Differences in virulence between the two clades of *Klebsiella pneumoniae* ST258

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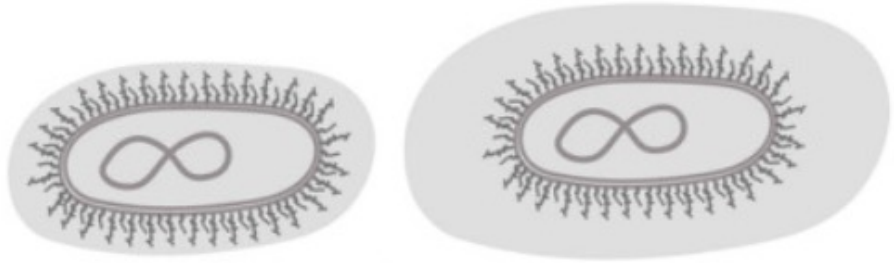
Klebsiella pneumoniae (KP)

- Gram-negative bacteria
- Two pathotypes: Classical and Hypervirulent

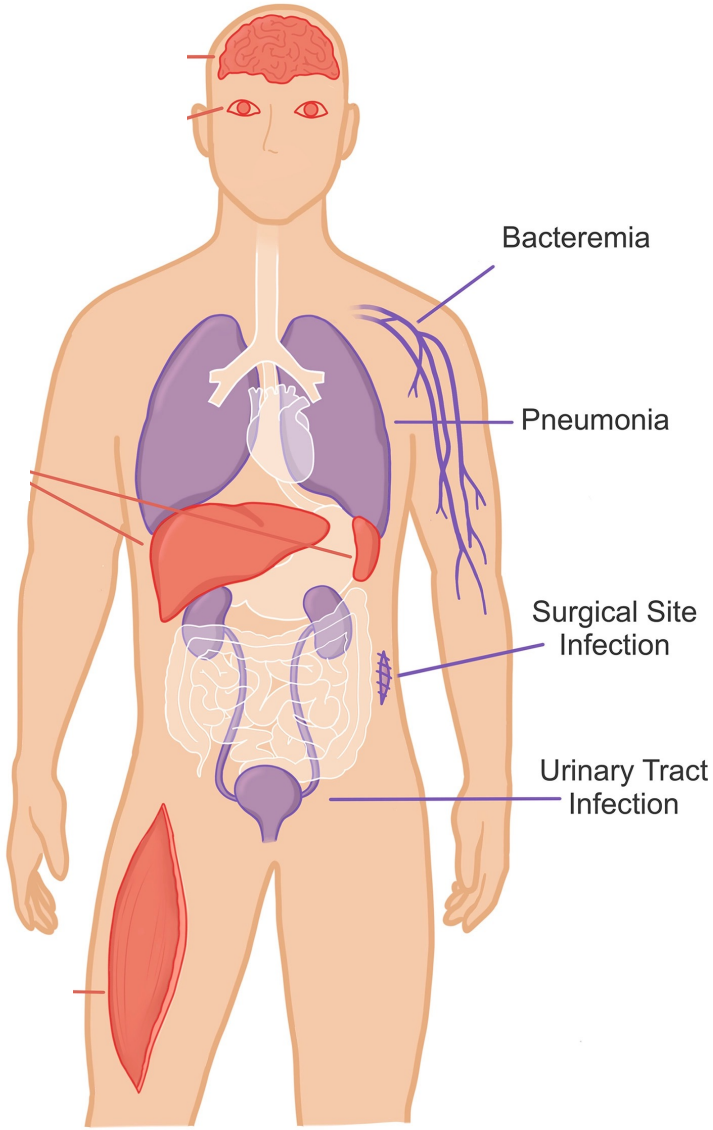
Sanchez-Lopez, et al. ID Cases 2019.



	Classical KP	Hypervirulent KP
Relative Capsule Size	++ Normal level	
Mucoviscosity	+	
Primary Infection Source	Hospital-acquired	
Antibiotic Resistance	Multidrug resistance (ESBL and CR)	

Adapted from Opoku-Temeng et al. J Innate Immun. 2022

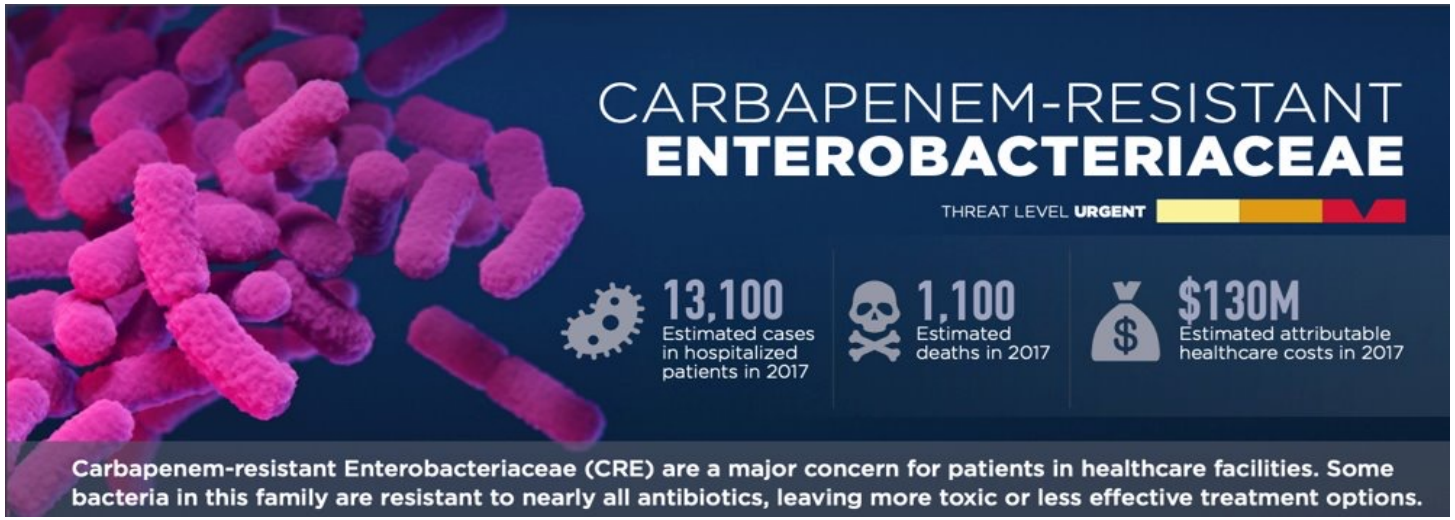
Classical and Hypervirulent Kp



Gonzalez-Ferrer et al. Infection and Immunity 2021

Klebsiella pneumoniae (KP) → Sequence Type 258

- Classical strain of KP that is extensively multidrug resistant
- Epidemic strain often responsible for outbreaks of carbapenem-resistant KP in hospitals



