Supplemental Material

Pathogenic *Acinetobacter* species have a functional type I secretion system and contact-dependent inhibition systems

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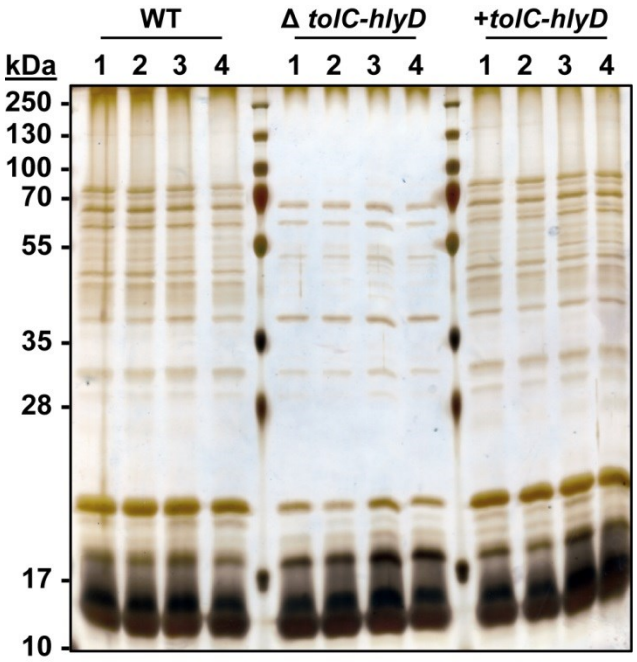

Figure S1. One dimensional SDS-PAGE analysis of secretome samples utilized for quantitative proteomics. Secreted proteins from the wildtype M2, the T1SS mutant, and the complemented strain were purified as described in the experimental methods section. A 10 microliter aliquot of each sample was then analyzed by SDS-PAGE followed by silver staining. The bottom band, likely LOS, demonstrates equal loading between each sample.

