

## A close-up photograph of a microscope objective lens, showing its metallic body and glass lens element. The background is blurred, showing a laboratory setting with various equipment.



**NEW YORK STATE**  
**Department of Health**  
**Wadsworth Center**

## Real-Time PCR and WGS Results

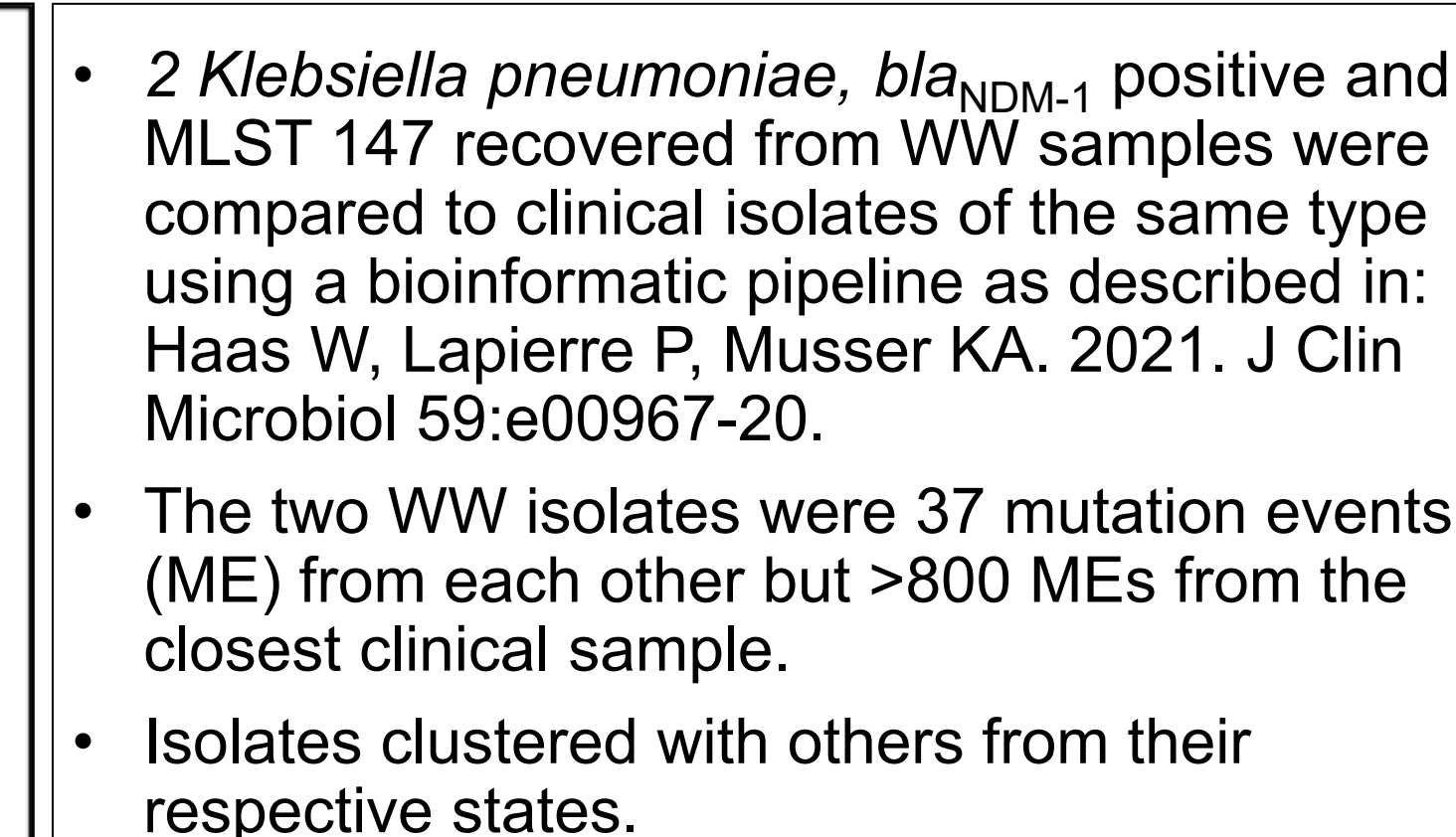
- High Speed vs Low Speed WW pellets tested by the WC AR LDT real-time PCR assays**
- 
- | Assay                        | Result       | Paired High Speed | Paired Low Speed | Unpaired Low Speed | Total |
|------------------------------|--------------|-------------------|------------------|--------------------|-------|
| <i>bla</i> <sub>KPC</sub>    | Positive     | 6262              | 48               | 172                | 6262  |
|                              | Negative     | 0                 | 0                | 0                  | 0     |
|                              | Inconclusive | 0                 | 0                | 0                  | 0     |
| <i>bla</i> <sub>NDM</sub>    | Positive     | 24                | 28               | 16                 | 68    |
|                              | Negative     | 32                | 28               | 30                 | 90    |
|                              | Inconclusive | 6                 | 6                | 2                  | 14    |
| <i>bla</i> <sub>OXA-48</sub> | Positive     | 6262              | 48               | 172                | 6262  |
|                              | Negative     | 0                 | 0                | 0                  | 0     |
|                              | Inconclusive | 0                 | 0                | 0                  | 0     |
| <i>bla</i> <sub>VIM</sub>    | Positive     | 33                | 36               | 41                 | 110   |
|                              | Negative     | 27                | 25               | 5                  | 57    |
|                              | Inconclusive | 2                 | 1                | 2                  | 5     |
| <i>bla</i> <sub>IMP</sub>    | Positive     | 55                | 57               | 47                 | 159   |
|                              | Negative     | 3                 | 4                | 0                  | 7     |
|                              | Inconclusive | 4                 | 1                | 1                  | 6     |



