

Characterization of Wastewater Treatment-Adapted *Klebsiella pneumoniae*: Implications for Environmental Antimicrobial Resistance (AMR) and Pathogenicity

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World Congress of Epidemiology 2024

September 26, 2024

Oral Abstract



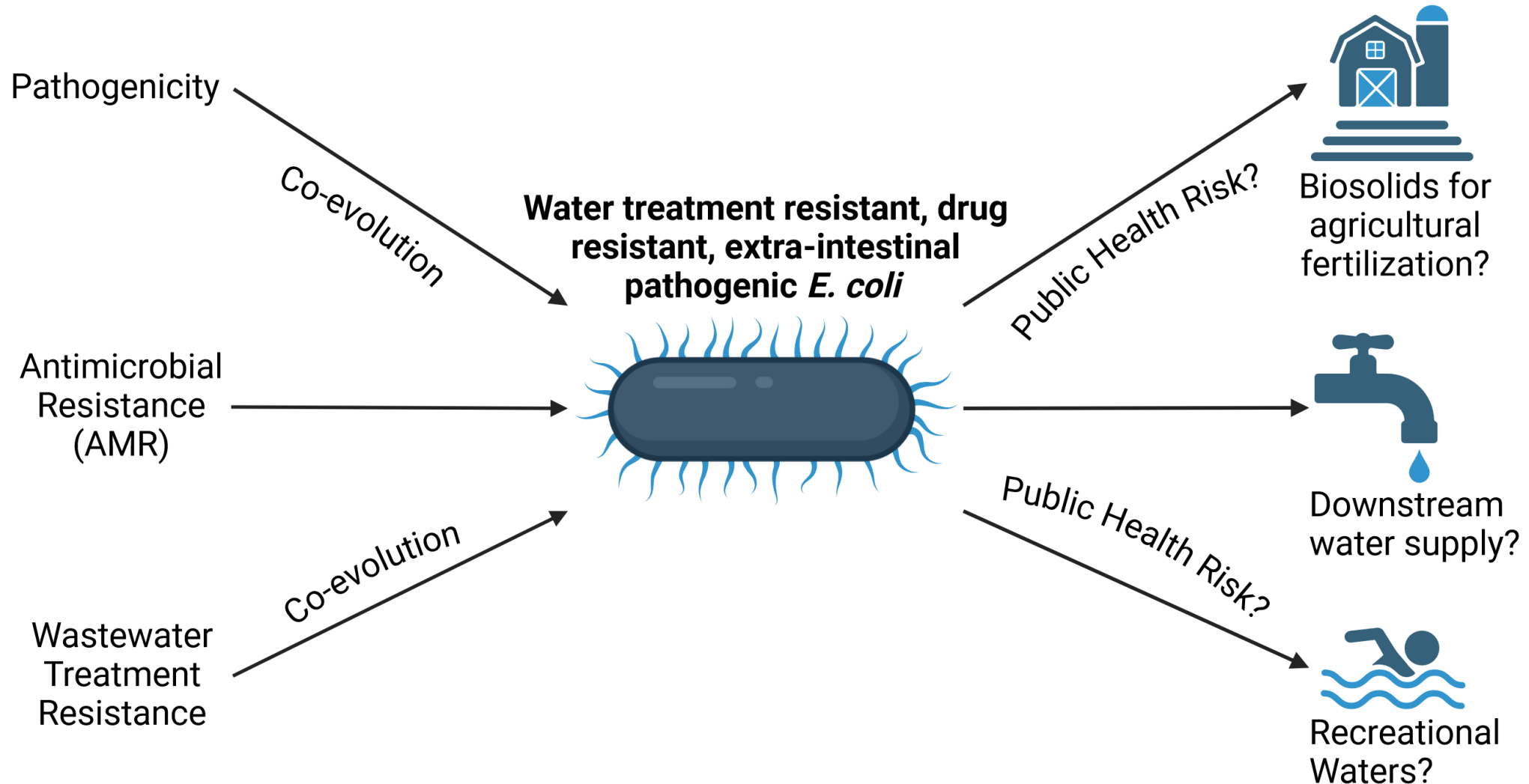
**Water treatment and disinfection are critical pillars
of infectious disease prevention**