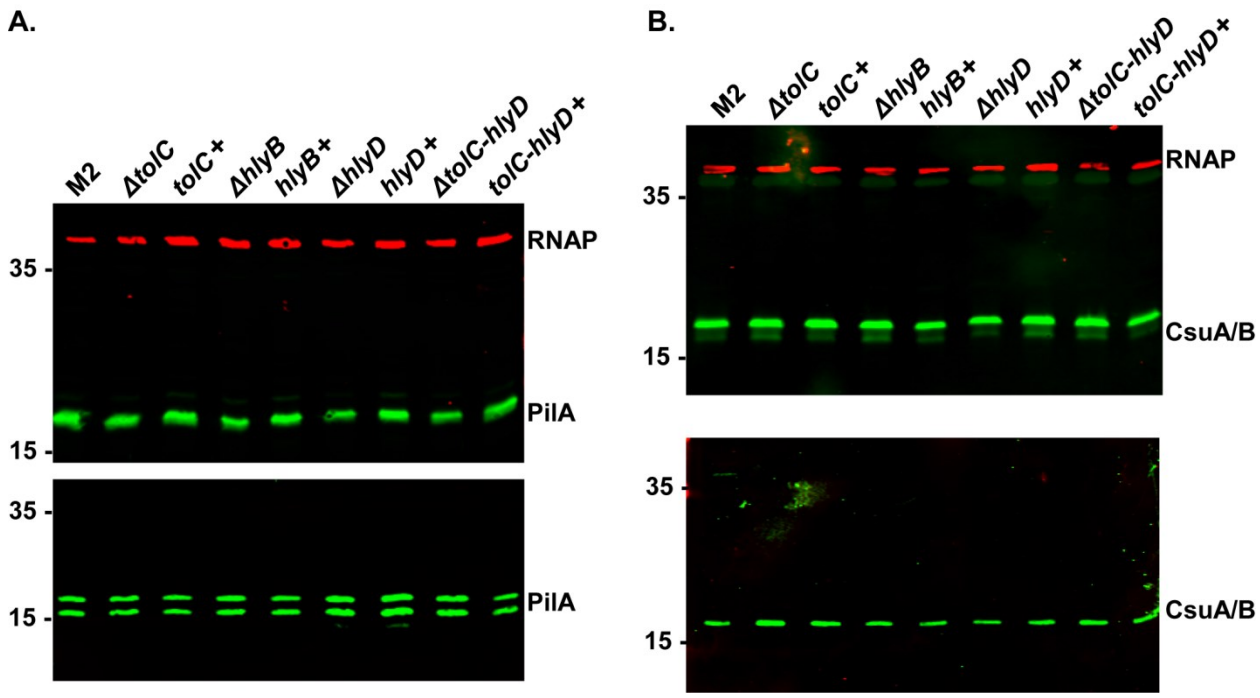

Figure S2. Western blot analysis for PilA and CsuA/B whole cell expression and surface presentation. Top Panel. Whole cell samples were analyzed by western blot for both RNA polymerase and PilA (A) and CsuA/B (B) expression. All strains had equal levels of expression. **Bottom panel.** Sheared protein fractions were analyzed by western blot for both RNA polymerase and PilA (A) or CsuA/B (B) expression. No cellular lysis was detected as indicated by the absence of RNA polymerase. PilA expression was found to be presented on the surface in equal amounts across strains. The presence of two PilA bands is indicative of multiple glycoforms previously characterized (1).

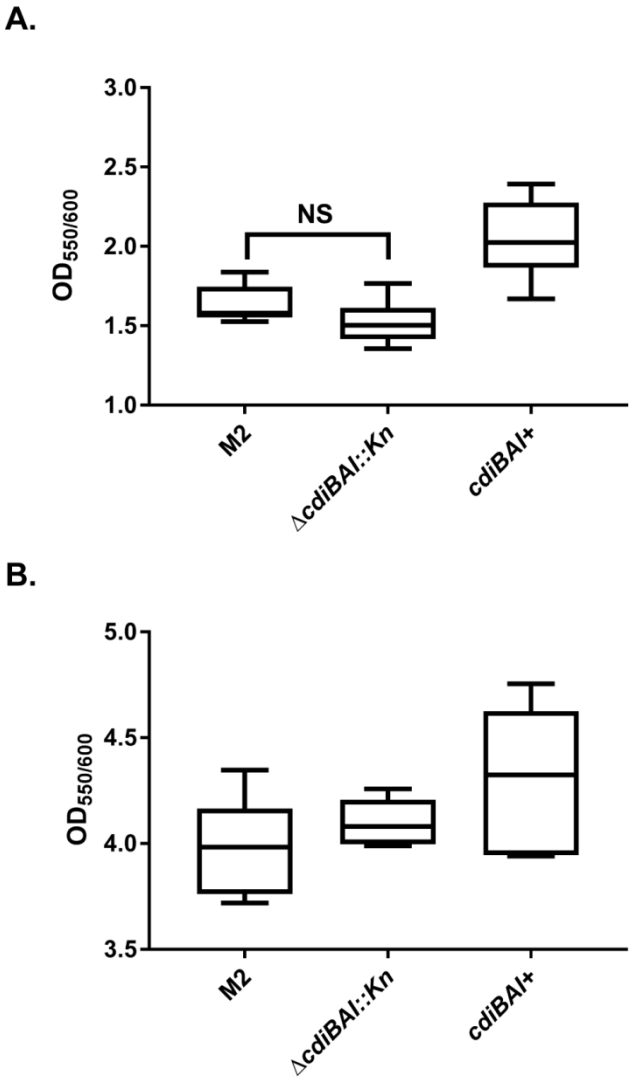

Figure S3. Biofilm analysis of M2 *cdi* full locus mutant and complemented strain. Biofilm formation was assessed by crystal violet staining. Plates were statically incubated at 37°C for 3h **(A)** or 6h **(B)**. After the designated time, an aliquot of the bacterial culture was removed for OD_{600} readings. Wells were subsequently washed and strained for 10 mins with 0.1% crystal violet. Excess crystal violet was removed by washing and solubilized by the addition of 30% acetic acid. Biofilm formation was assessed by comparing the absorbance ratio at 550nm for crystal violet and 600nm for bacteria optical density. There was no statistical difference between the parental strain and the *cdi* full locus mutant at either time points. There was a statistically significant difference between the complemented strain and both the parent and wildtype at the 3 hour time point.