### Carbapenemase Gene Positive Isolates Recovered from WW Pellets

- | AR Gene Variant and Species         | Number of Isolates | Number of Distinct MLSTs | AR Gene Variant and Species                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Number of Isolates | Number of Distinct MLSTs |
|-------------------------------------|--------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|--------------------------|
| <i>bla</i> <sub>KPC-2</sub>         | 29                 | 19                       | <i>bla</i> <sub>NDM-1</sub>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 3                  | 2                        |
| <i>Aeromonas</i> species            | 6                  | 1                        | <i>Klebsiella pneumoniae</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 3                  | 2                        |
| <i>Citrobacter freundii</i> complex | 4                  | 3                        | <i>bla</i> <sub>NDM-5</sub>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 5                  | 2                        |
| <i>Enterobacter cloacae</i> complex | 6                  | 3                        | <i>Escherichia coli</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 5                  | 2                        |
| <i>Escherichia coli</i>             | 1                  | 1                        | <i>bla</i> <sub>OXA-181</sub> ( <i>bla</i> <sub>OXA-48</sub> like)                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1                  | 1                        |
| <i>Klebsiella</i> species           | 8                  | 7                        | <i>Escherichia coli</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 1                  | 1                        |
| <i>Raoultella ornithinolytica</i>   | 4                  | 4                        | <i>bla</i> <sub>OXA-244</sub> ( <i>bla</i> <sub>OXA-48</sub> like)                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 2                  | 2                        |
| <i>bla</i> <sub>KPC-3</sub>         | 42                 | 29                       | <i>Escherichia coli</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 2                  | 2                        |
| <i>Aeromonas</i> species            | 2                  | 2                        | <ul style="list-style-type: none"> <li>123 carbapenemase gene positive isolates were identified from 24 WW samples: KPC (116), NDM (8), OXA-48 (4), VIM (0), IMP(0)</li> <li>83 isolates underwent WGS analysis using the WC AR WGS Pipeline which identified: AR gene variants (7), MLSTs (48)</li> <li>Species were identified by MALDI-TOF MS</li> <li>A known circulating clinical strain of <i>Klebsiella pneumoniae</i>, <i>bla</i><sub>NDM-1</sub> positive and MLST 147 was identified in the WW samples (Figure 3).</li> </ul> |                    |                          |
| <i>Citrobacter freundii</i> complex | 3                  | 2                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Enterobacter cloacae</i> complex | 10                 | 9                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Escherichia coli</i>             | 1                  | 1                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Klebsiella</i> species           | 22                 | 12                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Kluyvera</i> species             | 1                  | 1                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Raoultella ornithinolytica</i>   | 3                  | 2                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>bla</i> <sub>KPC-4</sub>         | 1                  | 1                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |
| <i>Klebsiella</i> species           | 1                  | 1                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                    |                          |

## WGS Relatedness Analysis



## Conclusions

- WW samples throughout NYS contain multiple carbapenemase genes, within a variety of different organisms, MLSTs, AR genes and variants detected. Some of the samples were found to have the same MLSTs and AR gene variant as those found in clinical strains previously sequenced at WC but are not closely related.
- The percentage of WW samples positive for AR genes does not align with known clinical strain positivity for these genes.
- There is little difference between high and low speed ultracentrifugation for WW pellets by either the AR genes detected by real-time PCR or ONT 16S sequencing. Only low speed samples will be tested moving forward.
- ONT 16S sequencing of WW pellets demonstrated limited clinical/surveillance utility as clinically relevant bacteria are often present at very low frequencies in these sample preparations. However, ONT 16S sequencing can serve as an early screening tool for TSB enrichments to prioritize samples for targeted isolation and characterization.
- Additional work is needed to optimize testing methodology to detect carbapenemase genes in WW as well as additional comparisons to clinical data to determine if this testing could provide an improved public health impact and reduce the spread of AR.

## Acknowledgements

- We thank the Wadsworth Center Applied Genomic Technologies Cluster for whole genome sequencing of our samples.
- This work was supported by the Cooperative agreement number NU50CK000516 funded by the centers for Diseases Control and Prevention and the New York State Department of Health, in collaboration with Syracuse University and partners.

Workflow made in Microsoft PowerPoint