

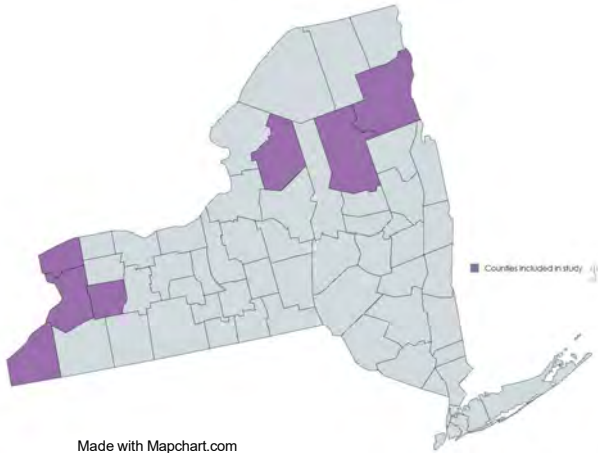
Wastewater Surveillance for Carbapenemase Genes During a Regional Outbreak of NDM-Producing *Klebsiella pneumoniae* in New York State

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Overview

- In 2024, a regional outbreak of NDM-producing *Klebsiella pneumoniae* was detected in New York State (NYS). Response efforts included testing of clinical isolates and isolates from positive colonization screenings at impacted healthcare facilities.
- The Wadsworth Center (WC), as a National Wastewater Surveillance System Center of Excellence, tests wastewater (WW) samples for a multitude of microbial targets including antimicrobial resistance (AR) genes such as the carbapenemase genes *bla*_{NDM}, *bla*_{OXA-48}, and *bla*_{VIM}.
- These genes are found on mobile genetic elements that can be transferred between bacterial species and pose concern for patients in healthcare facilities.
- This outbreak was determined to be caused by multilocus sequence type (MLST) 45 *Klebsiella pneumoniae* (KPN) carrying *bla*_{NDM-5} provided an opportunity to evaluate the use of real-time PCR and digital PCR (dPCR) to detect carbapenemase genes in municipal WW.
- Samples were collected from multiple wastewater treatment plants (WWTPs) from counties in the outbreak region and in an unaffected region.



Methods

- 24-hour composite samples were collected from influent flow at 8 WWTPs across 7 NYS counties.
- 250mL of WW influent were received at WC and concentrated using Nanotrap® Microbiome A Particles then nucleic acid was extracted on the KingFisher™ Apex.
- DNA was tested using QIAGEN dPCR assays on the QIAcuity® Four and WC developed multiplex real-time PCR assays¹ on the QuantStudio™ 5 to detect *bla*_{NDM}, *bla*_{OXA-48}, and *bla*_{VIM}.
- 15 mL of influent from selected *bla*_{NDM} positive WW samples were concentrated by centrifugation at 3,200g to 1.5mL and 50µL were inoculated into Tryptic Soy Broth (TSB), TSB with 10µg ertapenem discs, and TSB with 10µg meropenem discs and incubated overnight at 37°C.
- Broths positive for *bla*_{NDM} by real-time PCR were plated onto CHROMagar™ mSuperCARBA™ culture medium and incubated overnight at 37°C.
- Various isolated colonies were tested for carbapenemase genes with NG-Test® CARBA 5, and positive colonies were plated onto Tryptic Soy Agar with 5% Sheep Blood (BAP) and incubated overnight at 37°C.
- Positive isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and then frozen in TSB with 30% glycerol.
- A subset of carbapenemase-positive isolates underwent whole genome sequencing (WGS) on the Illumina™ NextSeq™ and were analyzed using in-house developed bioinformatic pipelines to 1) identify AR gene variants and MLST², and 2) determine relatedness among WW isolates and between WW isolates and clinical isolates sequenced at WC³.

