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APPLICATION NOTE

Wastewater surveillance using the Urinary Pathogen ID/AMR Panel

Broad pathogen detection and antimicrobial resistance (AMR) tracking with the MiSeq™ i100 Series

Exceptional breadth and depth of coverage of antimicrobial resistance genes (ARGs) enabled with enrichment with the Urinary Pathogen ID/ AMR Panel

Fast sequencing delivers results in < 12 hr for efficient wastewater surveillance

Simplified analysis with the DRAGEN™ Microbial Enrichment Plus app provides streamlined pathogen detection and characterization

Introduction

Antimicrobial resistance (AMR) has been described as a "silent pandemic."[1] By decreasing the effectiveness of available antimicrobials, AMR has the potential to indiscriminately influence the severity and clinical outcome of infections independent of underlying disease or other risk factors.[1] Expanding urbanization, global travel, and growing population density create ideal conditions for the transmission of infectious agents, and with them, antimicrobial resistance genes (ARGs).[2-3]

Many existing AMR surveillance programs are based on interactions with health care providers and, therefore, cannot effectively capture ongoing transmission of drug-resistant organisms outside of the health care setting. Furthermore, traditional growth-based methods for monitoring drug susceptibility cannot provide genotypic information about the organisms nor the ARGs themselves, or any information about organisms that are not cultivable in the laboratory using standard protocols.[3-4]

Wastewater surveillance includes a laboratory method that can help address these gaps and uncover the circulating "resistome" of a given community, including drug-resistant organisms and associated mobile genetic elements bearing ARGs (eg, plasmids) that may circulate in parallel.[3] Next-generation sequencing (NGS)–based wastewater surveillance of AMR has been shown to correlate with phenotypic resistance data from conventional isolate-based surveillance in various populations and settings.[4] Furthermore, wastewater surveillance can be useful for broad surveillance of known or novel threats that may be transmitted between people, nonhuman populations, such as companion animals or agricultural animals, and their shared environment in a "One Health" approach.[3]

Accurate and comprehensive detection of ARGs and associated bacteria in wastewater depends on the upfront enrichment of relevant genomic content by filtration or concentration techniques. Library preparation enrichment methods can help overcome the sequencing depth otherwise needed to comprehensively profile the resistome in wastewater samples.[5] By increasing the relative abundance of genomic content of interest in the library, enrichment also unlocks the potential for sequencing libraries from a biologically complex sample like wastewater on NGS benchtop instruments.

This application note demonstrates the detection and characterization of ARGs and associated bacterial pathogens in real-world wastewater samples using an NGS workflow that integrates the Urinary Pathogen ID/AMR Enrichment Kit, the MiSeq i100 Plus System, and onboard DRAGEN secondary analysis (Figure 1). The MiSeq i100 Series delivers same-day results for efficient wastewater surveillance with high-resolution data to inform public health response.

