatedness include Serratia marcescens, Klebsiella pneumoniae, Stenotrophomonas maltophilia, and Achromobacter xylosoxidans. These groups range in size from 2 to 15 patients. Pairwise genomic analysis confirmed at least 16 groups consistent with likely transmission events. To date, epidemiologic review has confirmed that 2 of these groups were plausible based on temporal and geographic relationships between patients. The remaining 14 groups are under investigation. **Conclusion:** WGS-based surveillance identified multiple transmission events that were not recognized by conventional IP methods, highlighting the added value of WGS for hospital surveillance. This is especially true in the case of pathogens that are commonly found in hospitals. Integrating WGS with genomic epidemiology and hospital metadata can enhance patient safety and reduce healthcare costs. These findings indicate that reliance on traditional surveillance alone may underestimate the true burden of hospital outbreaks, particularly those caused by common Gram-negative respiratory pathogens in pediatric populations.

Characteristic	Total	*P. aeruginosa*	*A. xylosoxidans*	*S. marcescens*	*K. pneumoniae*	*S. maltophilia*	Other
Clinical Samples Collected	494	—	—	—	—	—	—
Clones Sequenced	684	564	27	46	7	15	25
Genomic Clusters Identified	58	50	3	3	1	1	0
Clusters with Transmission Evidence (# of isolates)	—	15 (185)	1 (9)	2 (20)	0	0	—

Table 1. Distribution of Bacterial Isolates and Sequencing Derived Clusters

This table reports the total number of clinical samples processed, the number of isolates sequenced, and the resulting genetically related clusters identified across Gram-negative species. Clusters were defined using WGS and core-genome alignments

Antimicrobial Stewardship & Healthcare Epidemiology 2026;6(Suppl. S1):s186
doi:10.1017/ash.2026.10729

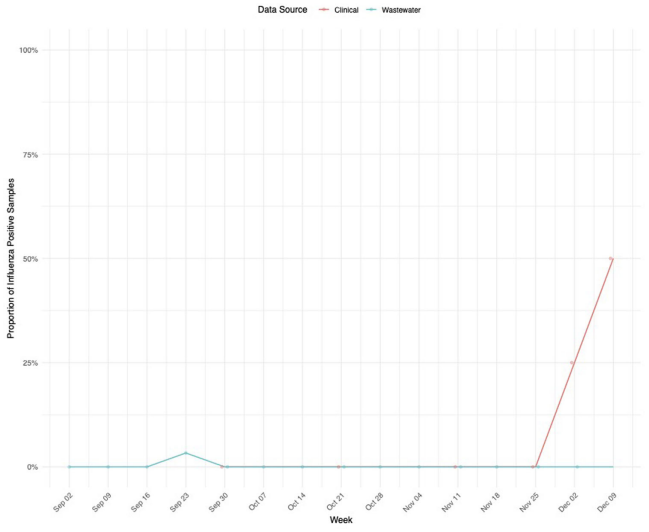
Presentation Type:
Poster Presentation
Subject Category: Surveillance
Evaluating Wastewater-Based Epidemiology in Healthcare Settings – Pilot‡
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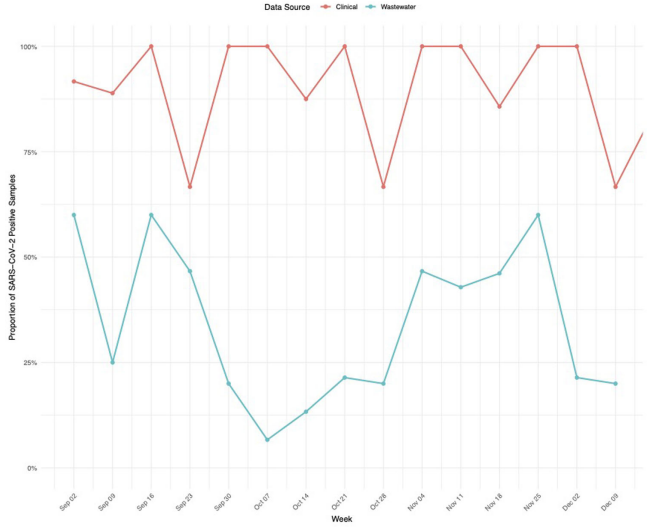
Purpose: Wastewater surveillance effectively monitors pathogens. This pilot study evaluated the feasibility and utility of hospital-level wastewater surveillance by integrating wastewater and electronic health record (EHR) data. Analyses focused on hospital-acquired infections (HAI) and temporal lags between wastewater and clinical detection. **Methods:** From August to December 2024, wastewater autosamplers operated across five hospital pavilions at Yale New Haven Hospital, collecting samples every five minutes during a 24-hour period three times per week. Samples were analyzed by dPCR for SARS-CoV-2, Influenza (A/B), and additional pathogens. Deidentified EHR data included admissions, diagnoses, and laboratory data. The primary focus was lab-confirmed HAI SARS-CoV-2 and Influenza. HAI was defined as infections diagnosed during hospitalization without evidence at admission. Clinical and wastewater data were used to calculate the proportion of positive samples, and correlation was assessed using Spearman's rank correlation coefficient (rho). Correlations were evaluated for lagged associations across 1–3-week lags. A sensitivity analysis was conducted by including all SARS-CoV-2 encounters. **Results:** Among 33,579 patient encounters, 62 SARS-CoV-2 and 74 Influenza HAI encounters were identified. This corresponded to 97 and 148 tests for SARS-CoV-2 and Influenza, respectively. Of 187 wastewater samples collected 60 (31.1%) and 1 (0.5%) were positive for SARS-CoV-2



