Supplemental Material

Supplemental Methods

The Multi-site Gram-negative Surveillance Initiative (MuGSI) is a part of the Centers for Disease Control and Prevention's Emerging Infections Program (https://www.cdc.gov/hai/eip/mugsi.html). MuGSI performs active population and laboratory-based surveillance for carbapenem-resistant Enterobacteriaceae isolated from sterile sites and urine, covering more than 15 million people in 8 states. Surveillance is performed in selected counties in the following states: Colorado, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee. One of the objectives of MuGSI is to describe the population-based incidence of carbapenem-resistant *Klebsiella pneumoniae*.

K. pneumoniae isolates collected through MuGSI between 2012 and 2015 were streaked out on blood agar plates (TSA with 5% sheep blood; Thermo Sci Remel). String test was performed with a 1μL inoculation loop on a single colony. A positive string test result was greater than 5mm in length.

For murine infections, C57BL/6 mice were infected intraperitoneally with 10^6 CFU of *K. pneumoniae* strains (CDC98 or 3 different ST258 strains isolated from the New York MuGSI site). Mice were provided with food and water *ad libitum* and monitored for signs of illness or weight loss. Mice exhibiting weight loss to below 80% starting weight were euthanized.

Antibiotic susceptibility testing was performed using the MicroScan WalkAway 96 plus® (Beckman Coulter, Inc., Brea, CA) and Neg Breakpoint Combo Panel Type 44. S, susceptible; I, intermediate; R, resistant.

Table S1. Antibiotic susceptibility profile of CDC98.

Antibiotic	Minimum Inhibitory Concentration (MIC)
Amikacin	I (32µg/mL)
Ampicillin/Sulbactam	R (≥32µg/mL)
Aztreonam	R (≥64µg/mL)
Cefazolin	R (≥64µg/mL)
Cefoxitin	R (16µg/mL)
Cefepime	R (4µg/mL)
Ceftazidime	R (≥64µg/mL)
Ceftriaxone	R (16µg/mL)
Gentamicin	S (≤1µg/mL)
Ertapenem	R (2µg/mL)
Levofloxacin	R (≥8µg/mL)
Meropenem	R (≥16µg/mL)
Piperacillin/Tazobactam	R (≥128µg/mL)
Tetracycline	S (2µg/mL)
Tigecycline	S (1µg/mL)
Tobramycin	R (≥16µg/mL)
Nitrofurantoin	R (256µg/mL)
Trimethoprim/Sulfamethoxazole	R (≥320µg/mL)

Table S2. Phenotypic and genetic traits of CR-hvKP strains CDC98 and K1.

	CDC98	K1
Origin	United States	China
Sequence type	ST258	ST11
Capsular type	K107	K47
Hypermucoviscous	+	+
pLVPKs	-	+
rmpA	-	-
rmpA2	-	+
Aerobactin receptor (*iutA)*	+ [c]	+ [p]
Salmochelin Receptor (*iroN)*	+ [c]	-
Carbapenemase	KPC-3 [c]	KPC-2 [p]

c – chromosomal, p – plasmid.

Supplemental Figure Legends

Figure S1. CDC98 is string test positive (>5mm).

Figure S2. Quantification of CDC98 string test results. The string test was performed by 6 independent, blinded testers on CDC98 as well as the indicated control, non-hypermucoviscous strains. Dotted line denotes 5 mm which is the cutoff for string test positivity.

Supplemental Movie Legend

Supplemental Movie S1. String test of CDC98.