CDC 2019

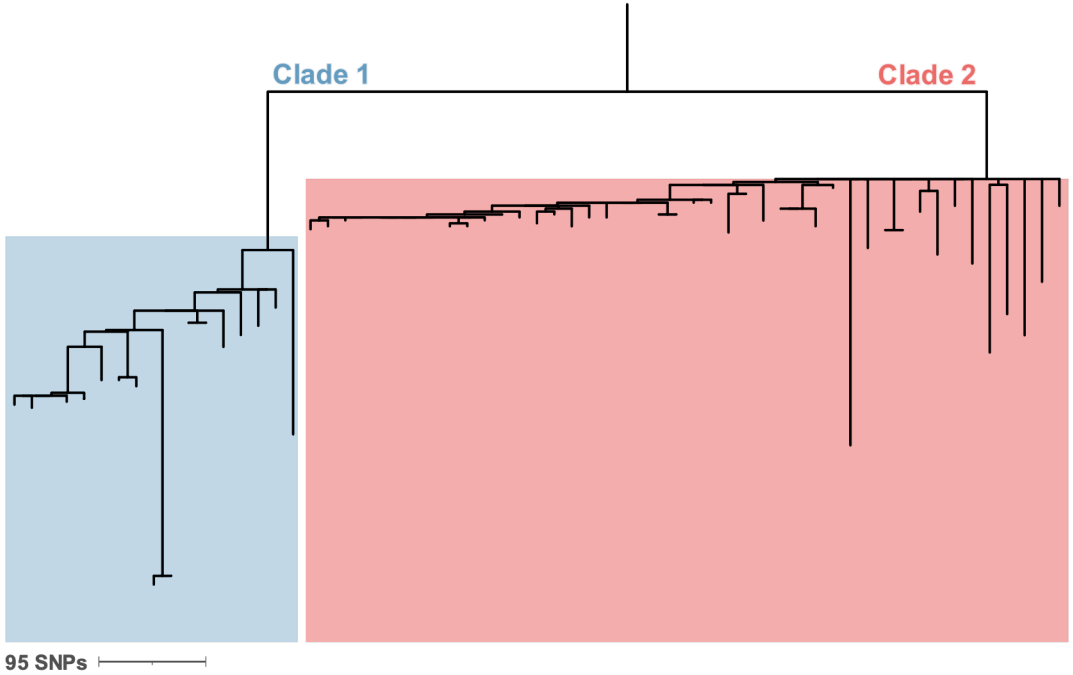
- Why has KP ST258 been more successful than other STs?

  → more characterization of this sequence type is needed

Marsh et al. mBio 2019.

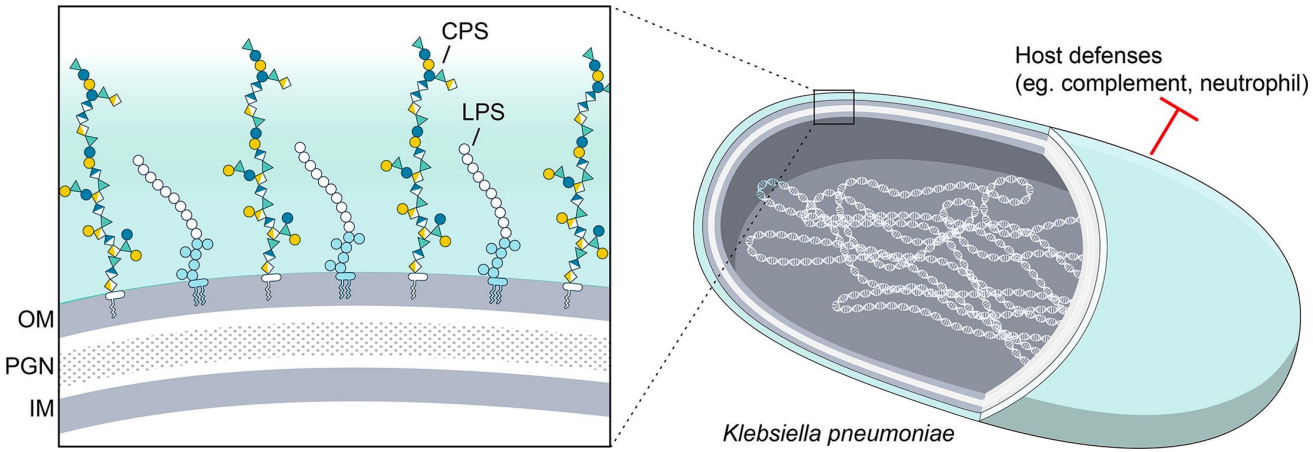
KP ST258 Clade 1 vs Clade 2

- Consists of two clades
- Much of the difference between the core genomes of the two clades is due to a ~215 kb region of divergence
  - This region contains the capsule locus

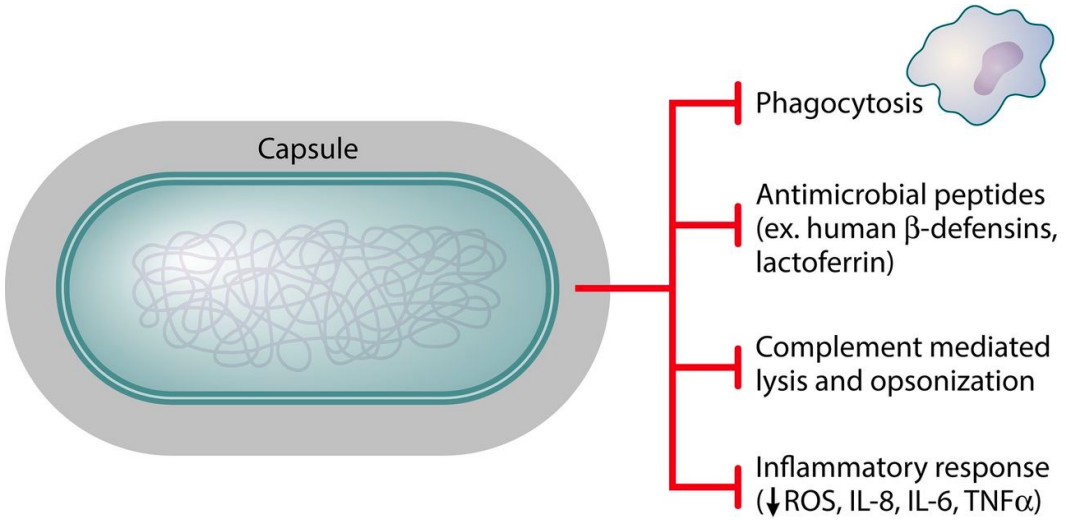


KP Capsule

- Capsule plays an important role in how KP interacts with the host



Opoku-Temeng, et al. Computational and Structural Biotechnology Journal 2019.



Paczosa and Mecsas. Microbiology and Molecular Biology Reviews 2016.