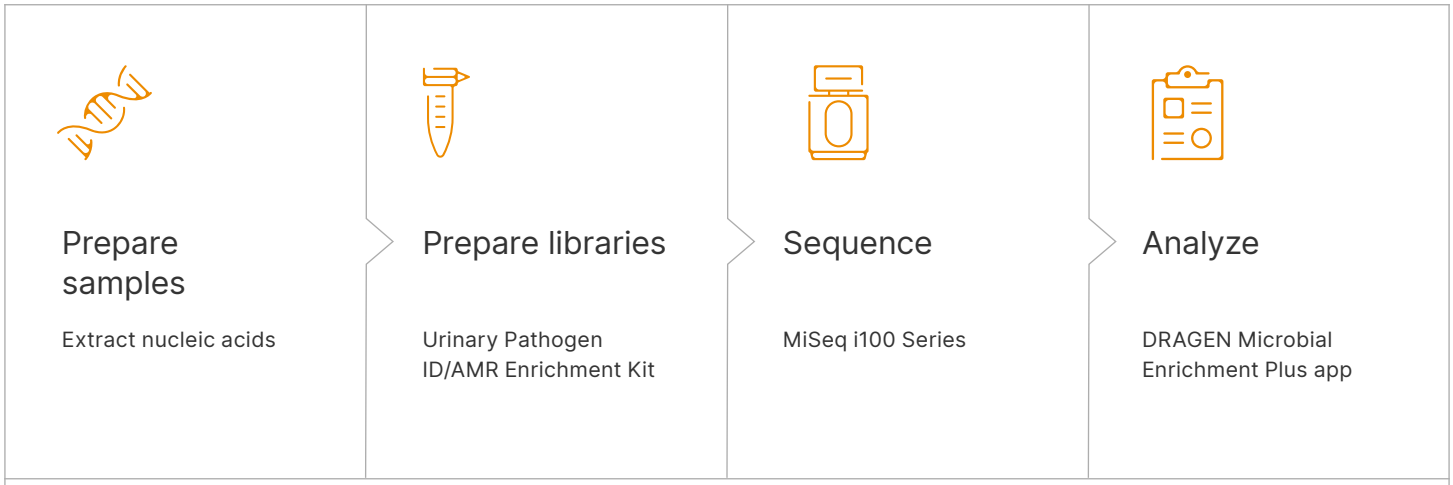

Figure 1: Comprehensive NGS workflow for wastewater surveillance

Methods

Samples

Raw wastewater samples were collected from wastewater treatment plants (WWTP) by Wisconsin State Laboratory of Hygiene (WSLH) (n = 27) and from student dormitories by Colorado State University (CSU) (n = 33); both sites are in the United States. Samples were collected from each site over multiple time points from November 4, 2022 to December 16, 2022. Samples from WSLH were prepared from 10–50 ml of sample wastewater by capturing and concentrating the microbes with Nanotrap Microbiome A Particles (Ceres Nanosciences, Inc., Catalog no. 44202). Nucleic acids were extracted using the Wizard Enviro Total Nucleic Acid Kit (Promega Corporation, Catalog no. A2991) according to the manufacturer's instructions. Samples obtained from CSU involved removal of solids via centrifugation at ~2000 × g, followed by capture and concentration of viruses with the CP Select Concentrating Pipette (InnovaPrep, Inc.). Nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Catalog no. 52904) according to the manufacturer's instructions.

Library preparation

Sequencing-ready libraries were prepared from a maximum volume of 30 µl of extracted total nucleic acid (TNA) input using the Urinary Pathogen ID/AMR Enrichment Kit, Set A (RUO) (96 indexes, 96 samples) (Illumina, Catalog no. 20090308). For comparison, shotgun metagenomics libraries were prepared using the same kit, omitting enrichment steps.

Sequencing

Prepared libraries were sequenced on the MiSeq i100 Plus System using a 25M flow cell with a run configuration of 2 × 151 bp.

Data analysis

After sequencing was complete, data were downsampled to 1M (enriched and unenriched) and 4M (unenriched only) clusters/fragments per sample using the FASTQ Toolkit App. Downsampled and nondownsampled data were analyzed using the DRAGEN Microbial Enrichment Plus app in BaseSpace™ Sequence Hub. This app can also be accessed onboard the MiSeq i100 Plus System.

Results

Sequencing metrics

The wastewater libraries were sequenced across three runs on the MiSeq i100 Plus System. Sequencing metrics across the three runs were high with > 94% reads greater than Q30 and of ≥ 87% reads passing filter (PF), indicating both high-quality data and consistent instrument loading concentrations. The total number of paired-end (PE) reads obtained exceeded the 50M specification of the flow cell, resulting in deeper coverage and potentially more confident identification of ARGs and microbes. For all three runs, the combined instrument run time and analysis times were under 10 hours (Table 1).

Table 1: Sequencing metrics for the MiSeq i100 Plus System

Sequencing run	No. of samples	Run time	Analysis time	Total no. PE read PF	% PF	% reads ≥ Q30
Run 1	21	6 hr 48 min	~40–45 min	68,795,096	87.00%	96.68%
Run 2	21	7 hr 12 min	~40–45 min	68,850,076	87.07%	94.72%
Run 3	18	7 hr 13 min	~40–45 min	70,374,364	89.00%	94.20%

Wastewater sample composition

Visual analysis tools within the DRAGEN Microbial Enrichment Plus app facilitate interpretation of enrichment performance at a glance. Sample composition plots show the proportion of targeted, untargeted, ambiguous, and unclassified reads. The percentage of reads in each category represents their proportion relative to the total number of reads in the sample (Figure 2A) or relative to the proportion of reads within the targeted or untargeted categories themselves (Figure 2B). For example, 52.1% of the total reads in the sample are classified as targeted AMR reads, and 33.1% of all the reads in the sample are untargeted bacterial (Figure 2A). In contrast, 98.7% of targeted reads are AMR reads, and 94.4% of all untargeted reads are classified as bacterial reads (Figure 2B).

