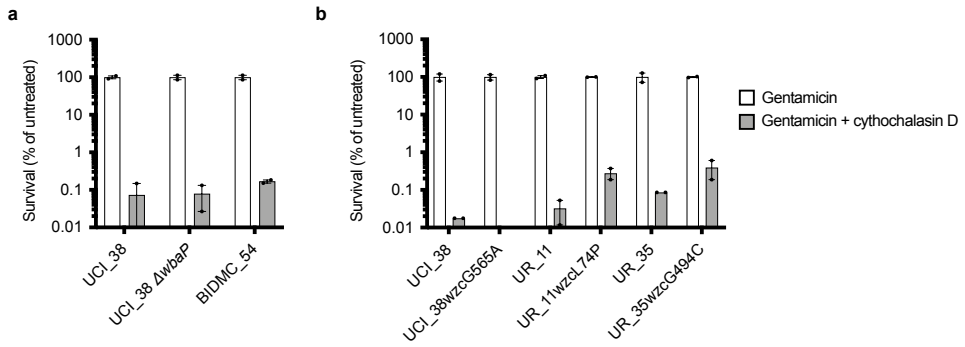

Supplementary Figure 1 | Cytochalasin D pretreatment of bladder epithelial cells and macrophages substantially reduces the recovery of clinical *K. pneumoniae* isolates from gentamicin and amikacin protection assays. 5637 Bladder epithelial cells and J774 macrophages were pretreated with cytochalasin D (1 μg/ml) for 30 minutes prior to infection with gentamicin and amikacin-susceptible *K. pneumoniae* isolates and mutants that had been used in gentamicin protection assays in this study. **a**, Cytochalasin D pretreatment substantially reduces the recovery of all tested *K. pneumoniae* strains from bladder epithelial cells in the gentamicin protection assay, confirming that gentamicin was able to effectively kill extracellular clinical isolates. **b**, Cytochalasin D pretreatment substantially reduces the recovery of *K. pneumoniae* from J774 macrophages in the gentamicin protection assay, confirming that gentamicin was able to effectively kill extracellular clinical isolates. Of note amikacin was used instead of gentamicin in the case of UR_35 because of gentamicin resistance. For a and b, the mean and SEM of n=2 independent experiments is shown.

Strain	Isolation site	ST	Clade	Collection	Isolation date	Mutation	*wbaP* truncation	Mucoidity	Bisample ID
UCI_61	urine	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN02581267
UCI_22	sputum	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN02356602
MGH_71	urine	258	Clade 1	Boston Prospective	Jun-13	*wbaP:ISKpn26*	359-383	hypomucoid	SAMN02581244
MGH_51	urine	258	Clade 1	Boston Prospective	Feb-13	*wbaP:ISKpn26*	331-383	hypomucoid	SAMN02581383
UCI_19	urine	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN02356599
UCI_63	wound	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN02581269
UCI_13	urine	258	Clade 1	Irvine Prospective	2012			mucoid	SAMN02138618
UCI_21	rectal	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN02356601
UCI_59	wound	258	Clade 1	Irvine Prospective	2013			mucoid	SAMN0258126
BIDMC_18B	urine	258	Clade 1	Boston Historical	Dec-10			mucoid	SAMN02138643
BIDMC_18C	urine	258	Clade 1	Boston Historical	Nov-10			mucoid	SAMN02138644
BIDMC_18A	urine	258	Clade 1	Boston Historical	May-12	*wzi → wcaH (deletion)*		hypomucoid	SAMN02138642
BIDMC_45	urine	258	Clade 1	Boston Prospective	Feb-13			mucoid	SAMN02356594
BIDMC_34	urine	258	Clade 1	Boston Prospective	Sep-12	*wzi → wzc (deletion)*		hypomucoid	SAMN02138665
MGH_29	respiratory	258	Clade 1	Boston Prospective	Sep-12			mucoid	SAMN02138569
BWH_22	urine	258	Clade 1	Boston Historical	Jun-12			mucoid	SAMN02138591
MGH_45	urine	1084	Clade 1	Boston Prospective	Oct-12	*act:ISKpn26*		mucoid	SAMN02138585
UCI_33	urine	258	Clade 2	Irvine Prospective	2013			mucoid	SAMN02356613
UCI_55	wound	258	Clade 2	Irvine Prospective	2013			mucoid	SAMN02581261
UCI_43	urine	258	Clade 2	Irvine Prospective	2013	*wbaP:IS1294 wzc:IS10R*	238-475	hypomucoid	SAMN02356623
UCI_44	urine	258	Clade 2	Irvine Prospective	2013	*wbaP:IS1294 wzc:IS10R*	238-475	hypomucoid	SAMN02356624
UCI_41	urine	258	Clade 2	Irvine Prospective	2013			mucoid	SAMN02356621
UCI_37	**urine**	**258**	**Clade 2**	**Irvine Prospective**	**2013**	***wbaP:IS1294***	**409-475**	**hypomucoid**	SAMN02356617
UCI_38	**urine**	**258**	**Clade 2**	**Irvine Prospective**	**2013**			mucoid	SAMN02356618
UCI_67	sputum	258	Clade 2	Irvine Prospective	2013	*ugp*Y502C		mucoid	SAMN02581273
UCI_1	sputum	258	Clade 2	Irvine Prospective	2012	*ugp*Y502C		mucoid	SAMN02138606
BIDMC_10	foot culture	258	Clade 2	Boston Historical	2009			mucoid	SAMN02138631
BIDMC_54	**urine**	**258**	**Clade 2**	**Boston Prospective**	**Jun-13**	***galF* → GTF1 *(deletion, IS1294)***		**hypomucoid**	SAMN02581275
BIDMC_42B	blood	258	Clade 2	Boston Prospective	Dec-12			mucoid	SAMN02356580
BIDMC_42A	blood	258	Clade 2	Boston Prospective	Dec-12			mucoid	SAMN02356579
BWH_36	bal	258	Clade 2	Boston Prospective	Sep-12			mucoid	SAMN02138599
BIDMC_7A	urine	258	Clade 2	Boston Historical	Apr-12			mucoid	SAMN02138627
BIDMC_7B	urine	258	Clade 2	Boston Historical	Apr-12			mucoid	SAMN02138628
BIDMC_13	**blood**	**258**	**Clade 2**	**Boston Historical**	**Nov-09**	**wzcG565S (GGT->AGT)**		**hypermucoid**	SAMN02138636
BIDMC_2A	blood	258	Clade 2	Boston Historical	May-10			mucoid	SAMN02138621
BIDMC_16	**blood**	**258**	**Clade 2**	**Boston Historical**	**Nov-09**	**wzcG565S (GGT->AGT)**		**hypermucoid**	SAMN02138639
BIDMC_5	blood	258	Clade 2	Boston Historical	Jul-08			mucoid	SAMN02138625
BIDMC_4	tissue, abdomen	258	Clade 2	Boston Historical	Sep-09			mucoid	SAMN02138624
BIDMC_32	**abscess**	**258**	**Clade 2**	**Boston Prospective**	**Aug-12**	***wzc*G565S(GGT->AGT);T567A (ACC->GCC)**		mucoid	SAMN02138662

