Phenotypic characterization of *Kingella kingae* isolates recovered from patients with endocarditis

Szirina Ismail[2], Daniel P. Morreale[1,2], Eric A. Porsch[2] and Joseph W. St. Geme III[1,2]

[1]Department of Microbiology, University of Pennsylvania, Philadelphia, PA

[2]Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia PA

Abstract

Kingella kingae is increasingly recognized as a leading cause of osteomyelitis, septic arthritis, and bacteremia among children between 6 months and four years of age. *K. kingae* has also been identified as an important cause of endocarditis among young children. *K. kingae* is commonly found in the oropharynx and can transition from a state of commensalism to invasive disease through the help of various colonization and virulence factors. The main factors are type IV pili, an adhesin called Knh, an RTX family pore forming toxin called RtxA, a polysaccharide capsule, and a galactan exopolysaccharide. Type IV pili and Knh facilitate bacterial adherence to the epithelium. The RtxA toxin is capable of lysing host cells. The surface polysaccharides aid in evasion of the immune system. Endocarditis is a severe clinical manifestation of *K. kingae* with a mortality rate of 16%. In this study we examined a collection of 12 isolates from patients with endocarditis to characterize their virulence determinants. We found that capsule was expressed in all strains, and the type a capsule predominates in the *K. kingae* endocarditis isolates. Twitching motility and adherence are type IV pili mediated phenotypes. Our results demonstrated that half of the strains show defects in twitching motility. Adherence to epithelial cells was variable, but most isolates demonstrated high levels of adherence. Lastly, we found that the *pamC1* allele, which encodes a type 1 galactan exopolysaccharide structure, is the dominant form in *K. kingae* endocarditis strains. Our results serve as a starting point for understanding how these endocarditis strains differ phenotypically from other *K. kingae* strains, and in the long-term will help us find novel ways to treat or prevent infective endocarditis caused by *K. kingae.*

Introduction

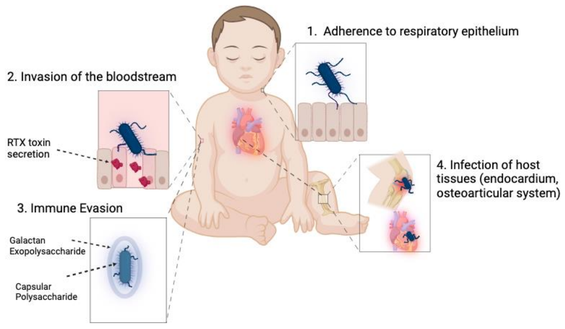

Figure 1: *K. kingae* pathogenesis. For *K. kingae* to produce invasive disease, it must first adhere to the respiratory epithelium to colonize the oropharynx. The bacterium must then breach the respiratory epithelial barrier and enter the bloodstream, which is mediated by the RtxA cytotoxin. As dissemination to sites of invasive disease occurs, the organism must be able to survive in the bloodstream, facilitated by the polysaccharide capsule and the galactan exopolysaccharide.

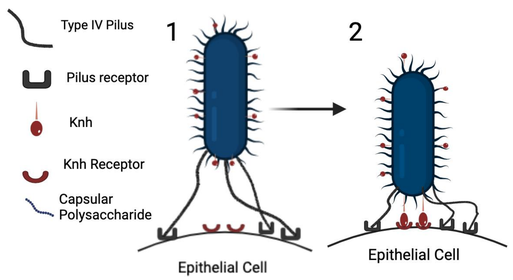

Figure 2: *K. kingae* adherence model. *K. kingae's* adherence to the respiratory epithelium is mediated by type IV pili and Knh and influenced by the polysaccharide capsule. Once pili have engaged their host cell receptor, pilus retraction pulls the bacterium into close contact with the host cell membrane. This action results in physical displacement of the polysaccharide capsule, exposing Knh to the host cell membrane. Unmasked Knh can then mediate high-affinity *K. kingae* interactions with human epithelial cells.