‡The abstract titles in this supplement have been corrected since original publication. A notice detailing the changes has been published at https://doi.org/10.1017/ash.2026.10802.

https://doi.org/10.1017/ash.2026.10730 Published online by Cambridge University Press





and Influenza respectively. Due to only a single detection of Influenza in the wastewater, correlation analysis was limited to SARS-CoV-2. A correlation test of the data found no statistically significant correlation between wastewater and clinical data when aligned temporally (rho: -0.21, p: 0.51). Lagged correlations between wastewater and clinical SARS-CoV-2 positivity were evaluated across 1–3-week temporal lags. These lagged correlations were not statistically significant, but rho increased in magnitude from a 1-week lag (rho: -0.04, p: 0.89) to a 3-weeks lag (rho: 0.46, p:0.21). The sensitivity analysis found no statistically significant correlation between wastewater and clinical positivity. **Conclusions:** Hospital-level wastewater surveillance shows potential as an early indicator of HAI SARS-CoV-2 infections, with exploratory trends suggesting a ~3-week lead time results in stronger associations between clinical and wastewater data. Although limited by small HAI sample sizes and a short wastewater sampling period, these findings support further evaluation in larger cohorts and highlights pathogen-specific limitations as observed for Influenza. Follow-up studies should employ longer wastewater sampling windows and further refine methods to account for community-associated SARS-CoV-2 contributions to hospital wastewater, an area of active investigation by our group.

Antimicrobial Stewardship & Healthcare Epidemiology 2026;6(Suppl. S1):s186–s187
doi:10.1017/ash.2026.10730

Table 1: Comparison of Cases Progressing to Invasive Infection versus Remaining Colonized

Risk Factor	Cases: Total N=17, N (%) or mean/SD	Controls: Total N=57, N (%) or mean/SD	P value
ICU	13 (76.5)	35 (61.4)	0.386
Renal failure	14 (82%)	34 (60%)	0.146
Dialysis	12 (71%)	20 (35%)	0.013
Liver failure	10 (59%)	9 (16%)	0.001
Heart failure	4 (26%)	14 (25%)	1.000
Solid organ transplant	9 (53%)	12 (21%)	0.016
Other MDROs	12 (71%)	5 (9%)	<0.0001
Ventilator	13 (76.5%)	37 (64.9%)	0.556
Tracheostomy	9 (53%)	8 (14%)	0.002
Indwelling Urinary Catheter	10 (58.9)	26 (45.6)	0.412
# OR procedures	2.5 (3.0)	1.9 (2.6)	0.470
# non-OR procedures	0.9 (1.8)	0.8 (1.6)	0.926
# Central Lines	1.5 (1.0)	1.0 (0.8)	0.081
# Drains	1.8 (1.8)	0.7 (0.9)	0.004
Time to colonization	32.2 (29.2)	21.5 (24.9)	0.212
LOS	80.7 (68.9)	40.4 (42.7)	0.021
Broad Gram-Negative Coverage*	0.6 (0.4)	0.5 (0.4)	0.116
Broad Gram-Positive Coverage*	0.5 (0.7)	0.2 (0.3)	0.429

*Defined as DOT/LOS

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Poster Presentation
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Risk Factors for Progression to Invasive Candida Auris Infection among Colonized Patients‡

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Background: Identified risk factors for Candida auris infection are generally markers of disease severity and/or medical vulnerability. A regional C. auris outbreak in 2022 led to extensive admission and point-prevalence testing in tertiary care hospital. We retrospectively reviewed charts to identify novel risk factors for progression from colonization to infection in a universally medically complex population. **Methods:** A case-control study was performed to compare patients developing invasive infection (cases) from those remaining colonization. Inclusion criteria were patients newly identified to be colonized with C. auris during a hospital admission from 1/2023 through 5/2025. Patients diagnosed with clinical infection as their first evidence of colonization were excluded. Risk factor variables included ICU status, other drug resistant organisms (MDROs), length of stay (LOS), time to colonization (days from admission to identification of C. auris), antimicrobial coverage with broadly acting agents, operative and other procedures, type/number of devices. Factors were compared between cases and controls using Fisher's Exact Tests for categorical variables and Mann-Whitney U Tests for continuous variables using SAS 9.4. **Results:** During the study, 74 patients became colonized and 17 went on to develop invasive infection. The following were associated with infection: colonization with other MDROs, tracheostomy, liver failure, dialysis (though not renal failure), transplant w/in last year (driven by liver, N=7), number of drains at colonization (table 1). Several factors associated with severity of illness (ICU status, antibiotic pressure) were not associated with progression to infection in this cohort. Discussion: In a medically complex population, several risk factors associated with illness severity were not associated with progression from C. auris colonization to infection, whereas liver disease and transplantation, along with other MDROs, tracheostomy, and number of drains were. Targeted interventions attempting to decolonize tracheostomy or drain sites, and/or liver disease patients in general may help prioritize infection prevention where it is most needed.

Antimicrobial Stewardship & Healthcare Epidemiology 2026;6(Suppl. S1):s187
doi:10.1017/ash.2026.10731

‡The abstract titles in this supplement have been corrected since original publication. A notice detailing the changes has been published at https://doi.org/10.1017/ash.2026.10802.

https://doi.org/10.1017/ash.2026.10730 Published online by Cambridge University Press