S2 Table. Plasmids and bacterial strains included in the study

Plasmid or strain	Relevant characteristic(s)	Reference/ Source
Plasmids		
pKD13	Contains kanamycin resistance gene from Tn5 flanked by FRT sites	(2)
pRSM3542	pKD13 containing *kan-sacB*	(3)
pFLP2	Encodes FLP recombinase	(4)
pGEM-T-Ez	General cloning plasmid	Promega
pRSM4063	pSMART-LCkan containing an empty mTn*7* element from pRSM3510 along with 2kb flanking DNA up and downstream of the *att*Tn*7* site in *A. nosocomialis* M2	(1)
pRSM3510	pKNOCK derivative with a mini-Tn*7* element containing a multiple cloning site	(3)
pRSM3510-*tolC*	pRSM3510 containing *tolC* with expression driven from predicted *tolC* promoter	This study
pRSM3510-*hlyB*	pRSM3510 containing *hlyB* with expression driven from predicted *tolC* promoter	This study
pRSM3510-*hlyD*	pRSM3510 containing *hlyD* with expression driven from predicted *tolC* promoter	This study
pRSM3510-*tolC-hlyB-hlyD*	pRSM3510 containing *tolC-hlyB-hlyD* with expression driven from predicted *tolC* promoter	This study
pSH1	plasmid pSH1 was constructed by replacing the multiple cloning site and promoter of pBAVMCS with the *araC* gene, P_{BAD} promoter, and Shine-Dalgarno from pMLBAD	This study
pSH1-*cdi_1*_19606	pSH1 containing F911_RS17425	This study
pSH1-*cdi_2*_19606	pSH1 containing F911_RS14340	This study
pSH1-*cdi_1*_1225	pSH1 containing *cdiI*_1 from *A. baumannii* 1225	This study
pSH1-*cdi_2*_1225	pSH1 containing *cdiI_2* from *A. baumannii* 1225	This study
Strains		
A. nosocomialis strain M2	Metro Health Systems Clinical Isolate	(5)
M2Δ*tolC-hlyD*::kan	M2 containing a deletion of the *tolC-hlyD* genes and replacement with a kanamycin resistance cassette	This study
M2Δ*tolC-hlyD*::kan (*tolC-hlyB-hlyD*)+	M2Δ*tolC-hlyD*::kan with a mini-Tn*7* element containing the *tolC-hlyD* genes driven off the predicted *tolC* promoter	This study
M2Δ*tolC*::kan-*sacB*	M2 containing a deletion of the *tolC* gene and replacement with a kan-*sacB* cassette	This study
M2Δ*tolC*::frt	M2 containing a deletion of the *tolC* gene and replacement with an frt scar	This study
M2Δ*tolC*::frt (*tolC*)+	M2Δ*tolC*::frt with a mini-Tn*7* element containing the *tolC* gene driven off the predicted *tolC* promoter	This study
M2Δ*hlyB*::kan-*sacB*	M2 containing a deletion of the *hlyB* gene and replacement with a kan-*sacB* cassette	This study
M2Δ*hlyB*::frt	M2 containing a deletion of the *hlyB* gene and replacement with an frt scar	This study
M2Δ*hlyB*::frt (*hlyB*)+	M2Δ*hlyB*::frt with a mini-Tn*7* element containing the *hlyB* gene driven off the predicted *tolC* promoter	This study
M2Δ*hlyD*::kan-*sacB*	M2 containing a deletion of the *hlyD* gene and replacement with a kan-*sacB* cassette	This study
M2Δ*hlyD*::frt	M2 containing a deletion of the *hlyD* gene and replacement with an frt scar	This study
M2Δ*hlyD*::frt (*hlyD*)+	M2Δ*hlyD*::frt with a mini-Tn*7* element containing the	This study