**What if pathogenic microorganisms were able to
develop resistance to treatment in a way that
compromised its effectiveness in this role?**



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ExPEC and the Co-evolution of AMR, Virulence and Wastewater Treatment Resistance

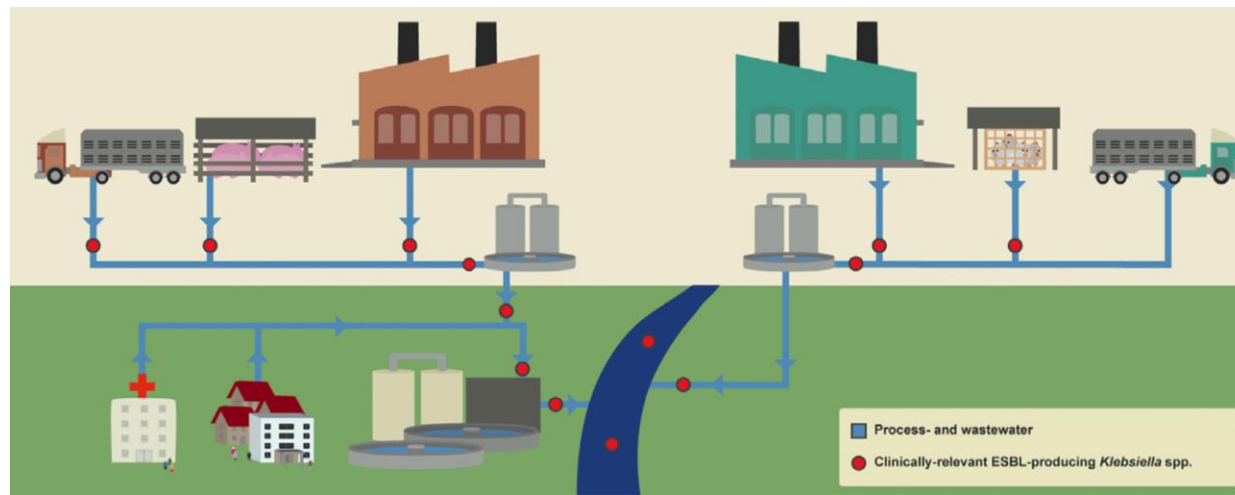


Similar Developments in Other Environmental Bacteria?

Not Just *E. coli*?

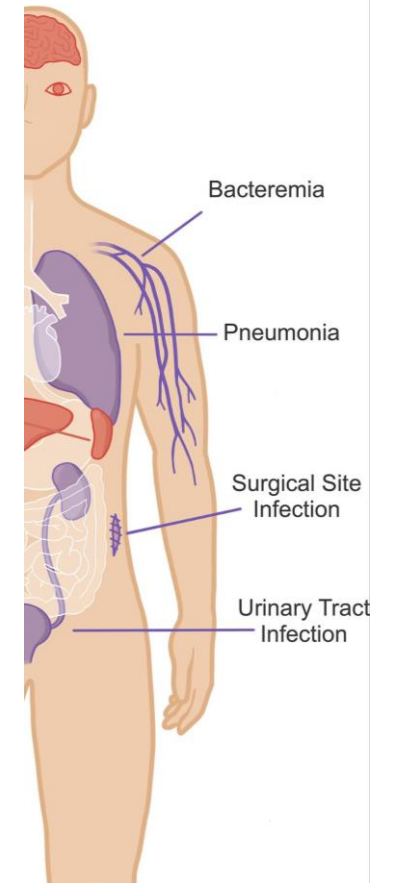
Klebsiella pneumoniae

- Potential candidate for co-evolution of AMR, treatment resistance and pathogenicity in the WWTP environment
 - Environmentally ubiquitous (including in wastewater)
 - Pathogen (UTIs, bloodstream infections, pneumonia, etc.) (opportunistic or hypervirulent)
 - Among the most significant global AMR threats (ESBL, carbapenemases, etc.)
- Strong evidence for multi-drug resistant (MDR) *K. pneumoniae* strains in WW
- Reports of certain strains surviving wastewater treatment and entering natural waterways



Adapted from Savin et al., 2022

Classical and Hypervirulent Kp



Adapted from Gonzalez-Ferrer et al., 2021

**Are pathogenicity and AMR co-evolving
with wastewater treatment resistance
in *K. pneumoniae* isolated from Alberta
WWTPs?**

Research Questions:

Question 1: Are *K. pneumoniae* strains from Alberta WWTPs able to resist stressors commonly associated with wastewater treatment?