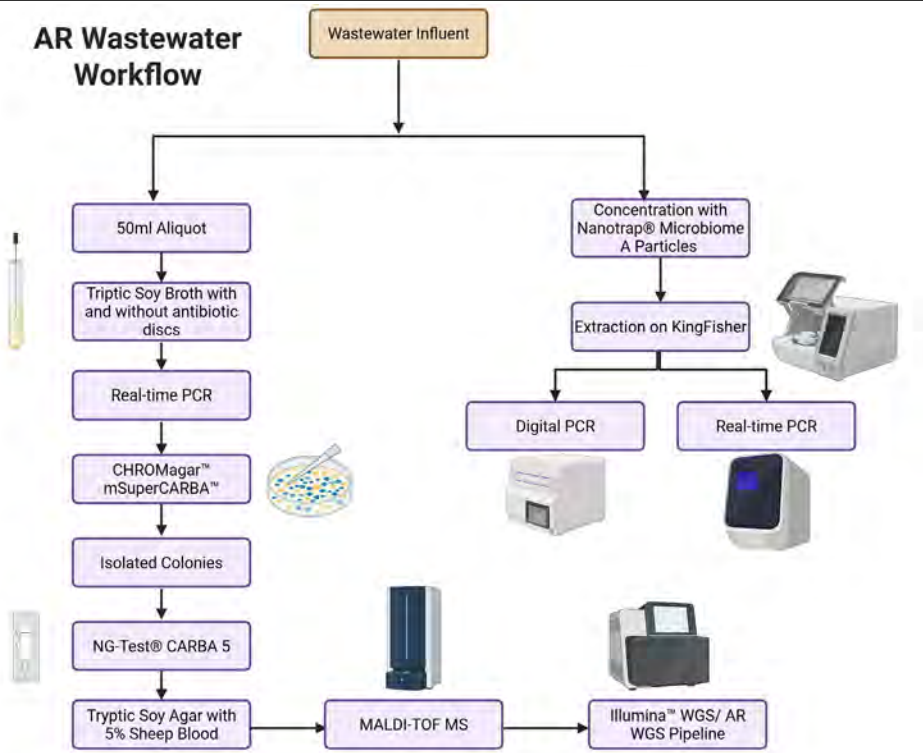


Figure 1.

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ACKNOWLEDGMENTS

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PCR and Culture Results

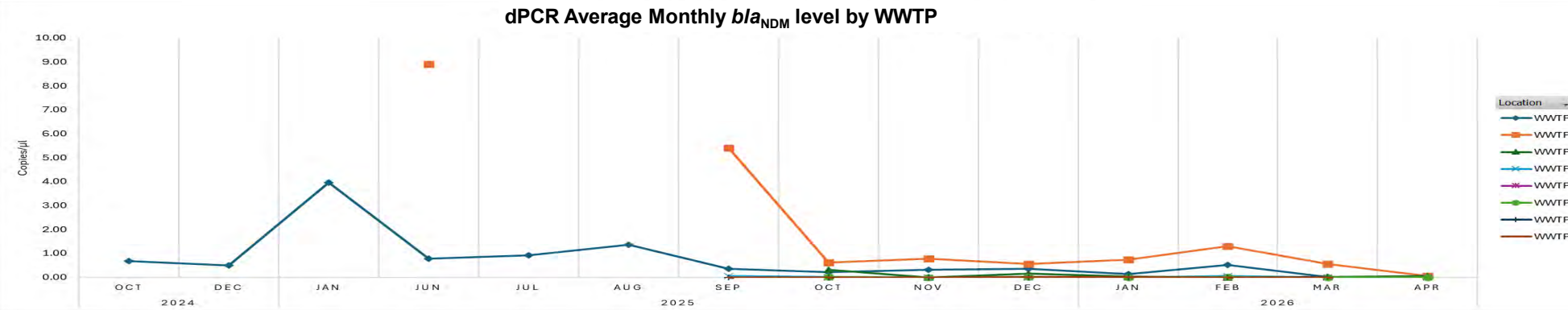


Figure 2.