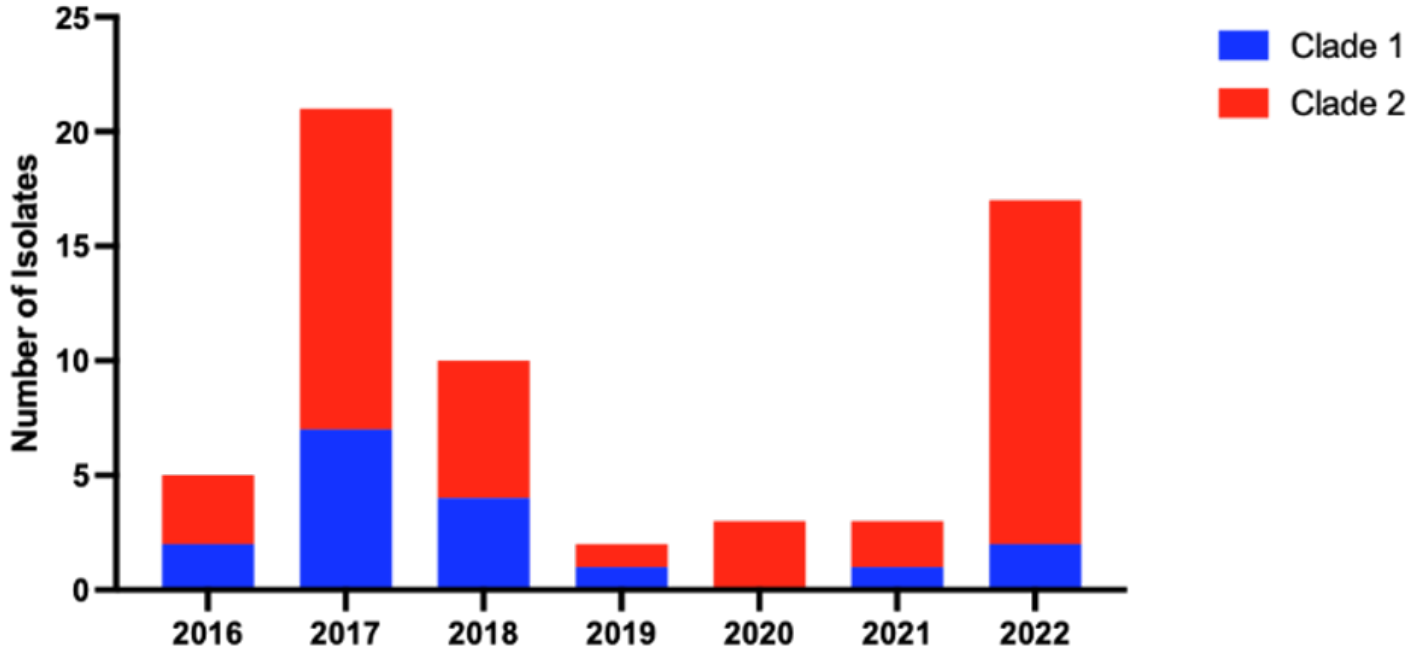
Project aim:

1. Genotypically and phenotypically characterize the two clades of KP ST258 at University of Pittsburgh Medical Center (UPMC)

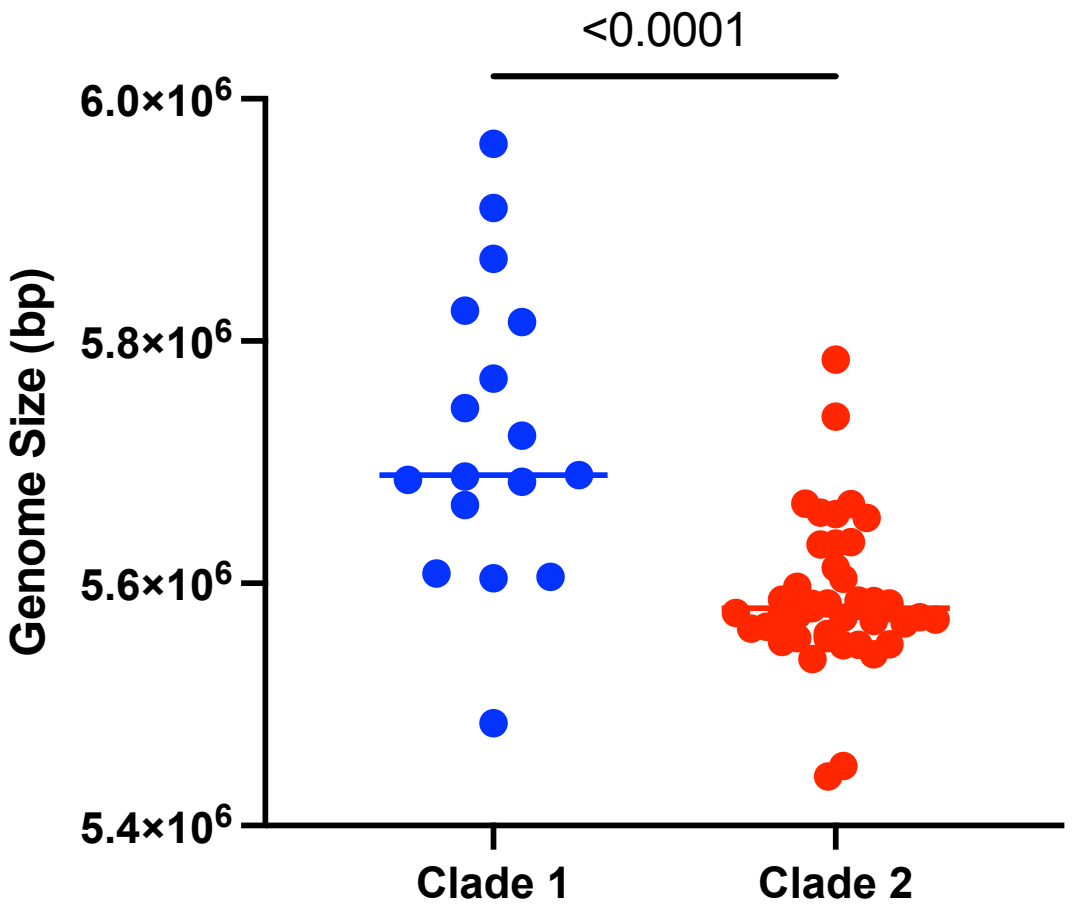
2. Compare and contrast the two clades in measures of virulence:
    1. Biofilm formation
    2. Resistance to serum killing
    3. Resistance to phagocytosis by macrophages
    4. Virulence in an *in vivo* model

Isolate collection

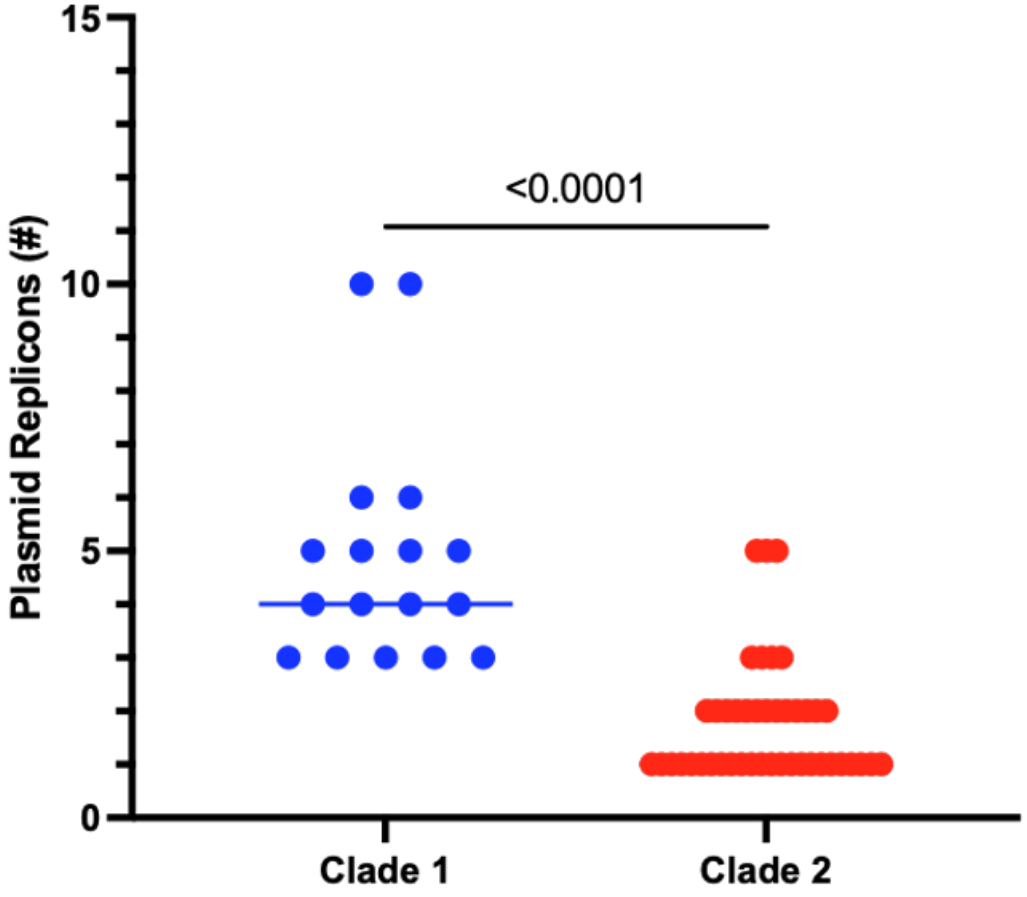
- Analyzed 61 KP ST258 isolates taken from UPMC between 2016-2022
  - 17 Clade 1 isolates
  - 44 Clade 2 isolates