BIDMC_12A	sputum	258	Clade 2	Boston Historical	Apr-12	*gnd*V407A		mucoid	SAMN02138633
BIDMC_12C	endotrachael	258	Clade 2	Boston Historical	May-12	*gnd*V407A		mucoid	SAMN02138635
BIDMC_12B	blood	258	Clade 2	Boston Historical	Apr-10	*gnd*V407A		mucoid	SAMN02138634
BWH_41	urine	258	Clade 2	Boston Prospective	Sep-12	*wbaP:ISKpn26; wzyC90R*	302-475	hypomucoid	SAMN02138603
BIDMC_1	bone tissue, toe	258	Clade 2	Boston Historical	Aug-12	*wzy*C90R		mucoid	SAMN02138620
MGH_59	urine	258	Clade 2	Boston Prospective	Apr-13	*wzy*C90R		mucoid	SAMN02581391
MGH_67	pleural tissue	258	Clade 2	Boston Prospective	May-13	*galF*A64E		mucoid	SAMN02581240
BIDMC_60	peritoneal fluid	258	Clade 2	Boston Prospective	Aug-13	*galF*A64E		mucoid	SAMN02581281
BIDMC_68	blood	258	Clade 2	Boston Prospective	Sep-13	*wbaP:ISKpn26; galFA64E*	279-475	hypomucoid	SAMN02581289
BWH_45	tissue	258	Clade 2	Boston Prospective	Sep-13			mucoid	SAMN02581255
BWH_47	tissue	258	Clade 2	Boston Prospective	Sep-13	*ugp*I37V		mucoid	SAMN02581301
BIDMC_14	urine	258	Clade 2	Boston Historical	Nov-09	*wbaP:ISKpn26*	445-475	hypomucoid	SAMN02138637
MGH_73	wound deep sinus	258	Clade 2	Boston Prospective	Jun-13	*wbaP (deletion)*	141-475	hypomucoid	SAMN02581246
MGH_31	tissue	258	Clade 2	Boston Prospective	Sep-12			mucoid	SAMN02138571
MGH_79	urine	258	Clade 2	Boston Prospective	Jul-13	*gtf2*G219V		mucoid	SAMN02581252

Supplementary Table 1 | Mutations in the capsule biosynthesis genes of clinical ST258 isolates obtained from the previously reported collection[13] are associated with hyper- and hypomucoidity. Strains investigated in this study are highlighted in bold and with a grey background. All clinical isolates are sorted in phylogenetic order according to a recent phylogenetic analysis [13]. Isolation site, date and collection type (prospective or historical) are indicated. Non-synonymous mutations and the resulting amino acid exchanges or deletions are indicated. Hospitals in which the strains were isolated are indicated in the strain name: BIDMC, Beth Israel Deaconess Medical Center (Boston, MA); BWH, Brigham and Woman's Hospital (Boston, MA); MGH, Massachusetts General Hospital (Boston, MA); UCI, University of California, Irvine, Medical Center (Irvine, CA). The genomes were all recently characterized[13] and are available in the NCBI database.