Central question

Are *K. kingae* isolates recovered from patients with endocarditis more virulent than other strains of *K. kingae?*

All endocarditis isolates express capsule

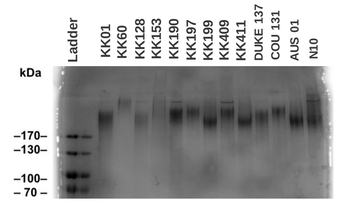

Figure 3. Capsule expression Gel. Bacteria were resuspended to an OD_{600} 0.8 in PBS, and capsules were extracted by incubating at 56 °C for 30 mins to disassociate the capsule from the bacteria. Extracted capsules were visualized by Alcian Blue staining after SDS-Page electrophoresis. This gel confirmed that all the endocarditis isolates expressed capsule. There are four structurally distinct capsule types (type a, b, c, d) which all differ in their sugar composition. Invasive *K. kingae* isolates are much more likely to express type a or type b. For endocarditis causing strains, the type a capsule is around two times more prevalent than the type b capsule.

Capsule Types

KK01	KK60	KK128	KK153	KK190	KK197	KK199	KK409	KK411	DUKE 137	COU 131	AUS 01	N10
a	c	a	a	a	a	b	a	b	a	a	b	b

Two endocarditis isolates are capable of aggregation

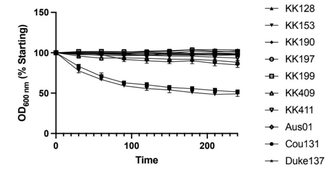

Figure 4. Aggregation Assay. Bacteria were resuspended bacteria to an OD_{600} 0.8 in heart infusion. The OD for each strain was measured every 30 mins for a 4-hour period. KK60 and KK153 displayed high levels of aggregation. Three independent biology replicates were performed.

All endocarditis isolates encode PamC1

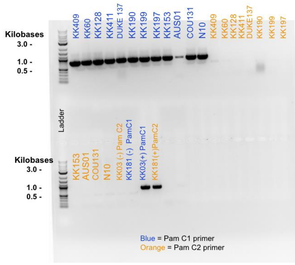

Figure 8. Exopolysaccharide type gel.
K. kingae produces an exopolysaccharide called the galactan. Due to the joint ability of the polysaccharide capsule and the exopolysaccharide to prevent opsonin deposition, *K. kingae* is able to effectively resist serum killing. The galactan exopolysaccharide is synthesized by the *pamABCDE* gene locus. In the *K. kingae* population there are two *pamC* alleles. Strains that contain the *pamC1* allele express the type 1 galactan, and strains that contain the *pamC2* allele express the type 2 galactan. To determine whether the *K. kingae* endocarditis isolates contain *pamC1* or *pamC2. W*e conducted a PCR analysis and found that all the strains contain *pamC1*. Strain KK03 was used as a *pamc1* positive control and KK181 was used as *pamc2* positive control.

Endocarditis isolates have variable twitching motility

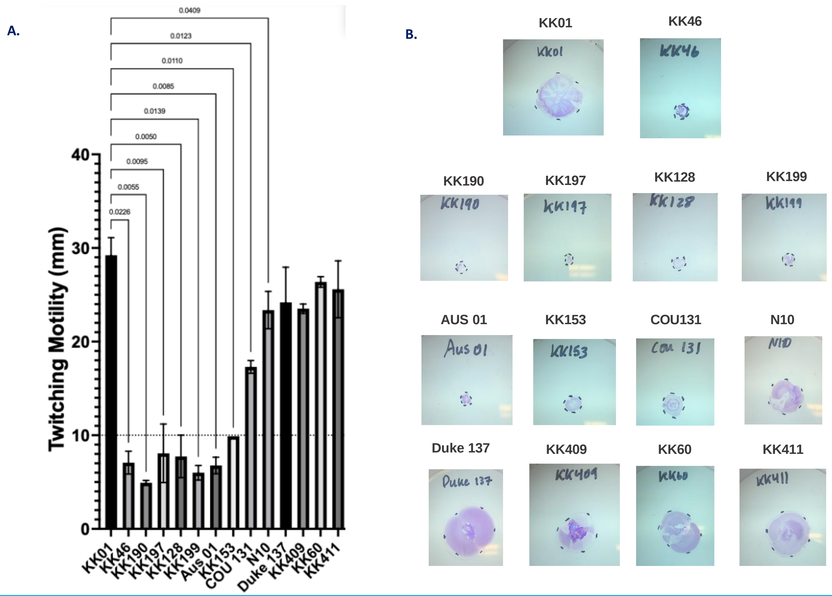

Figure 5 (A): Twitching motility assay. Bacteria were resuspended to OD_{600} 0.8 in 3 mL of PBS. A modified agar plate stab assay was performed. Twitching-zone diameters were measured in triplicate and averages were calculated from three independent experiments. The positive control is KK01, which expresses pili and can twitch. The negative control is KK46 (KK01 *ΔpilA1*), which does not express pili and thus cannot twitch. Any strain which was near or below the twitching motility of KK46, was considered not twitching. **(B) Twitching motility plates.** In order to quantify the twitching motility of each strain, twitching diameter measurements were taken from three different angles, then averaged to find the twitching motility for that plate. Shown are the twitching motility plates for all the isolates.