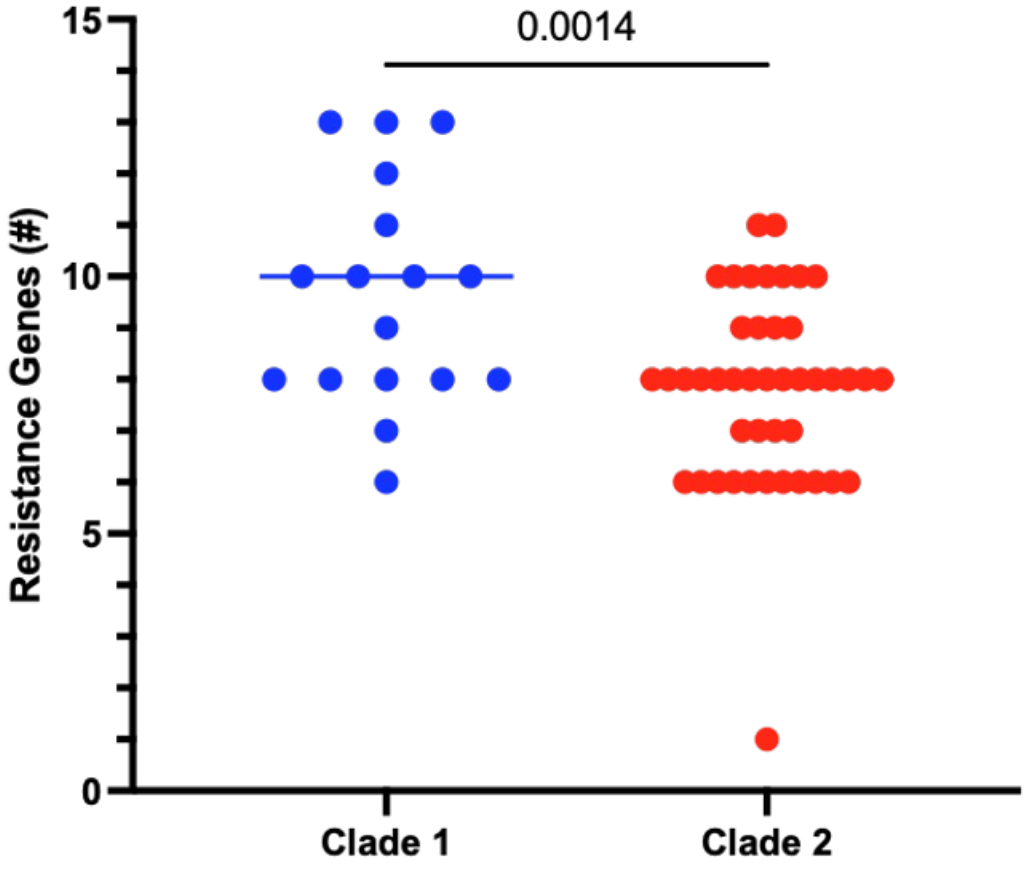


Clade 1 has a larger genome on average than Clade 2

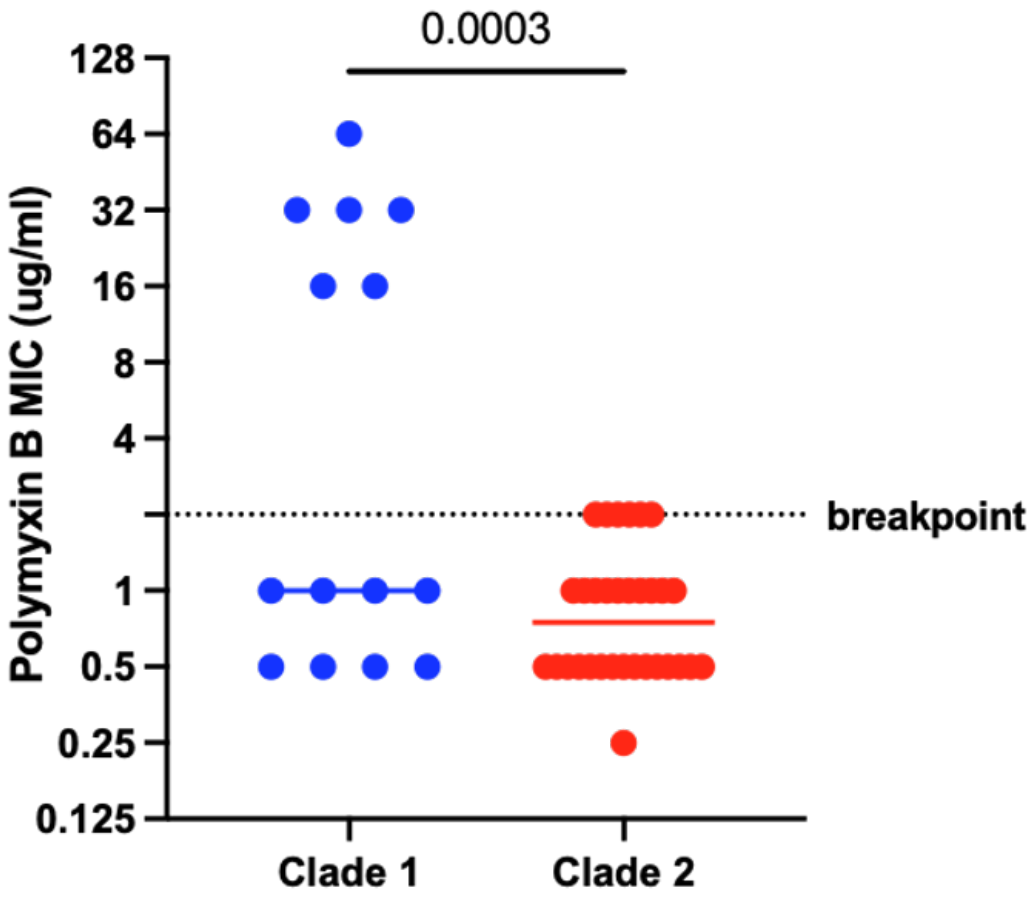


Clade 1 isolates have more plasmids and antibiotics resistance genes than Clade 2 isolates