Strain	Yersiniabactin	YbST	Aerobactin	AbST	Salmochelin	SmST	rmpA	rmpA2
Kleb_pneu_BIDMC_10_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_12A_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_12B_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_12C_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_13_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_14_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_16_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_18A_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_18B_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_18C_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_1_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_2A_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_32_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_34_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_42a_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_42b_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_45_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_4_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_54_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_5_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_60_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_68_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_7A_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BIDMC_7B_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BWH_22_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BWH_36_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BWH_41_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_BWH_45_V1.genome	ybt 17; ICEKp10	247	-	0	-	0	-	-
Kleb_pneu_BWH_47_V1.genome	ybt 17; ICEKp10	251	-	0	-	0	-	-
Kleb_pneu_MGH_29_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_31_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_45_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_51_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_59_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_67_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_71_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_MGH_73_V1.genome	ybt 17; ICEKp10	251	-	0	-	0	-	-
Kleb_pneu_MGH_79_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_13_V1.genome	-	0	-	0	-	0	-	-

Kleb_pneu_UCI_19_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_1_V1.genome	ybt 17; ICEKp10	272	-	0	-	0	-	-
Kleb_pneu_UCI_21_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_22_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_33_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_37_V1.genome	ybt 17; ICEKp10	266	-	0	-	0	-	-
Kleb_pneu_UCI_38_V1.genome	ybt 17; ICEKp10	267	-	0	-	0	-	-
Kleb_pneu_UCI_41_V1.genome	ybt 17; ICEKp10	267	-	0	-	0	-	-
Kleb_pneu_UCI_43_V1.genome	ybt 17; ICEKp10	264	-	0	-	0	-	-
Kleb_pneu_UCI_44_V1.genome	ybt 17; ICEKp10	264	-	0	-	0	-	-
Kleb_pneu_UCI_55_V1.genome	ybt 17; ICEKp10	268	-	0	-	0	-	-
Kleb_pneu_UCI_59_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_61_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_63_V1.genome	-	0	-	0	-	0	-	-
Kleb_pneu_UCI_67_V1.genome	-	0	-	0	-	0	-	-

Supplementary Table 2 | The previously reported collection of ST258 isolates[13] does not include isolates harboring the *rmpA/A2* and aerobactin genes. Other virulence factors found in hypervirulent strains are also listed (the siderophores yersiniabactin and salmochelin). The presence of colibactin correlated with the presence of yersiniabactin. All strains shown were recently screened for the presence of hypervirulence genes in a recent study on the global prevalence of hypervirulence-associated genes[12]. Integrative conjugative element (ICE) and yersiniabactin (ybt) type and the resulting sequence type (YbST) indicated.

Source	Isolation site	Total CFUs	*wzc* mutation	% Hypercapsule
Mouse	Abscess 1	$5x10^4$ / ml	G565R	7
Mouse	Abscess 2	$3x10^4$ / ml	A535E	3

Supplementary Table 3 | *wzc* mutants emerge from mice infected with UCI_38. Hypercapsule mutants were isolated from 2 out of 13 abdominal abscesses formed in the outer peritoneum and abdominal wall of 25 transurethrally infected TLR4-deficient mice (C3H/HeJ), which were used as hosts because they prolonged infections caused by UCI_38 (Extended Data Fig. 4f). The hypercapsule mutants could be easily identified by their hypermucoid colony morphology and a positive string-test. At least two colonies were analyzed for the presence of mutations in *wzc*. The first abscess (abscess 1) harbored a subpopulation of hypercapsule mutants that represented 7% of the total abscess population and contained the same amino acid position in *wzc* (G565R) that occurred in the hypervirulent, clinical isolate BIDMC_13. The other abscess (abscess 2) harbored a subpopulation of hypercapsule mutants that represented 3% of the total abscess population and contained a A535E mutation in *wzc* (which we also have confirmed to be hypercapsule conferring, Extended Data Figs 3b,c). The mutant populations could not be detected in the inoculum of 10^7 wild-type UCI_38 by visual inspection of at least 500 colonies grown from the inoculum (detection limit 0.2%), and by deep sequencing of *wzc*, suggesting that the mutants had at the very least been positively selected during the infection and possibly generated *in vivo*.