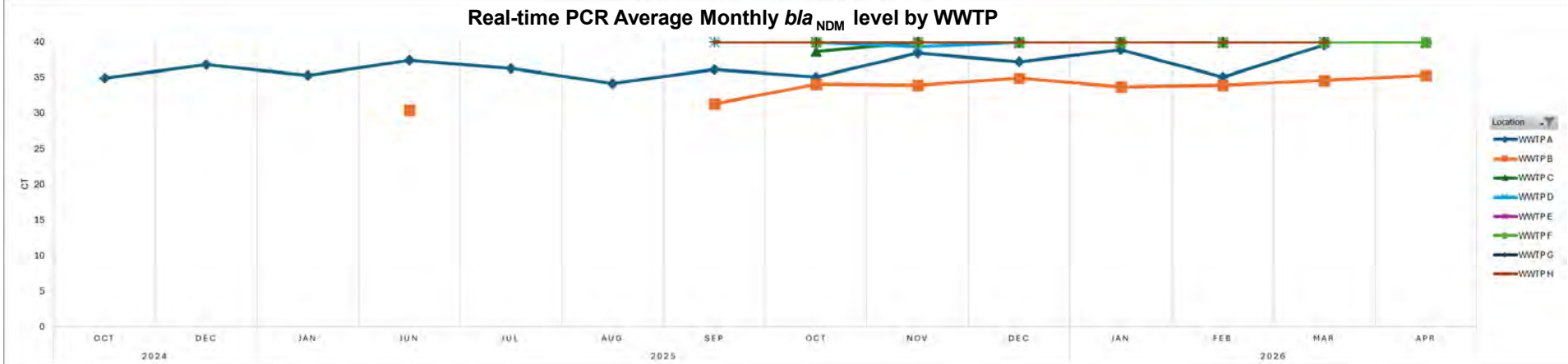


Figure 3.

- 261 WW samples were tested from 8 WWTP in 7 counties by dPCR and real-time PCR for *bla*_{NDM}, *bla*_{OXA-48}, and *bla*_{VIM}.
- Collection dates and frequency of collection varied by WWTP. The copies/µL (for dPCR) or cycle threshold (CT) (for real-time PCR) were averaged together for each WWTP by month.
- bla*_{NDM} levels using dPCR and real-time PCR assays provided similar detection trends.

Table 1. dPCR vs Real-Time PCR WWTP <i>bla</i> _{NDM} Positivity Rates		
<i>bla</i> _{NDM} Positivity	% of WW Samples Positive for <i>bla</i> _{NDM} by dPCR	% of WW Samples Positive for <i>bla</i> _{NDM} by Real-Time PCR
WWTP A*	73% (44/60)	68% (41/60)
WWTP B*	96% (49/51)	100% (51/51)
WWTP C	14% (3/21)	5% (1/21)
WWTP D	4% (1/26)	4% (1/26)
WWTP E	0% (0/23)	0% (0/23)
WWTP F	0% (0/25)	0% (0/25)
WWTP G	7% (2/28)	7% (2/28)
WWTP H	0% (0/27)	0% (0/27)

* = WWTP in county with outbreak

- bla*_{NDM} positivity rates were compared across 8 WWTPs in 7 counties. WWTP A and B, which are both located in the county where the outbreak originated, had higher positivity rates compared with the other WWTPs.
- WWTP A is located near a healthcare facility that was an early epicenter of the outbreak.

Table 3. dPCR vs Real-Time PCR WWTP <i>bla</i> _{VIM} and <i>bla</i> _{OXA-48} Positivity Rates				
Carbapenemase gene /Method	% of WW Samples Positive for <i>bla</i> _{VIM} by dPCR	% of WW Samples Positive for <i>bla</i> _{VIM} by Real-Time PCR	% of WW Samples Positive for <i>bla</i> _{OXA-48} by dPCR	% of WW Samples Positive for <i>bla</i> _{OXA-48} by Real-Time PCR
WWTP A	70% (42/60)	13% (8/60)	98% (59/60)	97% (58/60)
WWTP B	86% (44/51)	12% (6/51)	98% (50/51)	98% (50/51)
WWTP C	100% (21/21)	62% (13/21)	100% (21/21)	95% (20/21)
WWTP D	100% (26/26)	96% (25/26)	96% (25/26)	100% (26/26)
WWTP E	9% (2/23)	4% (1/23)	83% (19/23)	83% (19/23)
WWTP F	12% (3/25)	0% (0/25)	88% (22/25)	88% (22/25)
WWTP G	100% (28/28)	96% (27/28)	96% (27/28)	93% (26/28)
WWTP H	63% (17/27)	7% (2/27)	100% (27/27)	100% (27/27)

- Positivity rates for *bla*_{VIM} varied across the WWTPs and assays.
- Low levels of *bla*_{VIM} were detected by dPCR in samples that were negative for *bla*_{VIM} by real-time PCR.
- bla*_{OXA-48} was detected in almost all samples across the WWTPs and assays.