The Wastewater *K. pneumoniae*:

Chlorination, Isolation, Identification, Sequencing:

- Raw sewage samples from 10 Alberta WWTPs
- Sewage treated with chlorine
- Bacteria isolated and identified using biochemical methods
- Underwent whole-genome sequencing to allow for bioinformatic analysis

Result:

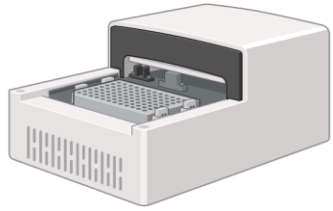
21 chlorine-tolerant isolates

**belonging to the *Klebsiella*
pneumoniae species complex**

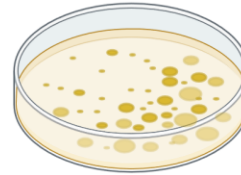


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Another Treatment-Associated Stressor: Heat



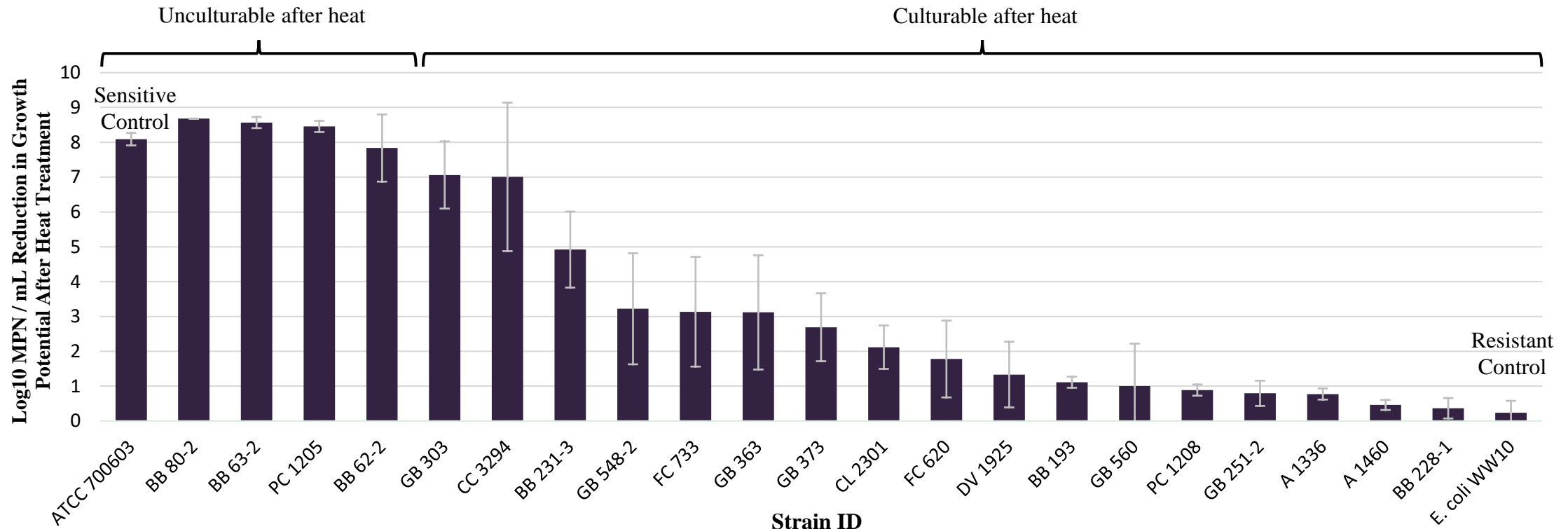
58°C for 5 minutes in Thermal Cycler



Growth measured (log Most Probable Number / mL), subtracted from growth in unheated samples