Strain	Biosample	Geographic location	Isolation_source	Collection_date	MLST	KPC	Wzc mutation
BIDMC 13	SAMN02138636	USA: Boston, MA	**Blood**	2009	ST-258	blaKPC-3	G565S
BIDMC 16	SAMN02138639	USA: Boston, MA	**Blood**	2009	ST-258	blaKPC-3	G565S
BIDMC 32	SAMN02138662	USA: Boston, MA	Abscess	2012	ST-258	blaKPC-3	G565S
CHS124	SAMN03280267	USA: North Carolina	Drainage	2014	ST-258	blaKPC-3	L74H
CHS133	SAMN03280276	USA: North Carolina	Rectal swab	2014	ST-258	blaKPC-3	L74H
CHS135	SAMN03280278	USA: North Carolina	Rectal swab	2014	ST-258	blaKPC-3	L74H
CHS142	SAMN03280285	USA: North Carolina	Urine	2014	ST-258	blaKPC-3	G565A
CHS147	SAMN03280289	USA: North Carolina	Rectal swab	2014	ST-258	blaKPC-3	L74H
CRK0067	SAMN06812607	USA: Ohio	**Blood**	2013	ST-258	blaKPC-3	G565C
EuSCAPE_IT005	SAMEA3538566	Italy	Urine	2013	ST-512	blaKPC-3	L74P
EuSCAPE_IT024	SAMEA3538585	Italy	Urine	2013	ST-512	blaKPC-3	G565D
EuSCAPE_IT096	SAMEA3729931	Italy	**Blood**	2013	ST-512	blaKPC-3	G565C
KP04C62	SAMN05730643	Italy: Turin	**Blood**	2011	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_07	SAMN06335643	Switzerland:Geneva	**Blood**	2015	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_08	SAMN06293083	Switzerland:Geneva	Hematoma	2015	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_09	SAMN06293086	Switzerland:Geneva	Urine	2015	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_10	SAMN06293090	Switzerland:Geneva	Feces	2015	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_11	SAMN06293102	Switzerland:Geneva	Feces	2015	ST-512	blaKPC-3	L74P
KPN_KPC_HUG_13	SAMN06293116	Switzerland:Geneva	**Blood**	2015	ST-512	blaKPC-3	L74P
MNCRE53	SAMN06067844	USA:Minnesota	Wound	2016	ST-258	blaKPC-2	L74H
30660/NJST258_1	SAMN03081502	USA	Urine	2010	ST-258	blaKPC-3	A531G
39911	SAMN06218077	USA	**Blood**	2013	ST-258	blaKPC-3	P533T
41959	SAMN06218063	USA	**Blood**	2013	ST-258	blaKPC-3	P533T
44817	SAMN06218033	USA	**Blood**	2013	ST-258	blaKPC-3	A531P
44830	SAMN06218119	USA	**Blood**	2013	ST-258	blaKPC-3	DelY703-G704
45708	SAMN06218113	USA	**Blood**	2013	ST-258	blaKPC-3	DelY703-G704
ATCC BAA-1705	SAMN02471470		Urine		ST-258	blaKPC-2	T538A
CHS 08	SAMN02581310	USA: North Carolina	Urine	2013	ST-258	blaKPC-3	Y714L
CHS 80	SAMN02581382	USA: North Carolina	Urine	2013	ST-258	blaKPC-3	A531G
CHS123	SAMN03280266	USA		2014	ST-258	blaKPC-3	S532N
CHS98	SAMN03280241	USA		2014	ST-258	blaKPC-3	A531G
CRK0008	SAMN06812666	USA: Unknown	**Blood**	2016	ST-258	blaKPC-3	A531V
CRK0013	SAMN06812661	USA: Michigan	**Blood**	2014	ST-258	blaKPC-3	A531V
CRK0104	SAMN05956150	USA: North Carolina	Urine	2014	ST-258	blaKPC-3	P533S
CRK0168	SAMN05956086	USA: Ohio	Urine	2013	ST-258	blaKPC-3	Y714H
CRK0174	SAMN05956080	USA: Ohio	Urine	2012	ST-258	blaKPC-3	Y714H
CRK0211	SAMN08579700	USA: Ohio	Urine	2013	ST-258	blaKPC-3	Y714H
EuSCAPE_IL011	SAMEA3729925	Israel	Urine	2013	ST-512	blaKPC-3	D561V
EuSCAPE_IL069	SAMEA3729926	Israel	**Blood**	2013	ST-512	blaKPC-3	D559E

EuSCAPE_IL079	SAMEA3538536	Israel	Urine	2014	ST-512	blaKPC-3	P533R

Supplementary Table 4 | Hypercapsule mutants have emerged repeatedly in ST258 clade 2 and are geographically diverse based on the NCBI database. 966 genomes were analyzed for the presence of *wzc* mutations. *wzc* SNPs occurring in amino acid positions that were confirmed to confer a hypercapsule in this study are highlighted in red. Mutations, occurring in close proximity to confirmed hypercapsule conferring mutations (Extended Data Table 3b), in essential regulatory elements of Wzc (Walker A and extended Walker A' site, and the tyrosine cluster, Extended Data Figure 3e) are highlighted in yellow. Hypercapsule mutants found in outbreaks are highlighted in grey. A *wzc*L74P mutant caused an outbreak at a hospital in Geneva (Switzerland) [31], while a L74H mutant caused an outbreak in North Carolina (USA)[32]. ST512 is a single locus variant of ST258 clade 2.