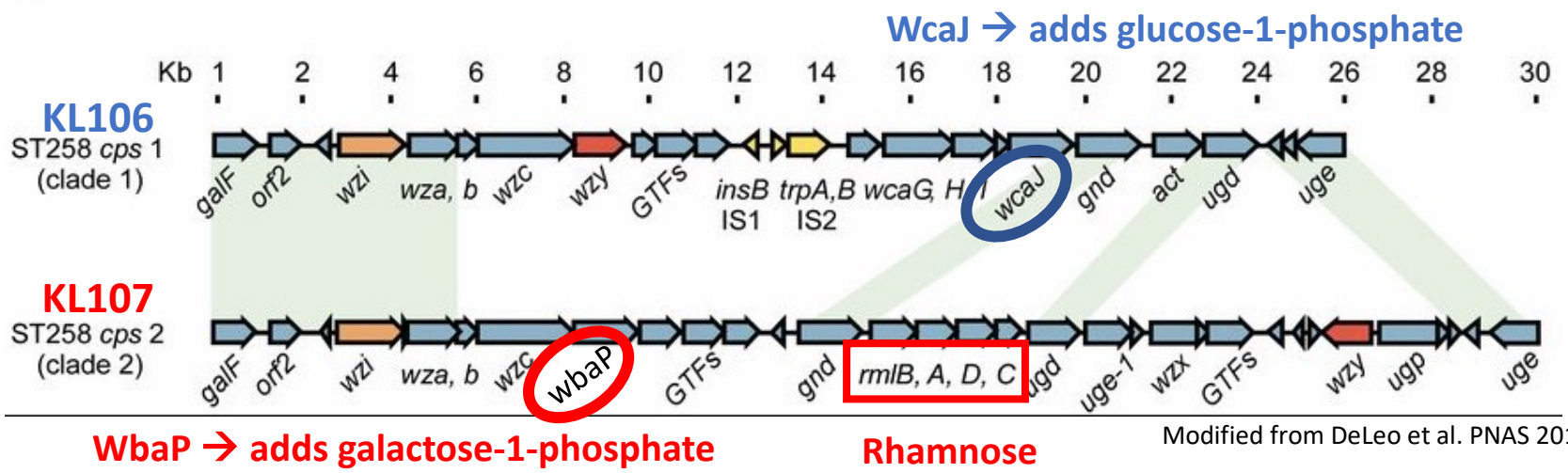




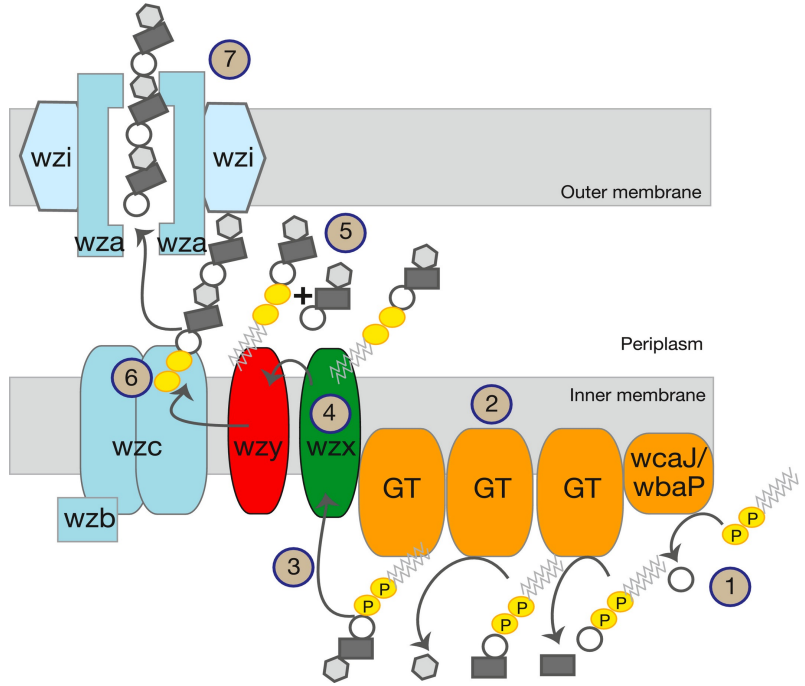
More Clade 1 isolates are resistant to polymyxin B than Clade 2 isolates



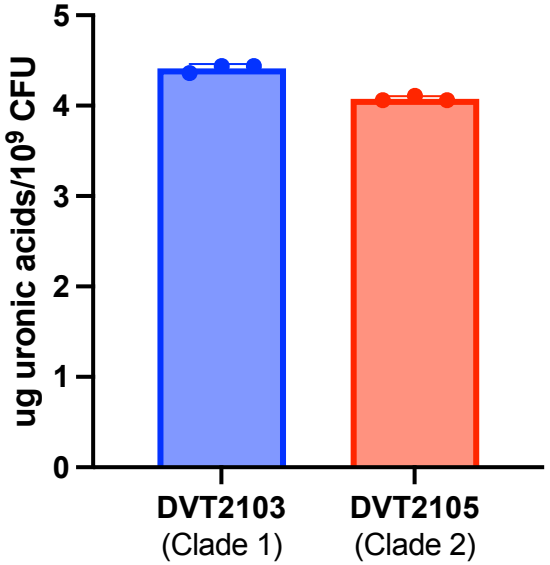
Capsule composition of Clade 1 and Clade 2



Modified from DeLeo et al. PNAS 2014



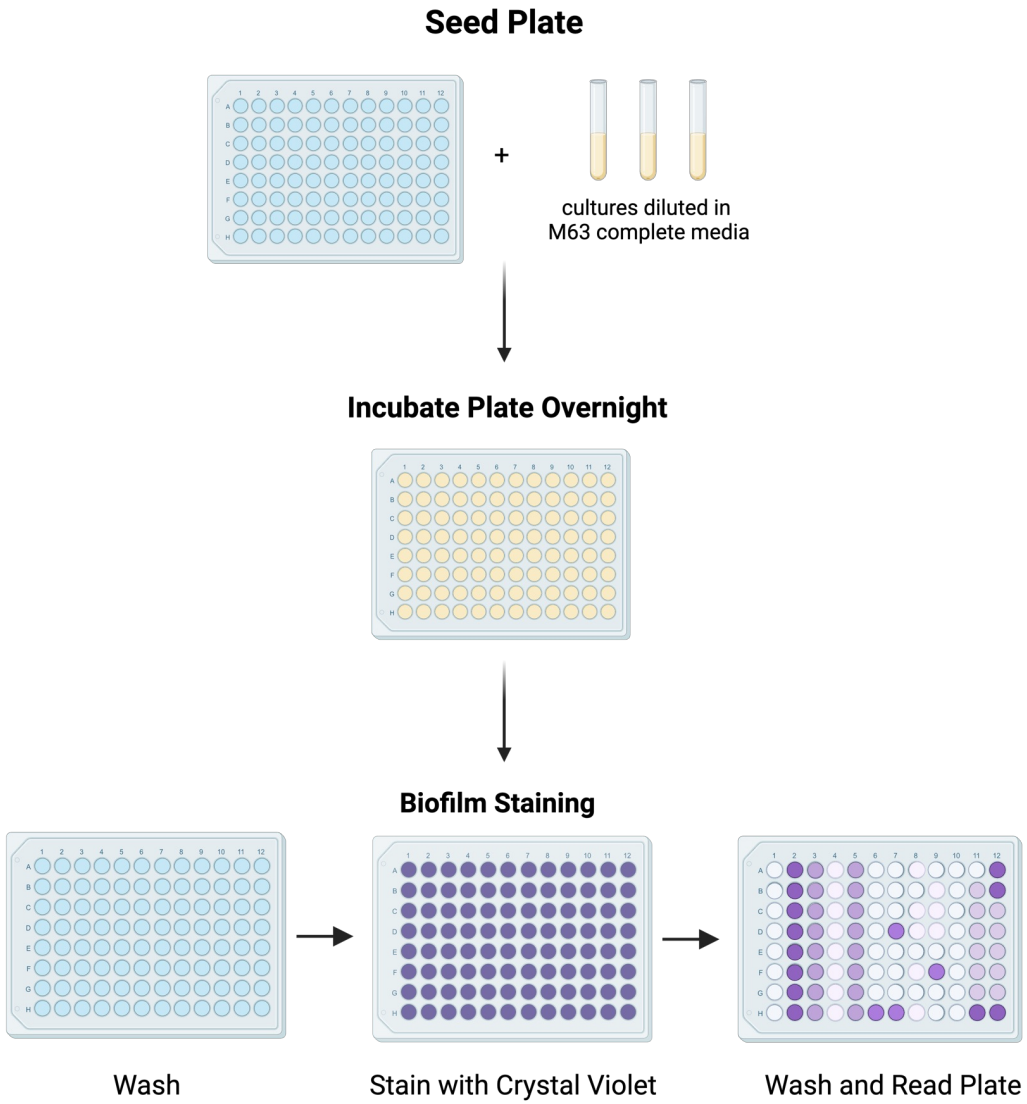
Rendueles. Molecular Microbiology, 2020.