Endocarditis isolates are capable of variable adherence to epithelial cells

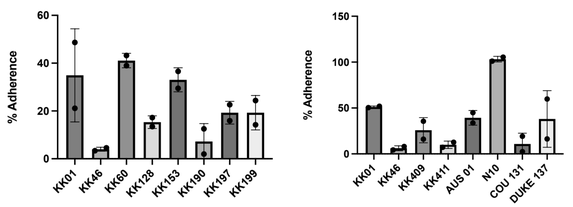

Figure 6. Quantitative adherence assay using Detroit-562 cells. Bacteria were resuspended to an OD_{600} 0.8 in BHI. Detroit-562 cells were fixed and infected with bacteria. Inocula were plated on chocolate agar and incubated at 37 °C. Adherent bacteria were released from the wells, diluted depending on their adherence level, then plated on chocolate agar and incubated at 37 °C. Shown is the graph of percent adherence for all strains, including the positive control KK01, and the negative control KK46. Two independent biological replicates were performed.

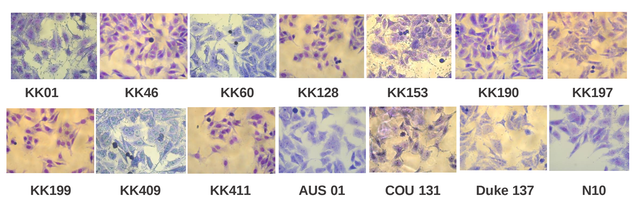

Figure 7. Chang cell Giemsa adherence assay. Bacteria were resuspended to an OD_{600} 0.8 in BHI. Chang cell monolayers were fixed and infected with bacteria. Wells were then Giemsa stained, and cover slips were mounted on a slide and imaged at 400X. Shown in this figure are images of the adherence slides for all the strains, including the positive control KK01, and the negative control, KK46.

Conclusions

- All isolates contain the *pamC1* allele, indicating they express the type 1 galactan exopolysaccharide structure.
- All isolates express capsule, and 2/3 express the type a capsule.
- Half of the isolates showed defects in twitching motility.
- Adherence to epithelial cells was highly variable, ranging from >10% of bacterial cells adhering to the monolayer, to >90% of bacterial cells adhering to the monolayer.
- Most isolates did not aggregate, but two strains, KK60 and KK153, displayed very high levels of aggregation.

Future directions

- Piliation status is correlated with adherence and twitching motility. Given the variability in adherence and twitching motility, we will also examine piliation and Knh expression.
- We plan to continue assessing adherence by conducting a quantitative adherence assay using Chang cells, as well as determining adherence to other cell lines such as endothelial cells and cells from the osteoarticular system.
- The RtxA toxin is necessary for epithelial barrier breach. We will conduct hemolysis assays to quantify the level of toxin expressed in these strains.

Acknowledgements

Thank you to Taylor Yount, Valeria Vigo, Kevin Hernandez, and Eva Agostino for their help and support in this project.

References

•Muñoz VL, Porsch EA, St Geme JW 3rd. *Kingella kingae* Surface Polysaccharides Promote Resistance to Human Serum and Virulence in a Juvenile Rat Model. Infect Immun. 2018, May 22.
•Porsch EA, Kehl-Fie TE, St Geme JW 3rd. Modulation of *Kingella kingae* adherence to human epithelial cells by type IV Pili, capsule, and a novel trimeric autotransporter. 2012, Oct 23.
•Starr KF, Porsch EA, Seed PC, Heiss C, Naran R, Forsberg LS, Amit U, Yagupsky P, Azadi P, St Geme JW 3rd. *Kingella kingae* Expresses Four Structurally Distinct Polysaccharide Capsules That Differ in Their Correlation with Invasive Disease. 2016, Oct 19.