Klebsiella Isolates from WWTPs Over Time: MLST and *bla*_{NDM} Gene Variant Results

Table 2.

Isolate ID	Species	MLST	Carbapenemase Gene	WWTP	Collection Date
Isolate 1	<i>Klebsiella oxytoca</i>	58	<i>bla</i> _{NDM-5}	WWTP A	7/22/2025
Isolate 2	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	7/22/2025
Isolate 3	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	8/7/2025
Isolate 4	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	7/31/2025
Isolate 5	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	8/5/2025
Isolate 6	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	7/15/2025
Isolate 7	<i>Klebsiella pneumoniae</i>	188	<i>bla</i> _{NDM-5}	WWTP A	7/29/2025
Isolate 8	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	7/29/2025
Isolate 9	<i>Klebsiella pneumoniae</i>	45	<i>bla</i> _{NDM-5}	WWTP A	12/11/2025
Isolate 10	<i>Klebsiella pneumoniae</i>	147	<i>bla</i> _{NDM-5}	WWTP B	3/10/2026
Isolate 11	<i>Klebsiella pneumoniae</i>	147	<i>bla</i> _{NDM-5}	WWTP B	2/19/2026

- Select WWTP samples were isolated in culture, and *bla*_{NDM}-positive *Klebsiella* isolates were sequenced to identify the *bla*_{NDM} gene variant and MLST.
- 11 *Klebsiella* isolates positive for *bla*_{NDM} were isolated from 2 WWTPs that are in the outbreak county.
- All 11 isolates carried *bla*_{NDM-5} and multiple MLSTs were identified including 7 MLST 45 (the outbreak strain MLST; shown in blue highlight).

*Klebsiella pneumoniae bla*_{NDM-5} MLST 45 Clinical Isolates and Wastewater Isolates Over Time

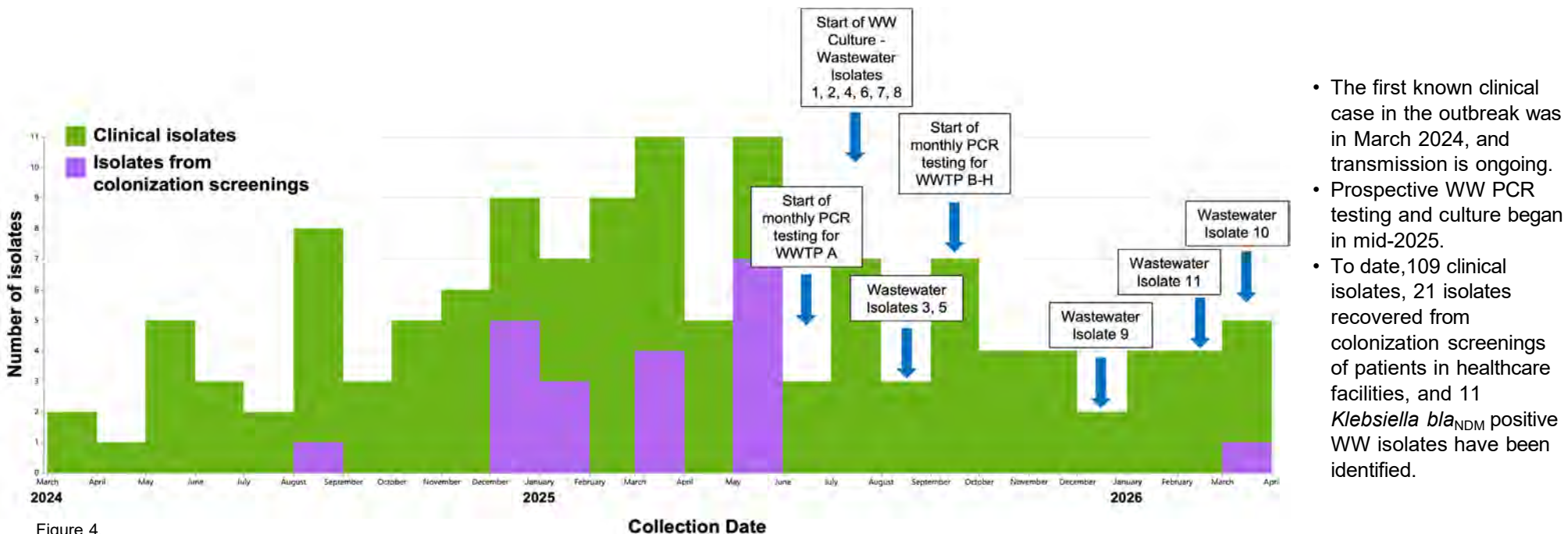


Figure 4.