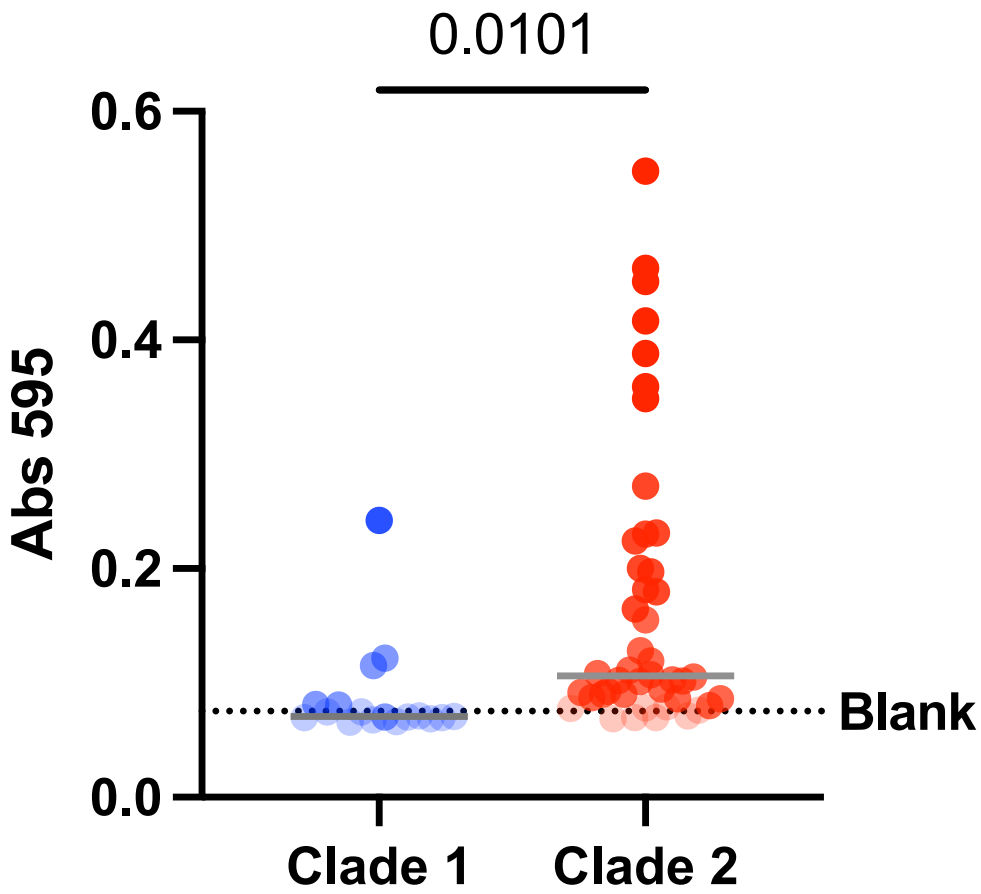


7.9% difference in capsule quantity

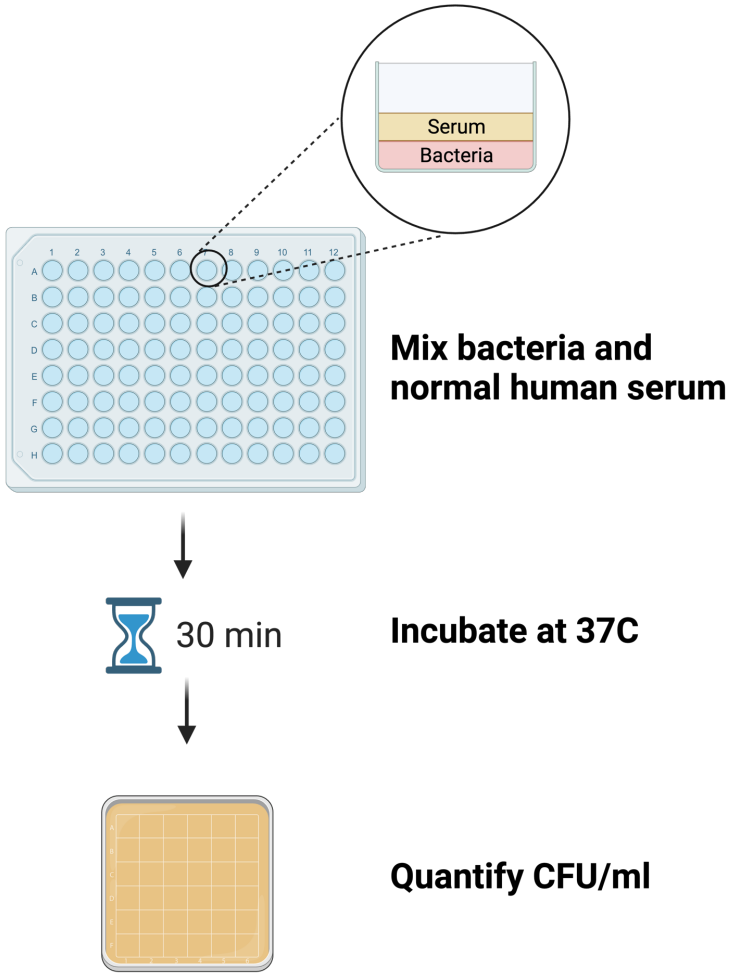
Sugar Composition	DVT2103 Clade 1 (KL106)	DVT2105 Clade 2 (KL107)
Glucose + Glucuronic Acid	86.1%	-
Galactose + Galacturonic Acid	13.9%	33.8%
Rhamnose	-	66.2%