Strain	Biosample	Geographic location	Isolation source	Collection date	MLST	KPC	WbaP	Protein Coordinates of Reference
CRK0256	SAMN08579655	USA: Ohio	Urine	2012	ST-258	blaKPC-2	0	
CRK0163	SAMN05956091	USA: Michigan	Urine	2013	ST-258	blaKPC-3	0	
BIDMC 54	SAMN02581275	USA: Boston	Urine	2013	ST-258	blaKPC-3	0	
kpneu042	SAMEA4916114	Switzerland		2010	ST-512	blaKPC-3	0	8-51,53-473
kpneu056	SAMEA4916131	Switzerland		2012	ST-512	Not detected	0	8-476
kpneu001	SAMEA4916025	Switzerland		2013	ST-512	blaKPC-3	0	7-87,94-418,424-476
OC217	SAMN04075791	USA: Chicago	Blood	2010	ST-258	blaKPC-3	0	39-476
EuSCAPE_IT086	SAMEA3515115	Italy	Wound	2013	ST-512	blaKPC-3	0	27-476
	SAMN06917607	Italy	Urine	2012	ST-512	blaKPC-3	0	11-476
CRK0008	SAMN06812666	USA	Blood	2016	ST-258	blaKPC-3	0	14-476
CRK0047	SAMN06812627	USA: Ohio	Blood	2013	ST-258	blaKPC-3	0	14-476
EuSCAPE_IT335	SAMEA3538703	Italy	Urine	2013	ST-512	blaKPC-3	0	207-476
MGH153	SAMN04521902	USA: Boston	Urine	2015	ST-258	Not detected	0	181-476
kpneu026	SAMEA4916078	Switzerland		2012	ST-512	blaKPC-3	0	7-418,357-450,450-476
kpneu024	SAMEA4916075	Switzerland		2013	ST-512	blaKPC-3	0	7-87,94-418,421-476
EuSCAPE_IT030	SAMEA3538591	Italy	Urine	2013	ST-512	blaKPC-3	0	21-476
MNCRE82	SAMN06107577	USA:MN	Urine	2013	ST-258	blaKPC-2	0	27-249,309-476
kpneu051	SAMEA4916126	Switzerland		2011	ST-258	blaKPC-19	0	8-51,53-75,75-287,288-450,449-476
1520(3-B)	SAMN05301552	USA	BAL	2015/2016	ST-258	blaKPC-31	28	1-28,31-476
	SAMN06917643	Italy	Rectal swab	2013	ST-512	blaKPC-3	29	1-29,33-476
kpneu054	SAMEA4916129	Switzerland		2014	ST-512	blaKPC-3	34	1-34,31-375,370-387,387-476
PEC-KPC	SAMN09269478	Spain: Madrid		2013	ST-258	blaKPC-3	66	1-66,65-476
UHKPC69	SAMN02142026				ST-258	blaKPC-3	77	1-77,80-476
kpneu053	SAMEA4916128	Switzerland		2013	ST-512	blaKPC-3	87	1-87,94-450,450-476
kpneu017	SAMEA4916064	Switzerland		2013	ST-258	blaKPC-3	87	1-87,94-476
1010363	SAMN10252239	Italy		2017	ST-512	blaKPC-3	91	1-91,88-476
kpneu049	SAMEA4916124	Switzerland		2014	ST-512	blaKPC-3	106	1-106,94-418,424-476
MNCRE64	SAMN06107581	USA:MN	Wound	2012	ST-258	blaKPC-2	120	1-120
EuSCAPE_IT022	SAMEA3538583	Italy	Respiratory Tract	2013	ST-512	blaKPC-3	131	1-131,131-476
CRK0189	SAMN05956065	USA: Ohio	Urine	2012	ST-258	blaKPC-3	131	1-131,131-476
ST258_FL	SAMN03435903	USA: Orlando	Urine	2008	ST-258	blaKPC-3	133	1-133,131-476
BWH53	SAMN03280381	USA	Sputum	2014	ST-258	blaKPC-3	141	1-141,142-476
MGH 73	SAMN02581246	USA: Boston	Wound	2013	ST-258	blaKPC-3	141	1-141,142-476
UCI 44	SAMN02356624	USA: Irvine, CA	Urine		ST-258	Not detected	237	1-237,235-476
UCI 43	SAMN02356623	USA: Irvine, CA	Urine		ST-258	Not detected	269	1-269,235-476
BIDMC 68	SAMN02581289	USA: Boston	Blood	2013	ST-258	blaKPC-2	281	1-281,278-476
BWH 41	SAMN02138603		Urine		ST-258	Not detected	301	1-301,300-476
EuSCAPE_IT124	SAMEA3515153	Italy	Blood	2013	ST-512	blaKPC-3	340	1-340,339-476
KpVA-7	SAMEA3108423			2011	ST-258	blaKPC-3	340	1-340,340-476

kpneu039	SAMEA4916110	Switzerland		2012	ST-258	blaKPC-3	389	1-34,31-476
MNCRE74	SAMN06107587	USA:MN	Sputum	2012	ST-258	blaKPC-2	391	1-391,368-476
EuSCAPE_IT318	SAMEA3538686	Italy	Other (biopsy material)	2013	ST-512	blaKPC-3	396	1-396,396-476
TUM15670	SAMD00126355	Singapore			ST-258	blaKPC-2	406	1-406,411-476
CHS139	SAMN03280282	USA	BAL	2014	ST-258	blaKPC-3	410	1-410,419-476
DG6099	SAMN08399046	Italy: Florence	Rectal swab	2016	ST-512	blaKPC-3	410	1-410,419-476
DG6684	SAMN08399047	Italy: Florence	Rectal swab	2016	ST-512	blaKPC-3	410	1-410,419-476
DG7779	SAMN08399045	Italy: Florence	Rectal swab	2016	ST-512	blaKPC-3	410	1-410,419-476
CHS163	SAMN03280304	USA	Urine	2014	ST-258	blaKPC-3	410	1-410,419-476
UCI 37	SAMN02356617	USA: Irvine, CA	Urine		ST-258	blaKPC-3	412	1-412,384-476
AR_0012	SAMN04014853				ST-258	Not detected	418	1-418,421-476
kpneu004	SAMEA4916032	Switzerland		2014	ST-512	blaKPC-3	418	1-418,421-476
EuSCAPE_IT126	SAMEA3515155	Italy	Urine	2013	ST-512	blaKPC-3	419	1-419,419-476
kpneu020	SAMEA4916070	Switzerland		2014	ST-512	blaKPC-3	419	1-476
BIDMC 14	SAMN02138637		Urine		ST-258	blaKPC-3	444	1-444,444-476
	SAMN06917611	Italy	Perianal swab	2012	ST-512	blaKPC-3	463	1-463
MNCRE80	SAMN06107576	USA:MN	Urine	2013	ST-258	blaKPC-2	471	1-471