Reduction in growth compared to known heat-sensitive and heat resistant isolate to identify heat-resistant *K. pneumoniae*

Created with BioRender.com



**A chlorine-resistant and heat-resistant population
of *K. pneumoniae* exists in Alberta wastewater**

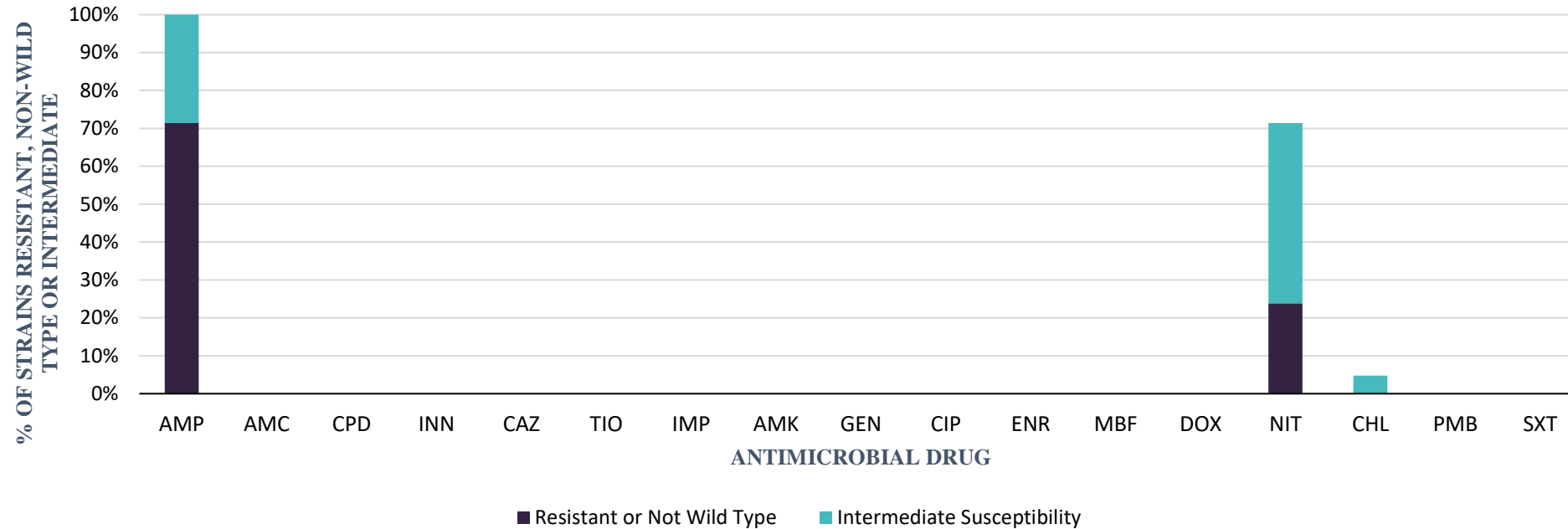
Research Questions:

Question 1: Are *K. pneumoniae* strains from Alberta WWTPs able to resist stressors commonly associated with wastewater treatment?

Question 2: Are *K. pneumoniae* strains from WWTPs able to resist antimicrobial drugs?

Phenotypic AMR:

Antimicrobial susceptibility assessed using VITEK2 Antimicrobial Susceptibility Testing