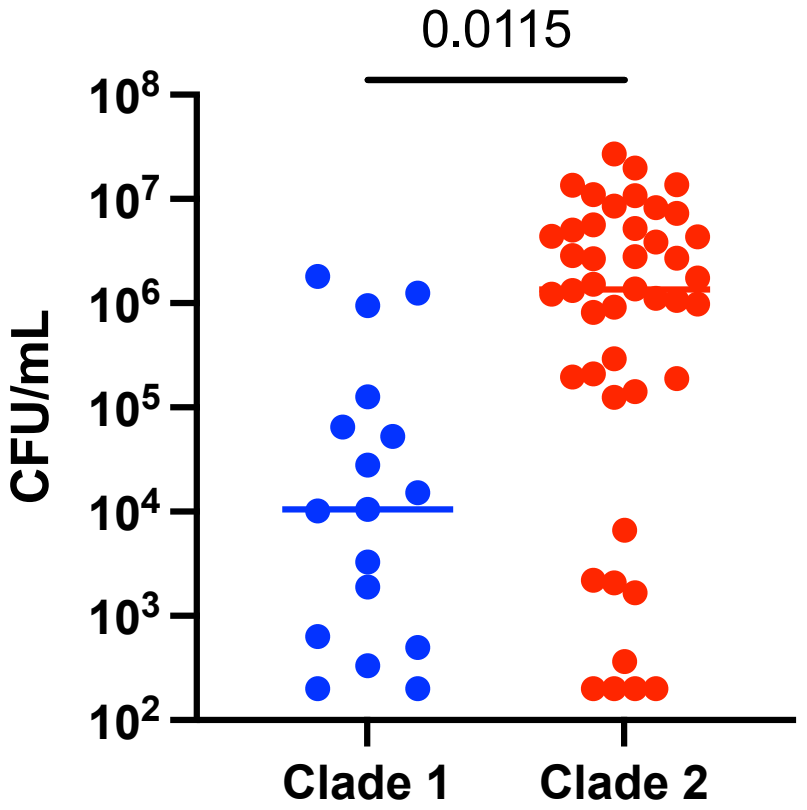
More Clade 2 isolates form biofilm than Clade 1 isolates



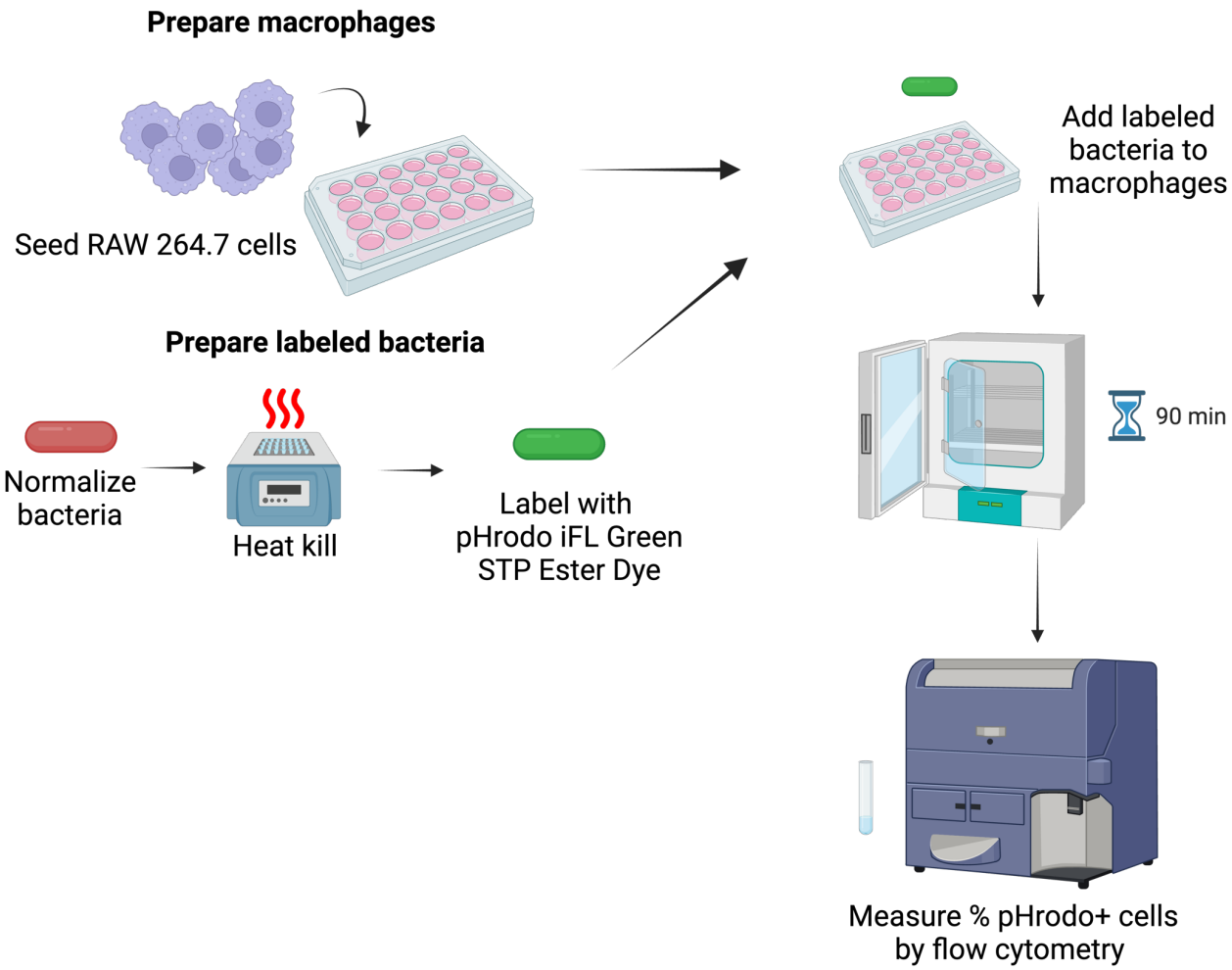


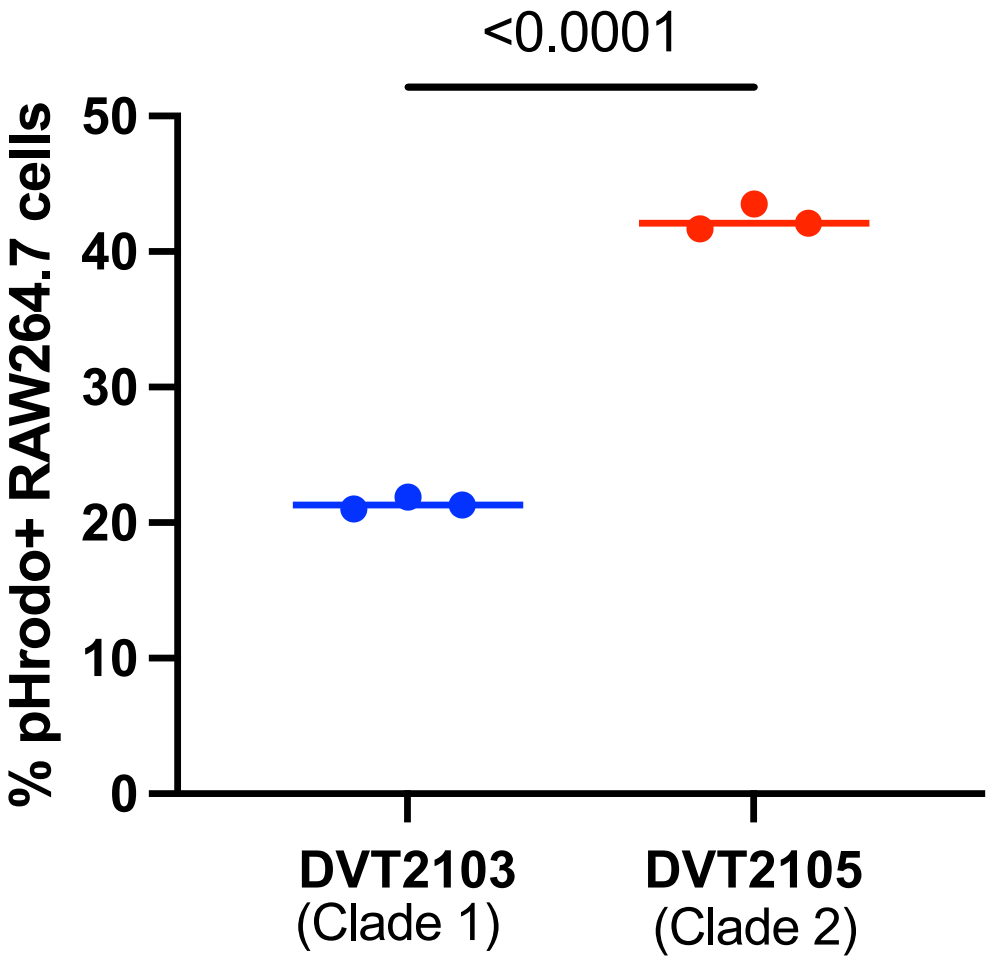
Clade 2 is more resistant to killing by serum than Clade 1