	hlyD gene driven off the predicted *tolC* promoter	
M2Δ*tolC-hlyD*::kan-*sacB*	M2 containing a deletion of the *tolC-hlyD* genes and replacement with a kan-*sacB* cassette	This study
M2Δ*tolC-hlyD*::frt	M2 containing a deletion of the *tolC-hlyD* genes and replacement with an frt scar	This study
M2Δ*tolC-hlyD*::frt (*tolC-hlyD*)+	M2Δ*tolC-hlyD*::frt with a mini-Tn7 element containing the *tolC-hlyD* genes driven off the predicted *tolC* promoter	This study
M2∆*pilD*::kan	*A. baumannii* strain M2 containing a deletion of *pilD* and replacement with a kanamycin resistance cassette	(6)
M2∆*pilD*::kan (*pilD*+)	M2∆*pilD* with a mini-Tn7 element containing the *pilD* gene transcribed from its predicted promoter	(6)
M2Δ*cdiBAI*::kan-*sacB*	M2 containing a deletion of the *cdiBAI* genes and replacement with a kan-*sacB* cassette	This study
M2Δ*cdiBAI*::frt	M2 containing a deletion of the *cdiBAI* genes and replacement with an frt scar	This study
M2Δ*cdiBAI*::frt (*cdiI*)+	M2Δ*cdiBAI*::frt with a mini-Tn*7* element containing the *cdiI* gene driven off the predicted *cdiB* promoter	This study
A. baumannii 19606	Type strain	ATCC
19606Δ*cdi_1*::kan	19606 containing a deletion of genes the *cdiBAI_1* genes (F911_RS17415 to F911_RS17425) and replacement with a kan cassette	This study
19606Δ*cdi_1*::frt	19606 containing a deletion of genes the *cdiBAI* genes and replacement with an frt scar	This study
19606Δ*cdi_1*::frt_Rif^R	19606Δ*cdi_1*::frt spontaneous rifampicin resistant clone	This study
19606Δ*cdi_1*::frt_Rif^R (*cdiI_1*)+	19606Δ*cdi_1*::frt_Rif^R with pSH1-*cdiI_1*	This study
19606Δ*cdi_2*::kan	19606 containing a deletion of genes the *cdiBAI_2* genes (F911_RS14340 to F911_RS14350) and replacement with a kan cassette	This study
19606Δ*cdi_2*::frt	19606 containing a deletion of genes the *cdiBAI* genes and replacement with an frt scar	This study
19606Δ*cdi_2*::frt_Rif^R	19606Δ*cdi_1*::frt spontaneous rifampicin resistant clone	This study
19606Δ*cdi_2*::frt_Rif^R (*cdiI_2*)+	19606Δ*cdi_1*::frt_Rif^R with pSH1-*cdiI_2*	This study
E. coli DH5α	General cloning strain	Invitrogen
E. coli EC100D	General cloning strain, *pir*$^+$	Epicentre
E. coli TOP10	General cloning strain	Invitrogen
E. coli HB101(pRK2013)	Conjugation helper strain	(7)

Table S2. Primers

Primer Name	Sequence
pGEM R	CGCCATGGCGGCCGGGAGCATG
pGEM F	ACTAGTGAATTCGCGGCCGCCTGCA
5' hlyD + 15bp pGEM F	CCGGCCGCCATGGCGgttattacgtgctgaatcac
5' hlyD + 15bp kan cassette R	acggatccccggaatcatgaattatccccctt
3' hlyD + 15bp kan cassette F	cagctccagcctacagaagcattacgtgaaagata
3' hlyD + 15bp pGEM R	CGCGAATTCACTAGTcggacggtctagttgca
P1 kan sacB F	attccggggatccgtcgacc
P2 kan sacB R	tgtaggctggagctgcttcg
M2 3' hlyD R	gtcatctgctaactcctcctcaa
M2 5' hlyD F	gatgtacgtcgtgatatgggatta
M2 hlyD mut check F	gaagtttcagccgacattgg
M2 hlyD mut check R	atctcaagcggtccatgtaaacta
M2 hlyD seq 1	gaacaatccgtgaaaacctga
M2 hlyD seq 2	acagcccaacaaaaacaagtaagc
kan sacB seq 1	gatggattgcacgcaggttctc
kan sacB seq 2	aatggccgcttttctggat
M2 hlyD seq 3	tgttcgctttaatgatgatggtga
M2 hlyD seq 4	gtcgagatcggggagaact
M2 hlyD internal F	caagaattagcgatgactgaacct
M2 hlyD internal R	cgaatatctaccgttgctaccat
tolC comp F + SpeI	cgcGAGCTCgctgaaggcacaccacaag
hlyD comp R + XmaI	gcgCCCGGGcgtgaaattaatagaagcgaacct
hlyD comp F + Phos	atgagcgaacaacaacaaga
tolC out R + Phos	cagccctttcaggaaagc
5' hlyB + 15bp pGEM F	CCGGCCGCCATGGCGggctgatgcttttaagtacactaca
5' hlyB + 15bp kan R	acggatccccggaattcatggttgtacttcgaacc
3' hlyB + 15bp kan F	cagctccagcctacacaagtaagccaaggggata
3' hlyB + 15bp pGEM R	CGCGAATTCACTAGTttatctttcacgtaatgcttctttc
hlyB comp F	atgatgacaaaaataaattaccagc
tolC seq 1	tgatatacgctgctaaaccaagtg
tolC seq 2	agcgaacgttttagtcagtgtgga
tolC seq 3	tgttaaaggctcggtcagtcag
hlyD out R	tcatggttgtacttcgaacc
hlyD out F	aaaacaagtaagccaaggggataa
hlyD seq 1 R	atgcccaaataaagaaaataacga
hlyD seq 1 F	ttgtgggaattggactcgtta
5' tolC + 15bp pGEM F	CCGGCCGCCATGGCGatgcagcaagctcttcaaca