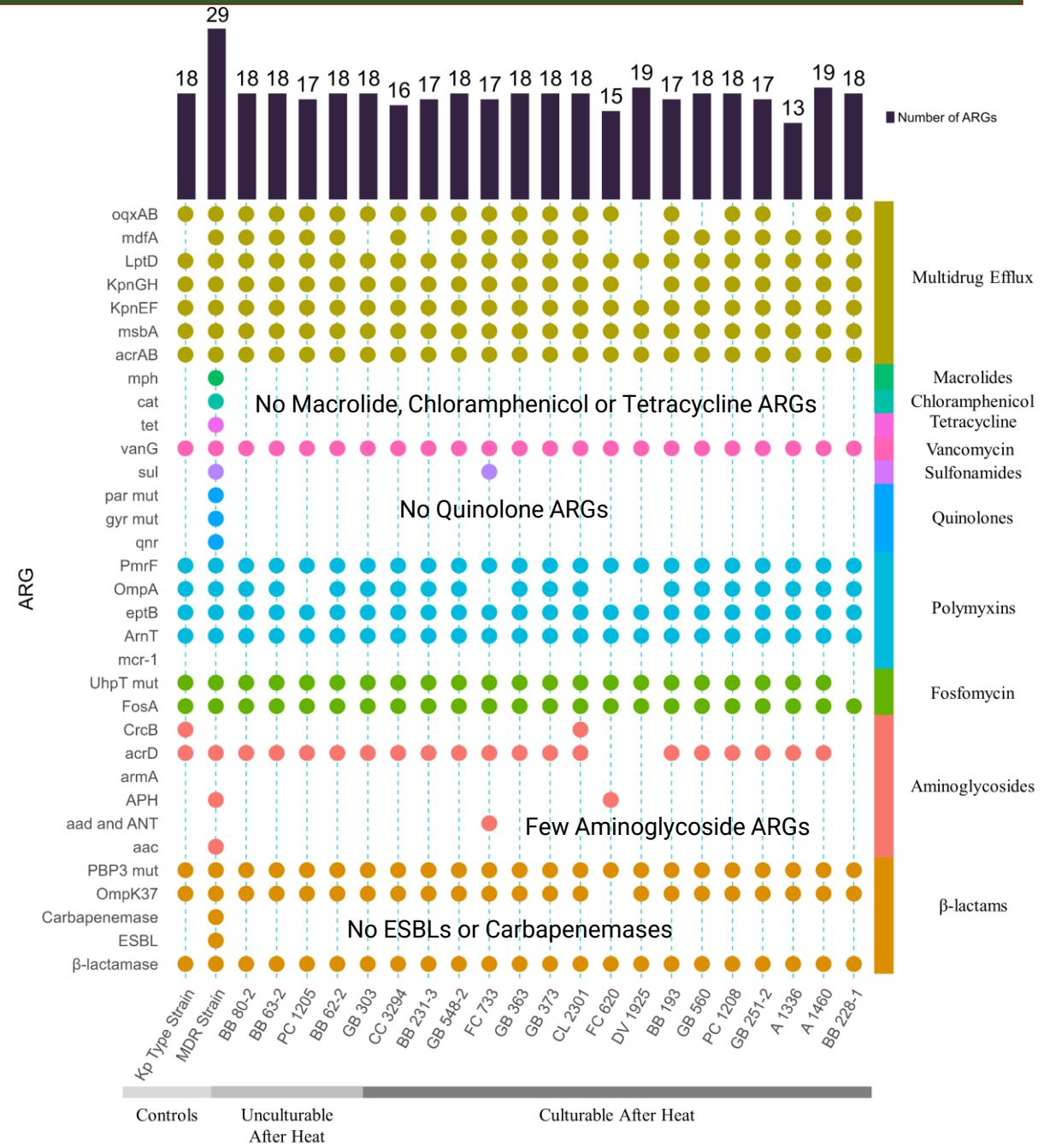
Tested Antimicrobials: ampicillin (**AMP**), amoxicillin/clavulanic acid (**AMC**), cefpodoxime (**CPD**), cefovecin (**INN**), ceftazidime (**CAZ**), ceftiofur (**TIO**), imipenem (**IMP**), amikacin (**AMK**), gentamicin (**GEN**), ciprofloxacin (**CIP**), enrofloxacin (**ENR**), marbofloxacin (**MBF**), doxycycline (**DOX**), nitrofurantoin (**NIT**), chloramphenicol (**CHL**), polymyxin B (**PMB**), trimethoprim/sulfamethoxazole (**SXT**).

Isolates were phenotypically susceptible to most tested antimicrobials

Antimicrobial Resistance Gene (ARG) Carriage:

- Screened isolate genomes for known antimicrobial resistance genes (ARGs)
- Comparison to clinical multi-drug resistant (MDR) and non-MDR genomes
- RGI software, CARD database (Alcock et al., 2023)

Isolates lacked key ARGs, those ARGs that were present largely did not contribute to a substantial AMR phenotype



Research Questions:

Question 1: Are *K. pneumoniae* strains from Alberta WWTPs able to resist stressors commonly associated with wastewater treatment?