Supplementary Table 5 | 966 genomes were analyzed for the presence of *wbaP* truncations and deletions. The length of the identified WbaP protein is shown in grey (based on open reading frame), as well as the WbaP coordinates of the reference sequence. ST512 is a single locus variant of ST258 clade 2.

	wzc SNP	wild type *wzc*
Blood	16	176
Other	16	402
Total	32	578
% Blood	**50%**	**30.45%**

P=0.0299

	wbap mutation	wild type *wbaP*
Urine	18	231
Other	11	350
Total	29	581
% Urine	**62%**	**39.80%**

P=0.020

Supplementary Table 6 | Capsule mutants are clinically associated with infection sites. ST258/512 genomes with known infection sites (610 of 966, n=610) were analyzed for associations of hypercapsule mutants with bloodstream infections and capsule-deficient mutants with urinary tract infections. Significance was calculated with a Fisher's exact test. 28 infection-unrelated specimens (rectal swabs, feces, cutaneous swabs and handrail) were excluded from the analysis.

Sample	Time from patient to initial testing	Maximum time at room temperature	% Mutants	Total CFUs at time of mutant detection	Mutant CFUs	Doubling time of normal capsule producing isolates in urine (minutes)	Doubling time of capsule mutants in urine (minutes)	Doubling time of normal capsule producing isolates in LB (minutes)	Doubling time of capsule mutants in LB (minutes)	Days since last detection from UTI
UR_1	0:05	3:07								
UR_2	1:24	8:01								
UR_3	0:14	6:32								
UR_4		7:09								
UR_5	0:12	3:19	99	$1.69x10^8$	$1.67x10^8$	52	65 (hyper), 71 (hypo)	47	42 (hyper), 43 (hypo)	83
UR_6	0:15	9:14								
UR_7	2:22	5:03								
UR_9	2:11	3:51								
UR_10	0:17	1:36								
UR_11	0:26	4:59	1	$3.1x10^7$	$3.1x10^5$	52	58	39	39	233
UR_12	0:51	1:02								
UR_13	1:28	4:19								
UR_14	1:28	4:11								
UR_15	0:25	7:16								
UR_35	1:24	7:43	46	$1.25x10^8$	$5.75x10^7$	52	51	42	38	7
UR_37	1:21	2:28								
UR_41	1:45	4:32								
UR_42	0:06	5:47								
UR_43	4:47	6:42								
UR_44	0:02	5:52								
UR_45	6:49	7:51								
UR_46	0:08	0:40								
UR_47	0:03	1:41								
UR_48	0:19	5:03								
UR_50	0:06	1:02								
UR_53	0:04	6:33								
UR_54	0:04	5:40								
UR_55	1:16	5:04								
UR_56	0:11	0:19								
UR_57	0:15	2:58								

Supplementary Table 7 | Capsule mutant subpopulations detected in patient specimens emerged *in vivo.* Specimens with capsule mutant subpopulations are highlighted in red. The specimens were initially analyzed for colony forming units (CFUs) and antibiotic susceptibility, as part of the routine diagnostic procedures at the Brigham and Women's Hospital in Boston. Every specimen harbored more than 100,000 bacteria per ml at the time of initial testing. The time from specimen isolation to initial plating for CFUs is indicated, as well as the maximum amount of time (hours) that the specimens were stored at room temperature (see Methods for more details). After the identification of *K. pneumoniae* and the detection of <100,000 CFUs, the specimens were analyzed for the presence of mutants (the percentage of mutant CFUs and overall CFU numbers detected in the specimen are shown). The capacity of the capsule mutants and the reference strains (normal capsule producing isolates from the specimens) to grow in urine from a healthy donor, as well in LB medium at room temperature (24 ºC) was determined (doubling time is shown and based on log growth phase). The capsule mutants did not display a growth advantage in urine or in LB medium, which taken together with the presence of at least 100,000 bacteria shortly after isolation from the patient excluded the possibility that the mutants could have emerged in the specimen. The patients with capsule mutant subpopulations all had a recurring UTI (days since the detection of *K. pneumoniae* isolates displaying the same antibiotic resistance profiles in urine specimens shown).

Culture ID	Recurrence (days)	Leukocytes	Catheterization	Notables
UR_15	270; 21	22	no	History of UTIs starting at young age
UR_47		122	62 days prior to isolation for 1 week	Mixed Gram-negative flora detected 14 days prior to UTI
UR_53		140	yes (51 days)	

Supplementary Table 8 | The capsule-deficient mutants from the prospective collection of urine specimens caused acute symptomatic UTIs. Days of potential recurrent infection based on prior isolation of an isolate with the same antibiotic susceptibility profile shown. The presence and number of leukocytes detected in the urinalysis is shown. UR_15 and UR_47 caused symptomatic infection, while no symptoms were noted for UR_53.