5' tolC + 15bp kan R	acggatccccggaatcatcagccctttcaggaaag
3' tolC + 15bp kan F	cagctccagcctacagggttcgaagtacaaccatg
3' tolC + 15bp pGEM R	CGCGAATTCACTAGTccaatgtcggctgaaacttc
hlyD out F V2	gaaagaagcattacgtgaaagataa
hlyD out R V2	ttatccccctttggcttacttgtttt
5' cdiB + 15bp pGEM F	CCGGCCGCCATGGCGgaaggagttggctatgc
5' cdiB + 15bp kan R	acggatccccggaatcatctagtatacagaatagtacatatagaa
P1 kan F	attccggggatccgtcgacc
P2 kan R	tgtaggctggagctgcttcg
3' cdiI + 15bp kan F	cagctccagcctacacatgaatttattgattattaatctt
3' cdiI + 15bp pGEM R	CGCGAATTCACTAGTcgccgattaattttaaaata
pSMART F	cccgggctgcaggaattc
pSMART R	gagctcatgcatgatcgaattagc
15 pb pSMART+M2 CDI F	cgatcatgcatgagctcagaggttttttgagggg
15 pb pSMART + M2 CDI R	ggaattcctgcagcccggggagaccttaaacaggtctctc
15bp CDI prom+M2cdiI F	tctgtatactagatgggggtaattattaaatgatgc
M2CDI prom R + 15bp cdiI	tttaataattacccccatctagtatacagaatagtacatatagaaac
15pb pSH+1225-1 cdiB F	GGAGGAATTCACCATtacgtacaattctgtctataaaatg
15 bp pSH+1225-1 cdiI F	GGAGGAATTCACCATatgtacaaattattaagttccg
1225-1 Cdi R+ 15bp pSH	CCGCTACTAGTATTAactaatcatcaaagtttatttaatg
15bp pSH+1225-2 cdiB F	GGAGGAATTCACCATatgcaaaacaaaatttttttttac
15bp pSH+1225-2 cdiI F	GGAGGAATTCACCATatgaagctgttaaatgaagc
1225-2 cdi R15bp pSH	CCGCTACTAGTATTAgcttccaaatttatcagcc
15bp pSH+19606-1 cdiB F	GGAGGAATTCACCATcgtacaattctgtatatgtgatg
15bp pSH+19606-1 cdiI F	GGAGGAATTCACCATaagtttagataatggagtagataatg
19606-1 cdi R+15bp pSH	CCGCTACTAGTATTAtaaacttattaatcattatgccc
15bp pSH+19606-2 cdiB F	GGAGGAATTCACCATtggtaaattaaaaataatattttatgc
15bp pSH+19606-2 cdiI F	GGAGGAATTCACCATtggagaaatatgtaaatgaagc
19606-2 cdi R+15bp pSH	CCGCTACTAGTATTAttatttttttgtttgtatctccc

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