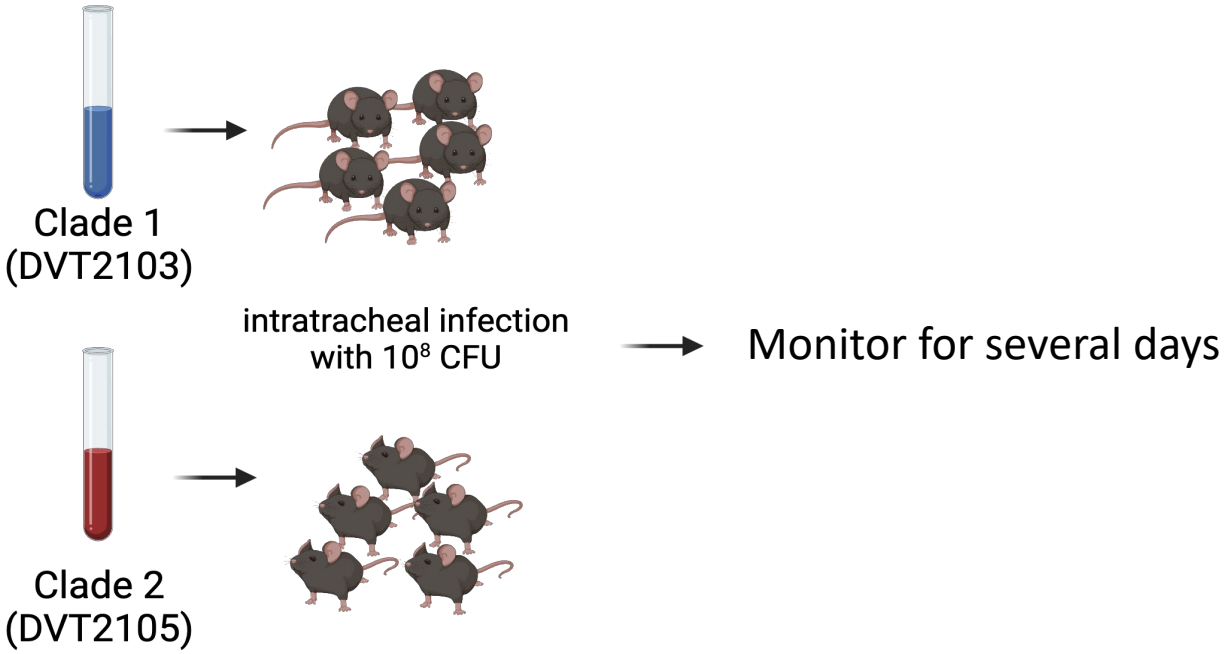


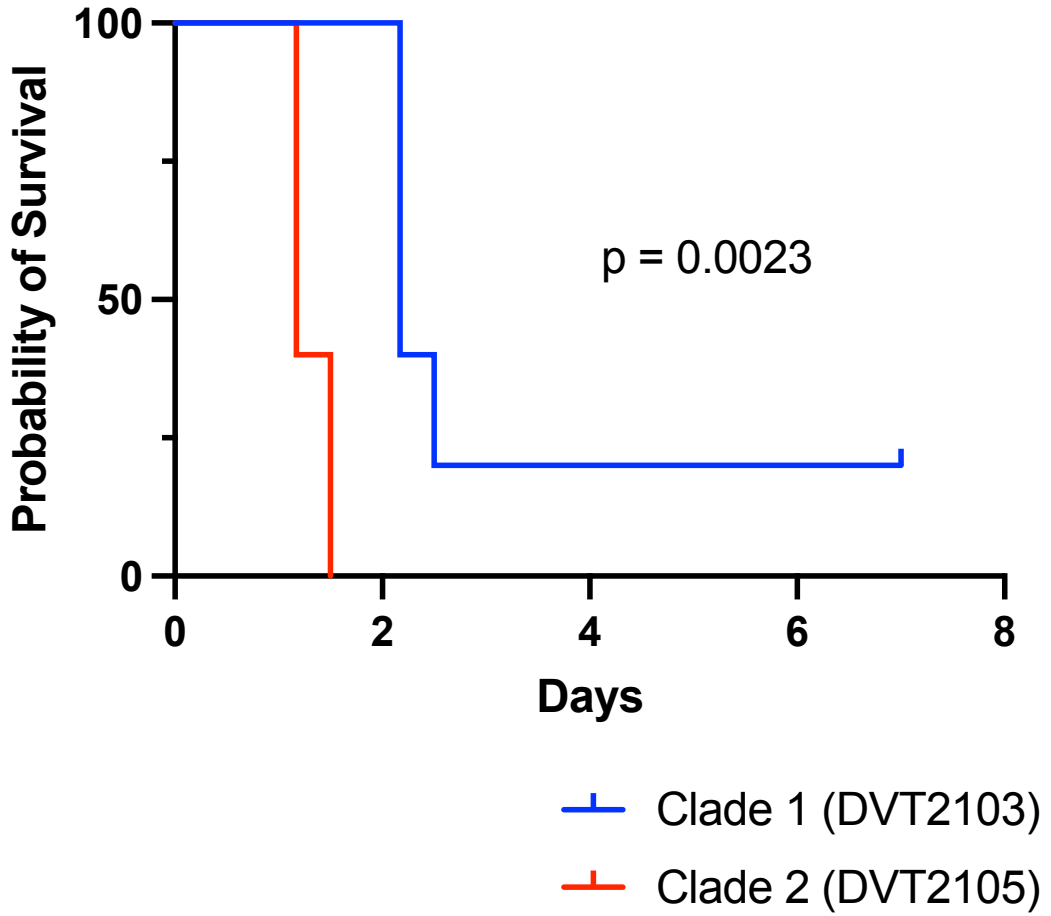
A Clade 2 isolate is phagocytosed more than a Clade 1 isolate





A Clade 2 isolate is more virulent in a pneumonia model than a Clade 1 isolate





Summary

	KP ST258 Clade 1	KP ST258 Clade 2

Future Directions

- Do the differences in capsule type mean that the two clades also have differences in metabolism?
- What role does rhamnose specifically play in the characteristics of Clade 2?
- If the capsules are swapped between the two clades, how does that impact their virulence?
- Are there differences in clinical outcomes between infections with Clade 1 vs Clade 2?
- Why is Clade 2 more resistant to killing by serum, yet more susceptible to phagocytosis?

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