Class	Antibiotic	UR_1	UR_2	UR_3	UR_4	UR_5*	UR_6	UR_7	UR_9*	UR_10	UR_11*	UR_12	UR_13	UR_14
Carbapenems	Meropenem	<= 0.25	<= 0.25	<= 0.25	<= 0.25	>= 16	<= 0.25	<= 0.25	<= 0.25	<= 0.25	<= 0.25	<= 0.25	<= 0.25	<= 0.25
	Ertapenem	<= 0.5	<= 0.5	<= 0.5	<= 0.5		<= 0.5	<= 0.5	<= 0.5	<= 0.5	<= 0.5	<= 0.5	<= 0.5	<= 0.5
Cephalosporins	Ceftriaxone	<= 1	<= 1	<= 1	<= 1	>= 64	<= 1	<= 1	>= 64	<= 1	>= 64	<= 1	>= 64	<= 1
	Ceftazidime	<= 1	<= 1	<= 1	<= 1	>= 64	<= 1	<= 1	16	<= 1	>= 64	<= 1	4	<= 1
	Cefazolin	<= 4	<= 4	<= 4	<= 4	>= 64	<= 4	<= 4	>= 64	<= 4	>= 64	<= 4	<= 4	<= 4
	Cefepime	<= 1	<= 1	<= 1	<= 1	>= 64	<= 1	<= 1	32	<= 1	8	<= 1	2	<= 1
Penicillins + beta-lactamase inhibitors	Amoxicillin/Clavulanate	4	4	<= 2	<= 2	>= 32	<= 2	<= 2	>= 32	<= 2	>= 32	<= 2	4	<= 2
	Piperacillin/Tazobactam	<= 4	8	8	<= 4	>= 128	<= 4	<= 4	32	<= 4	>= 128	<= 4	<= 4	<= 4
Fluoroquinolones	Ciprofloxacin	<= 0.25	0.5	<= 0.25	<= 0.25	>= 4	<= 0.25	<= 0.25	>= 4	<= 0.25	>= 4	<= 0.25	<= 0.25	<= 0.25
	Levofloxacin	<=0.12	1	<=0.12	<=0.12	>= 8	<=0.12	<=0.12	>= 8	<=0.12	>= 8	<=0.12	<=0.12	<=0.12
Aminoglycosides	Amikacin	<= 2	<= 2	<= 2	<= 2	>= 64	<= 2	<= 2	4	<= 2	>= 64	<= 2	<= 2	<= 2
	Gentamicin	<= 1	<= 1	<= 1	<= 1	>= 16	<= 1	<= 1	>= 16	<= 1	<= 1	<= 1	<= 1	<= 1
	Tobramycin	<= 1	<= 1	<= 1	<= 1	>= 16	<= 1	<= 1	>= 16	<= 1	>= 16	<= 1	<= 1	<= 1
Tetracyclines	Tetracycline	<= 1	4	<= 1	<= 1	>= 16	<= 1	<= 1	>= 16	<= 1	>= 16	<= 1	>= 16	2
Nitrofuran	Nitrofurantoin	128	256	64	64	>= 512	32	<=16	128	64	256	64	64	64
Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole	>=320	<= 20	<= 20	<= 20	>=320	<= 20	<= 20	>=320	<= 20	>=320	<= 20	>=320	<= 20

Class	Antibiotic	UR_15	UR_35*	UR_37	UR_41	UR_42*	UR_43	UR_44	UR_45	UR_46*	UR_47	UR_48	UR_50	UR_53
Carbapenems	Meropenem	<= 0.25	<= 0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25	<=0.25
	Ertapenem	<= 0.5	<= 0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Cephalosporins	Ceftriaxone	<=1	>= 64	<=1	<=1	>= 64	<=1	<=1	<=1	>= 64	<=1	<=1	<=1	<=1
	Ceftazidime	<=1	8	<=1	<=1	16	<=1	<=1	<=1	16	<=1	<=1	<=1	<=1
	Cefazolin	<=4	>= 64	<=4	<=4	>= 64	<=4	<=4	<=4	>= 64	<=4	<=4	<=4	<=4
	Cefepime	<=1	2	<=1	<=1	4	<=1	<=1	<=1	2	<=1	<=1	<=1	<=1
Penicillins + beta-lactamase inhibitors	Amoxicillin/Clavulanate	8	16	<=2	<=2	16	<=2	<=2	<=2	16	4	<=2	<=2	<=2
	Piperacillin/Tazobactam	16	16	8	<=4	16	<=4	<=4	<=4	32	64	<=4	<=4	<=4
Fluoroquinolones	Ciprofloxacin	>= 4	>= 4	<=0.25	<=0.25	>= 4	<=0.25	<=0.25	<=0.25	>= 4	<=0.25	<=0.25	<=0.25	<=0.25
	Levofloxacin	>= 8	>= 8	<=0.12	<=0.12	>= 8	<=0.12	<=0.12	<=0.12	>= 8	<=0.12	<=0.12	<=0.12	<=0.12
Aminoglycosides	Amikacin	<= 2	<= 2	<=2	<=2	4	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2
	Gentamicin	<=1	>= 16	<=1	<=1	>= 16	<=1	<=1	<=1	>= 16	<=1	<=1	<=1	<=1
	Tobramycin	<=1	>= 16	<=1	<=1	>= 16	<=1	<=1	<=1	8	<=1	<=1	<=1	<=1
Tetracyclines	Tetracycline	>= 16	>= 16	<=1	>= 16	<=1	<=1	2	<=1	>= 16	<=1	<=1	<=1	<=1
Nitrofuran	Nitrofurantoin	128	128	64	64	128	64	64	128	128	64	32	<=16	<=16
Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole	>=320	>=320	<=20	<=20	>=320	<=20	<=20	<=20	>=320	<=20	<=20	<=20	<=20