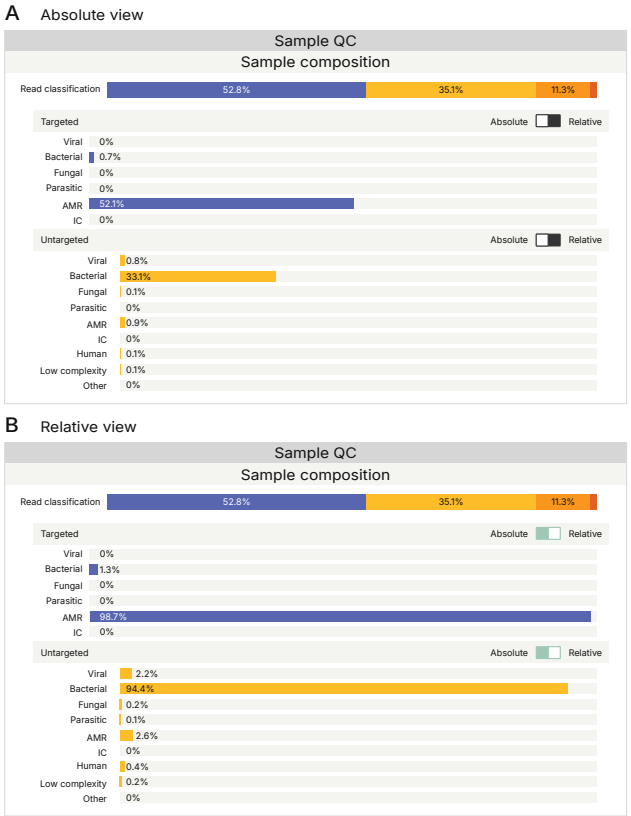

Figure 2: Composition of wastewater samples
The read composition was broken down into the following categories: targeted (blue), untargeted (yellow), ambiguous (orange), and unclassified (red). Sample composition displayed as (A) absolute shows the percentage of reads in each category relative to the total number of reads in the sample, and (B) relative shows the percentages relative to the proportion of reads within the targeted or untargeted categories themselves.

AMR detection in wastewater samples

The target enrichment design of the Urinary Pathogen ID/AMR panel is highly sensitive, outperforming shotgun metagenomics for identifying genes associated with AMR. The target enrichment approach also allows for greater analytical sensitivity at a lower number of total reads compared to shotgun metagenomics methods (Figure 3). Importantly, the DRAGEN Microbial Enrichment Plus app automatically flags relevant ARGs associated with extended-spectrum β-lactamase (ESBL) and carbapenemase genes. These genes are designated as critical priorities by global health agencies due to their role in multidrug resistance and rapid dissemination.[6-8] This enables users to focus on the most relevant ARGs in large data sets, providing a clear starting point for further analysis.

Analysis of results from sequencing wastewater samples with the Urinary Pathogen ID/AMR panel showed the proportion of AMR genes detected at each collection site, categorized by known association with a family of antimicrobial drugs (Figure 44). These findings highlight the prevalence of diverse AMR genes in various communities, inclusive of dense human populations (dormitory samples) and a "One Health" sample of a broad community (WWTP samples).

Microbe detection in wastewater samples

Analysis of sequencing results from wastewater samples using the Urinary Pathogen ID/AMR panel revealed the distribution of microbes across two collection sites, including gram-negative bacteria, gram-positive bacteria, and viruses (Figure 5). The majority of reads from both sites were associated with gram-negative bacteria, including typical gut-associated organisms and opportunistic pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Enrichment with the Urinary Pathogen ID/AMR panel also revealed the presence of genomic content from other bacterial and viral organisms. For example, dormitory samples showed a relatively higher proportion of gram-positive bacteria, including *Enterococcus* spp, *Staphylococcus* spp, *Streprococcus* spp, and acid-fast organisms were detected across both sites, although dormitory samples showed a higher proportion (Figure 5).