WGS Relatedness Analysis Results

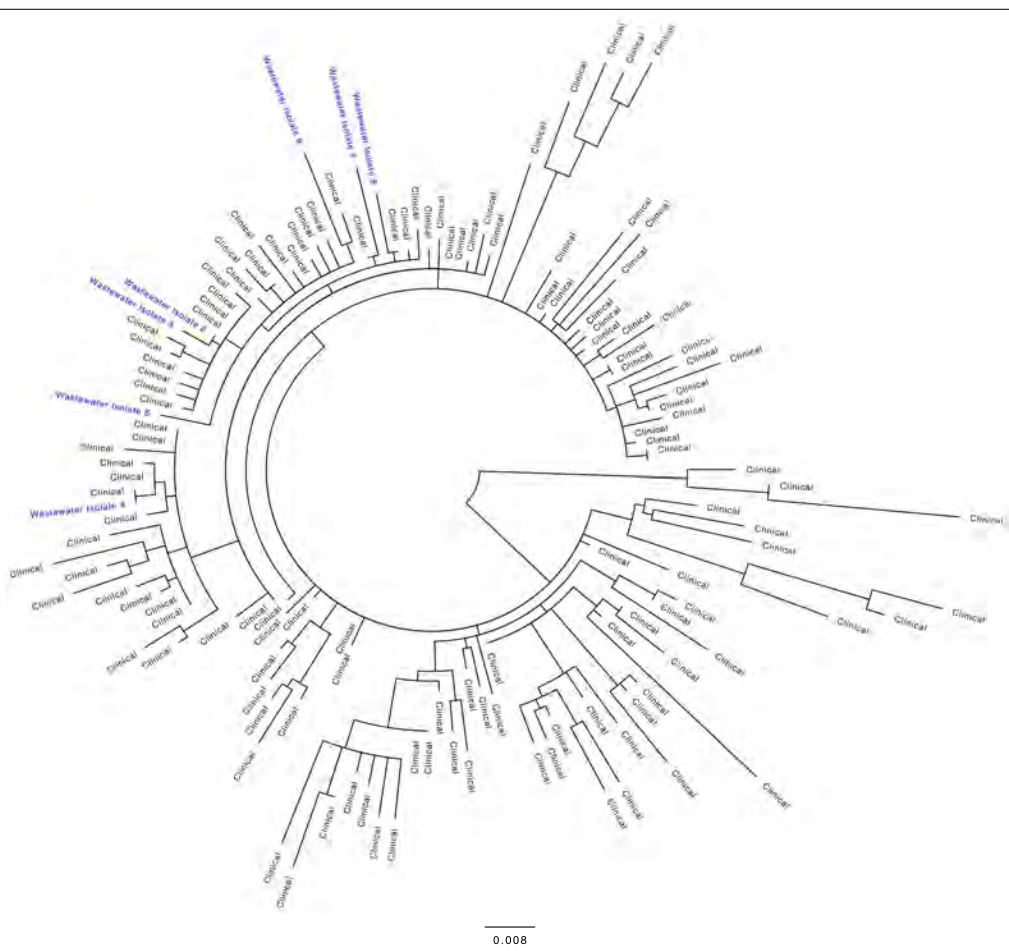


Figure 5.

- 7 *bla*_{NDM-5} MLST 45 WW isolates from WWTP A (in blue) were compared to clinical isolates from the outbreak.
- All 7 WW isolates clustered with the outbreak isolates and were each separated by ≤15 mutation events (ME) from an outbreak isolate and ≤35 MEs from each other.