Class	Antibiotic	UR_54	UR_55	UR_56	UR_57
Carbapenems	Meropenem	<= 0.25	<= 0.25	<=0.25	<=0.25
	Ertapenem	<= 0.5	<= 0.5	<=0.5	<=0.5
Cephalosporins	Ceftriaxone	<=1	<=1	<=1	>= 64
	Ceftazidime	<=1	<=1	<=1	16
	Cefazolin	<=4	<=4	<=4	>= 64
	Cefepime	<=1	<=1	<=1	2
Penicillins + beta-lactamase inhibitors	Amoxicillin/Clavulanate	<=2	<=2	<=2	8
	Piperacillin/Tazobactam	<=4	<=4	<=4	<=4
Fluoroquinolones	Ciprofloxacin	<=0.25	<=0.25	<=0.25	0.5
	Levofloxacin	<=0.12	<=0.12	<=0.12	1
Aminoglycosides	Amikacin	<= 2	<= 2	<=2	<=2
	Gentamicin	<=1	<=1	<=1	<=1
	Tobramycin	<=1	<=1	<=1	<=1
Tetracyclines	Tetracycline	>= 16	<=1	<=1	>= 16
Nitrofuran	Nitrofurantoin	64	32	64	128
Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole	<=20	<=20	<=20	>=320

Supplementary Table 9 | Urine specimens with capsule mutant subpopulations are associated with multidrug-resistant infections. 30 urine specimens were prospectively collected at the Brigham and Women's Hospital in Boston in 2018. Antibiotic susceptibility of the clinical isolates was determined using the automated broth microdilution method performed on a Vitek2 instrument (Biomerieux). The minimum inhibitory concentrations (MICs) are shown. Specimens with subpopulations of capsule mutants are highlighted in yellow, specimens with homogenous capsule-deficient subpopulations are highlighted in black. MICs determined to be intermediate or fully resistant are highlighted in red. The limit of detection of clones displaying altered mucoidity is 0.2-0.5%. Multidrug-resistance (MDR) is defined as resistance (including intermediate resistance) to at least 1 agent in all but 2 or fewer classes of antibiotics (similarly to a recent definition by the CDC and ECDC[65]), as determined in UR_5, UR_9, UR_11, UR_35, UR_42 and UR_46. (indicated with an asterisk). According to this definition, 20% of the isolates are MDR (6/30). 10% (3/30) of the specimens harbored homogenous hypomucoid populations, while another 10% (3/30) of the specimens harbored heterogenous subpopulations of capsule mutants. Subpopulations of capsule mutants occured in 50 % (3/6) of the MDR specimens.

Specimen	Comparison	SNPs	# shared positions	Identity	SNPs to reference	Mutation in cps operon
UR_5	normal vs hyper	44	5145900	99.999%	133 (normal),107 (deficient)	*wzc*A539D
UR_5	normal vs deficient	43	5149727	" "	133 (normal), 106 (deficient)	*wbaP*D435V
UR_11	normal vs hyper	45	5174997	" "	803 (normal), 779 (hyper)	*wzc*L74P
UR_35	Normal vs hyper	5	5282340	" "	965 (normal), 967 (hyper)	*wzc*G494C, G713*fs*

Supplementary Table 10 | High confidence SNPs observed between normal and capsule mutant subpopulations in urine specimens. The total number of identified SNPs is shown, as well as the sequence similarity (#of shared positions, identity) of representative capsule mutants vs normal capsule producing strains from each specimen

Name	Sequence
wzc_ST258-fw	CCAAGAGCTCTCATGTTTAACTCGATTCTAGTAGTTTGTA
wzc_ST258-rv	CGTCTGGTACCTTACTTATTATCTTTATAAGAATAGCCGTAATAATTA TAGCC
wzc_UR_5-fw	CCAAGAGCTCTCATGTTTAGTACAATATTAATTGTTTGCAC
wzc_UR_5-rv	CGTCTGGTACCCTATTTTTTATCTGAATATGAATAATCG
wzc_UR_11-fw	GGTGAGCTCCTAATCCATCAAGCTGGT
wzc_UR_11-rv	GCGGGTACCCCCTCTTGGATTTCACAT
wbaP_UCI_38-fw	GAGCTCGGTACCGAGTTATATGAGAGGTCACATGGCG
wbaP_UCI_38-rv	GCATGCCTGCAGTTAATATGCCCCGTCCTTTCTTAAC

Supplementary Table 11 | Primers used to clone *wzc* genes from clinical isolates, and *wbaP*, in pBAD33.

Specimen	Total reads	ST	Reference	Identifier	Kmer similarity score	% Reads mapped to reference
UR_5 (normal)	21,161,710	14	U25	GCA_001463165	0.97	87.6
UR_5 (hyper)	19,392,932	14	U25	GCA_001463165	0.97	91.3
UR_5 (hypo)	14,173,892	14	U25	GCA_001463165	0.97	79.5
UR_11 (normal)	28133162	258	KPNIH33	GCA_000775375	0.95	90.8
UR_11 (hyper)	34604838	258	KPNIH33	GCA_000775375	0.95	91.9
UR_35 (normal)	15896215	307	MGH_43	GCA_000567685	0.94	92.0
UR_35 (hyper)	14529272	307	MGH_43	GCA_000567685	0.94	93.8

Supplementary Table 13. Reference sequences used to identify SNPs in hyper- and hypomucoid subpopulations. The total number of reads obtained for each specimen, as well as the sequence type (ST) and the identified reference sequence (based on Kmer similarity) is shown.