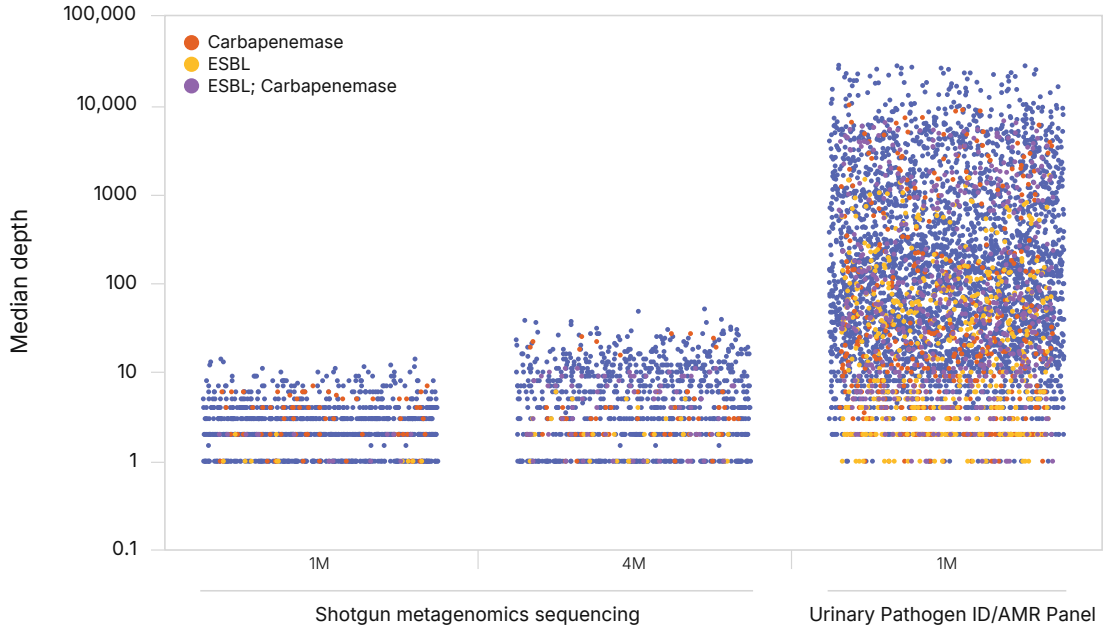

Figure 3: Comparison of AMR gene detection in Urinary Pathogen ID/AMR panel–enriched vs shotgun metagenomics libraries

After libraries were prepared and sequenced on the MiSeq i100 Plus System, the resulting FASTQ files were downsampled to 1M clusters or 2M PE reads (4M clusters or 8M PE reads for shotgun libraries) and analyzed with the DRAGEN Microbial Enrichment Plus app. A significant increase in the total number of ARGs was consistently detected in enriched libraries compared to shotgun libraries, both at comparable depth (1M clusters) and at 4× the sequencing depth for shotgun libraries. Genes associated with carbapenemases and extended-spectrum beta-lactamases (EBSLs) were identified using the analysis software.

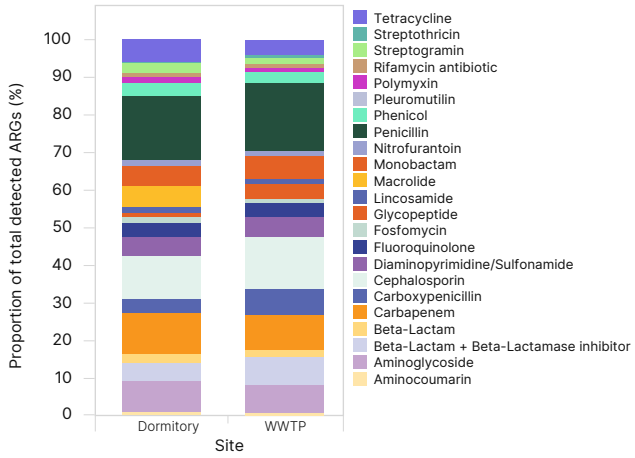

Figure 4: Resistomes of dormitory and WWTP samples

The distribution of AMR genes across 24 drug classes from the two collection sites (dormitory (n=33) and wastewater treatment plant (n=27) shows the proportion of resistance within each drug class, allowing for a comparative assessment of the resistome between the two sites. DRAGEN Microbial Enrichment Plus reports putative associations between a given AMR marker detection and one or more drug classes based on public metadata.