Question 2: Are *K. pneumoniae* strains from WWTPs able to resist antimicrobial drugs?

Question 3: What is the pathogenic potential of *K. pneumoniae* isolated from Alberta WWTPs (are they hypervirulent)?

Virulence Factor Screen

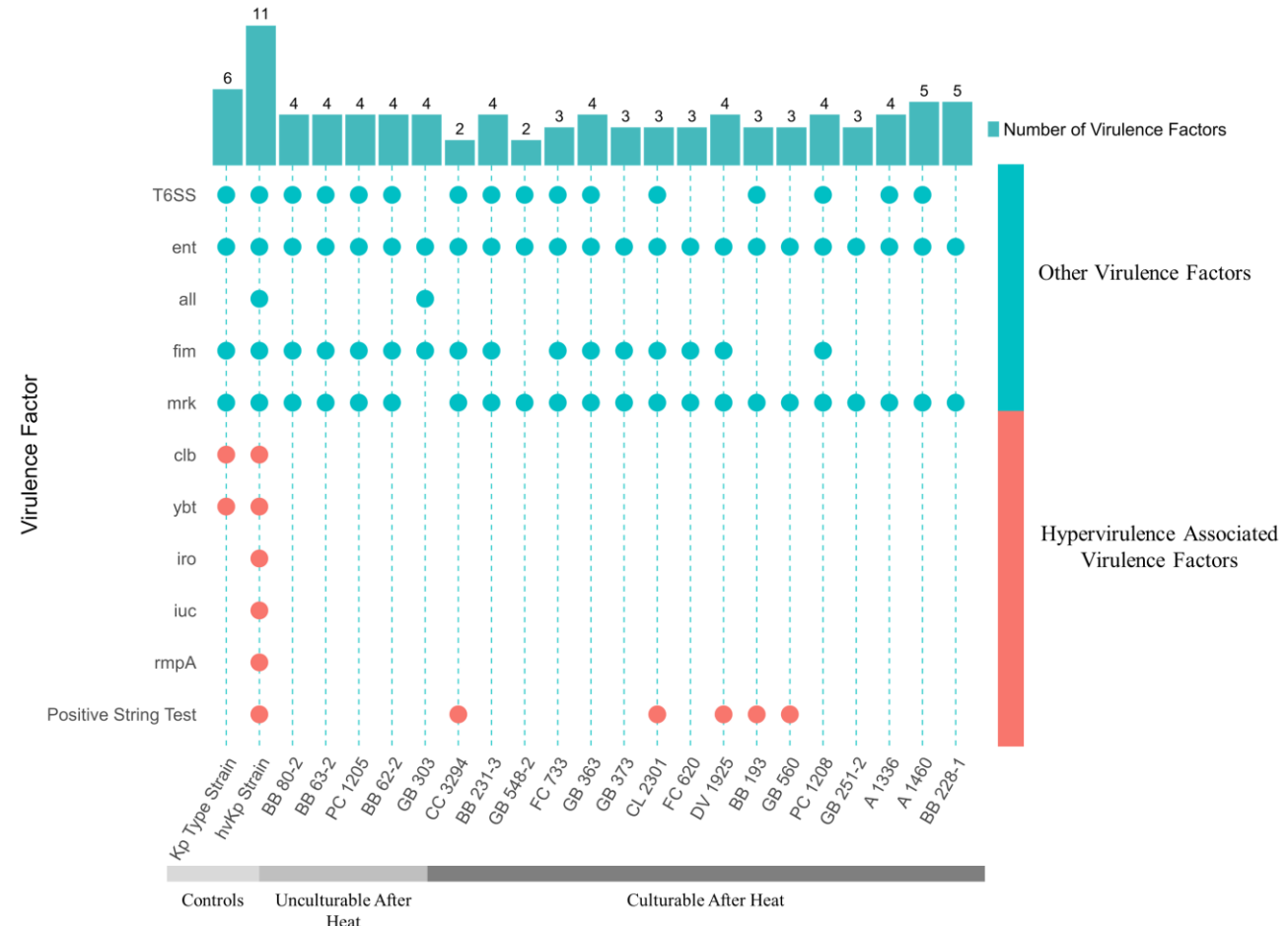
Methods:

- Screened isolate genomes for known *Kp* virulence factors (vfs)
- ABRicate software (Torstwen Seemann, 2014), Virulence Factor Database (VFDB) (Liu et al., 2022)
- Tested for vfs associated with classical, opportunistic pathogenicity and 'hypervirulent' pathogenicity
- Also conducted 'string test', simple phenotypic proxy for hypervirulence
- Compared to clinical hypervirulent and non-hypervirulent control strains

Results:

The only hypervirulence-associated virulence factor observed was a positive string test for 5 isolates (false positives?).

Virulence factors associated solely with opportunistic infections were more prevalent



Conclusions

- Chlorine-tolerant and heat-resistant strains of *K. pneumoniae* were isolated from Alberta sewage
- These isolates did not exhibit substantial AMR
- The isolates did not carry genes for hypervirulence, but could still be of clinical origin (opportunistic infection?)
- **In the strains included in this study, wastewater treatment resistance does NOT appear to have co-evolved with AMR or high virulence**
- Our sample represents a snapshot of Alberta sewage at the time of sampling (2011), it is not necessarily representative of trends over time

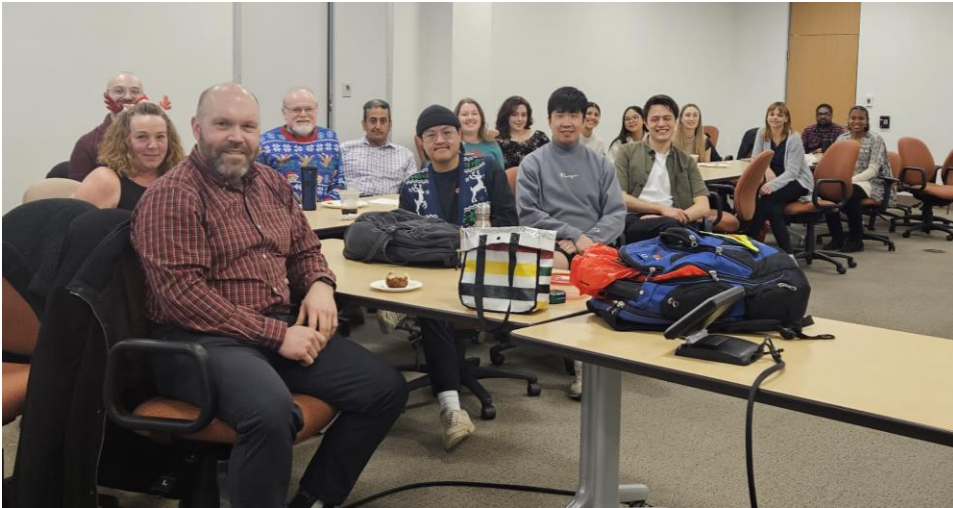
Next Steps:

- Phylogenomic comparison to published *K. pneumoniae* genomes may provide further insight into strain origin and pathogenicity
- Genome-wide association study to examine genetic basis of wastewater treatment resistance

Acknowledgements

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<https://www.heat-amr.com/>

Otto, Neumann and Hanington Labs:



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Human-Environment-Animal Transdisciplinary
Antimicrobial Resistance Research Group



Funding:

1. This research is part of the AMR – One Health Consortium, funded by the Major Innovation Fund program of the Ministry of Jobs, Economy and Trade + Ministry of Innovation and Technology, Government of Alberta.
2. Partial funding from an Alberta Innovates-NSERC Alliance grant, the Ministry of Jobs, Economy and Trade + Ministry of Innovation and Technology, and the Government of Alberta.
3. NSERC Discovery Grant.
4. NSERC Canada Graduate Scholarship – Master's award
5. University of Alberta Walter H. Johns Graduate Fellowship



Natural Sciences and Engineering
Research Council of Canada

Conseil de recherches en sciences
naturelles et en génie du Canada



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