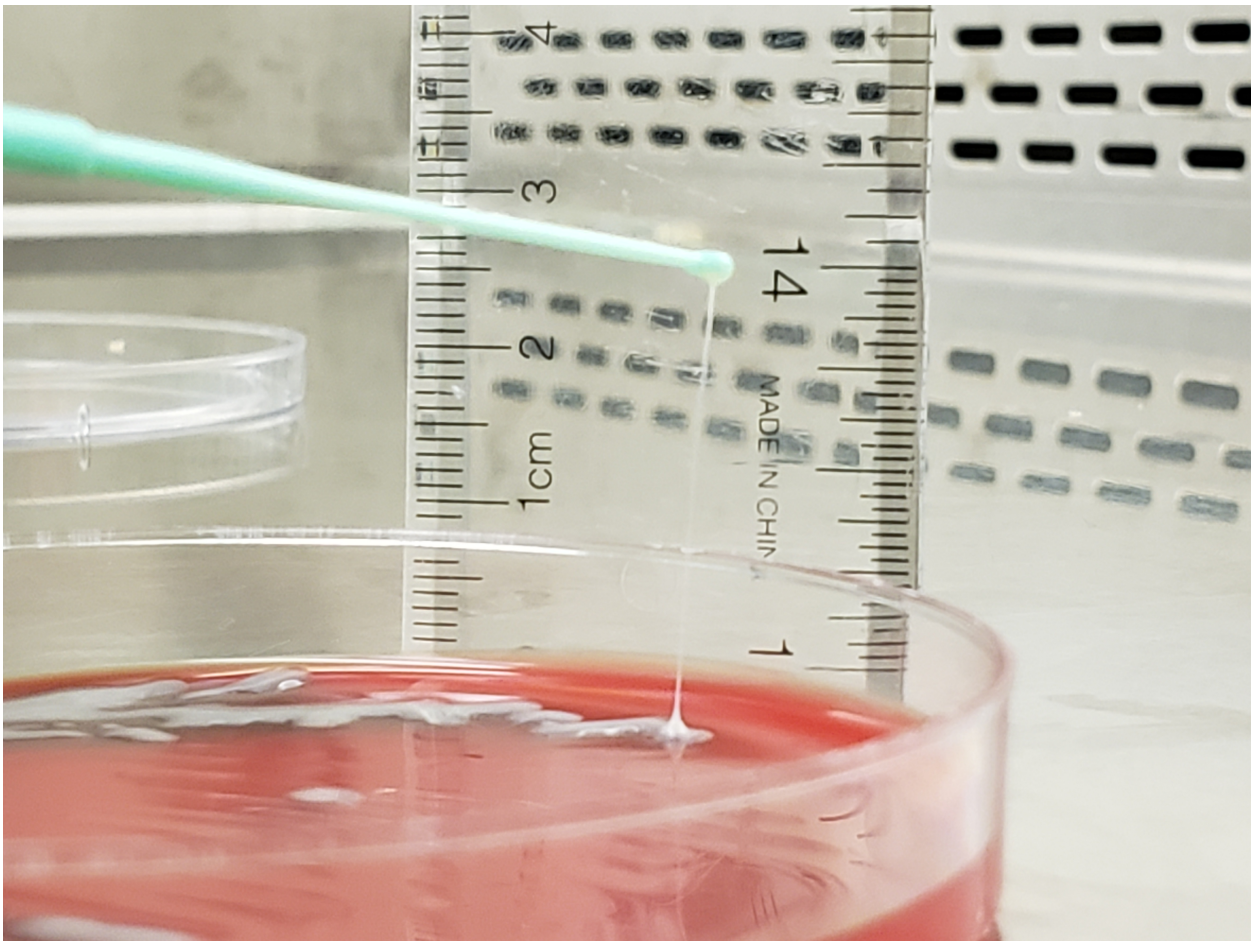

Figure S1. CDC98 is string test positive (>5mm).

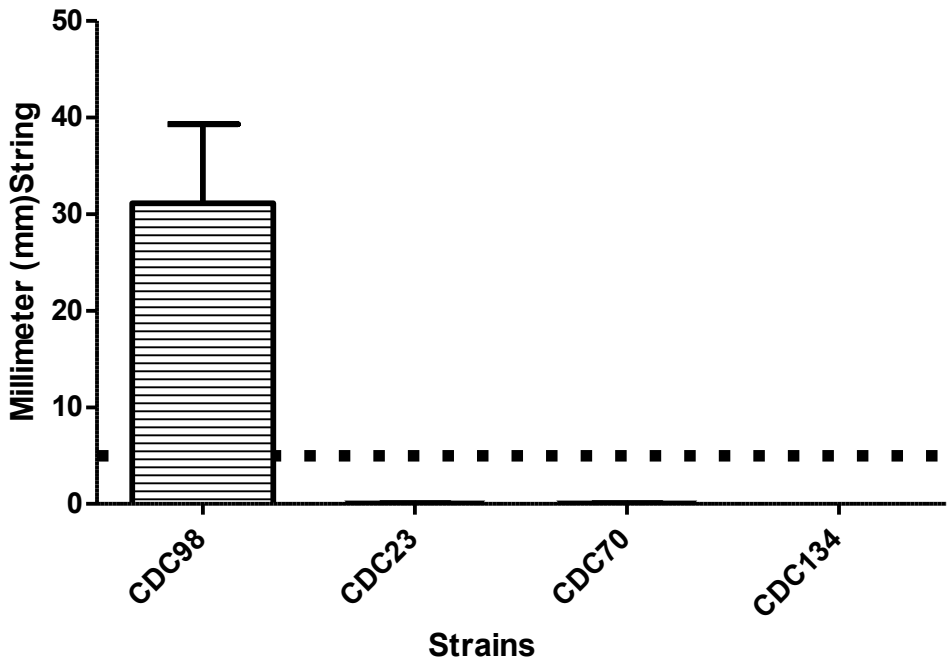

Figure S2. Quantification of CDC98 string test results. The string test was performed by 6 independent, blinded testers on CDC98 as well as the indicated control, non-hypermucoviscous strains. Dotted line denotes 5 mm which is the cutoff for string test positivity.