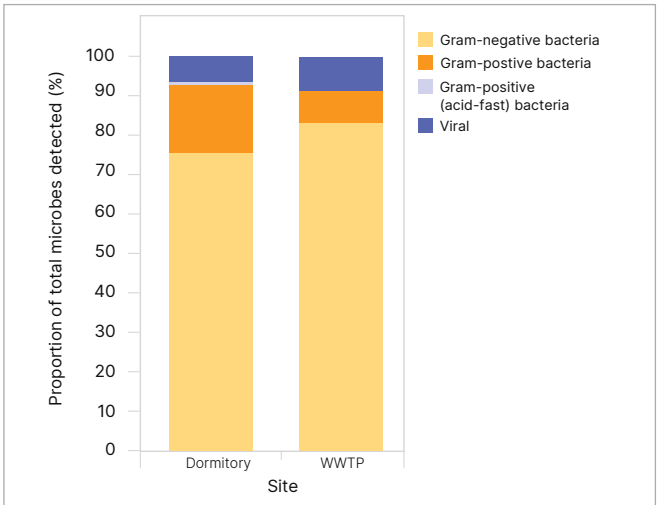

Figure 5: Microbe detection by collection site

The distribution of microbes detected from the two collection sites: dormitory (n=33) and wastewater treatment plant (n=27) highlights the differences in the prevalence of detected microbes at both sites. Samples from the dorms had a larger proportion of gram positive bacteria detected, including some acid fast bacteria. WWTP had more viral detections compared to dorms. Overall, the largest proportion of reads were associated with gram negative bacteria.

Samples from WWTPs exhibited greater viral diversity, likely reflecting the larger and more heterogeneous population from which the samples were collected from. Viral detections at both sites included JC polyomavirus and BK polyomavirus. Additionally, Human papillomavirus (HPV) was identified in the dormitory samples, and Adenovirus E was detected in the WWTP samples. These findings emphasize the focus of the Urinary Pathogen ID/AMR panel on detecting and quantifying bacteria and associated AMR genes relevant for human health, while also highlighting its capacity to capture broader microbial trends in complex sample types.

Summary

The MiSeq i100 Series combined with the Urinary Pathogen ID/AMR Enrichment Kit provides a fast, comprehensive workflow that enables detection and characterization of ARGs and associated bacterial pathogens directly from samples to support wastewater surveillance as part of public health efforts.

Learn more

MiSeq i100 Series

Urinary Pathogen ID/AMR Enrichment Kit

References

1. Antimicrobial resistance: a silent pandemic. *Nat Commun*. 15, 6198 (2024). https://doi.org/10.1038/s41467-024-50457-z
2. Reyes R, Ahn R, Thurber K, Burke TF. Urbanization and Infectious Diseases: General Principles, Historical Perspectives, and Contemporary Challenges. *Challenges in Infectious Diseases*. 2012;123-146. Published 2012 May 19. doi:10.1007/978-1-4614-4496-1_4
3. Vassallo A, Kett S, Purchase D, Marvasi M. The Bacterial Urban Resistome: Recent Advances. *Antibiotics (Basel)*. 2022;11(4):512. Published 2022 Apr 12. doi:10.3390/antibiotics11040512
4. Chau KK, Barker L, Budgell EP, et al. Systematic review of wastewater surveillance of antimicrobial resistance in human populations. *Environ Int*. 2022;162:107171. doi:10.1016/j.envint.2022.107171
5. Baba H, Kuroda M, Sekizuka T, Kanamori H. Highly sensitive detection of antimicrobial resistance genes in hospital wastewater using the multiplex hybrid capture target enrichment. *mSphere*. 2023;8(4):e0010023. doi:10.1128/msphere.00100-23
6. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Published 2017. Accessed April 22, 2025.
7. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol*. 2015;13(5):310-317. doi:10.1038/nrmicro3439
8. Kizny Gordon AE, Mathers AJ, Cheong EYL, et al. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature [published correction appears in *Clin Infect Dis*. 2017 Oct 15;65(8):1431-1433. doi: 10.1093/cid/cix563.]. *Clin Infect Dis*. 2017;64(10):1435-1444. doi:10.1093/cid/cix132

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