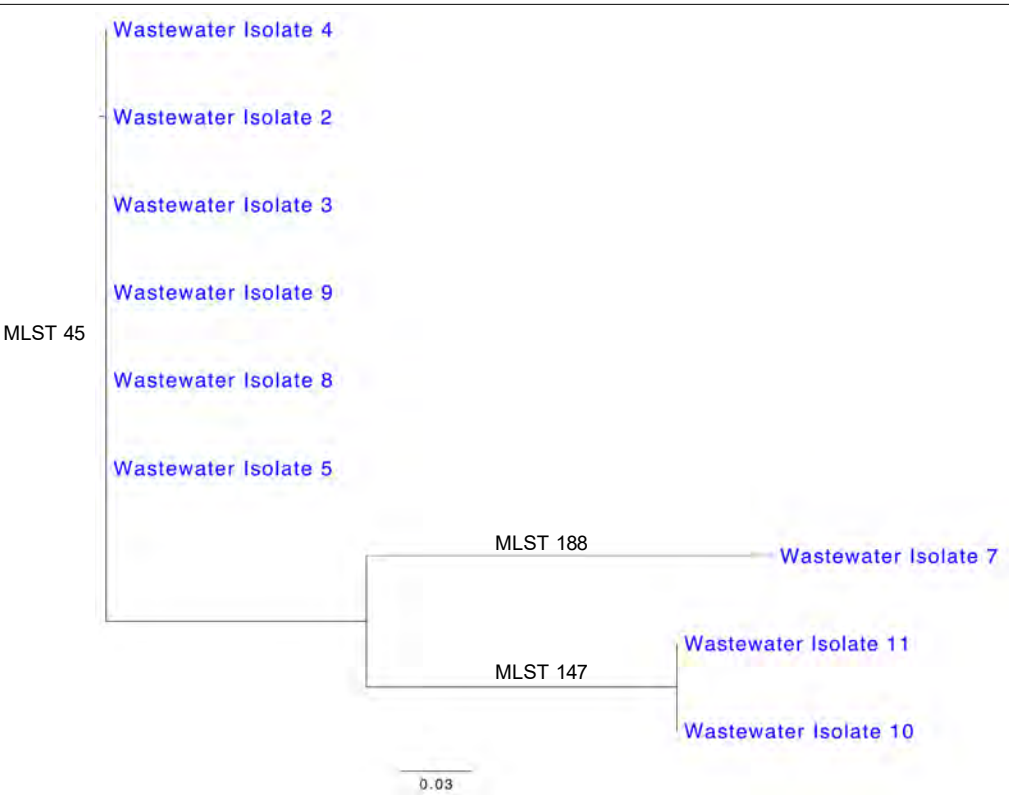


Figure 6.

- The 2 *bla*_{NDM-5}-positive MLST 147 WW isolates 10 and 11 from WWTP B were separated by 10 ME from each other.
- The *bla*_{NDM-5} MLST 188 WW isolate 7 from WWTP A clustered separately from all other WW isolates.
- Isolates 7 and 10/11 were separated by over 5,000 MEs from each other and from the MLST 45 outbreak isolates.

CONCLUSIONS

- WW samples collected from WWTPs within the outbreak region showed a higher *bla*_{NDM} positivity rate by both real-time PCR and dPCR than unaffected areas.
- This proof-of-concept study indicates that PCR on WW extracts could be useful for monitoring trends in carbapenemase gene presence during an outbreak in areas where that gene is not prevalent.
- The outbreak MLST 45 *K. pneumoniae* strain was isolated from one WWTP in the outbreak area, but unrelated *bla*_{NDM}-carrying *K. pneumoniae* strains were also isolated, indicating that *bla*_{NDM} positivity in WW samples did not always only correspond to the presence of the outbreak strain in this region.
- Bacterial isolation and WGS was essential to confirm the presence of the outbreak strain in WW.
- The high positivity rates of *bla*_{VIM} and *bla*_{OXA-48} in all WWTPs indicates that WW surveillance may have limited usefulness for monitoring the presence of these carbapenemase genes, though long-term monitoring, taking into account regional baseline levels, could be considered.
- Low levels of *bla*_{VIM} detected by dPCR in samples negative by real-time PCR could be due to increased sensitivity of the dPCR assay, PCR inhibition, or due to primer/probe differences between the assays.

References

- Prussing *et al.* 2021. *Antimicrob Agents Chemother* 65:10.
- Prussing *et al.* 2020. *Front Microbiol* 11:2007.
- Haas *et al.* 2021. *J Clin Microbiol* 59:10.