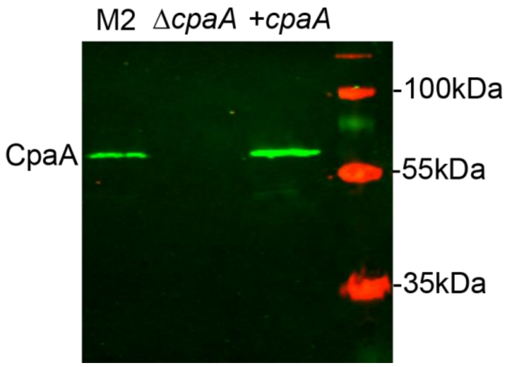

Figure S1-*A. nosocomialis* CpaA Secretion is Dependent upon *cpaA*. Supernatants from wildtype M2, Δ*cpaA*, and the complemented strains (*cpaA*+) were analyzed by Western blot analysis probing for CpaA and RNA polymerase for secretion of CpaA. CpaA is detected in the supernatants of wildtype M2 and the complemented Δ*cpaA* strain, indicating CpaA secretion is dependent upon *cpaA*. RNA polymerase was used as a lysis control. RNA polymerase is not detected; indicating CpaA is actively secreted by *A. nosocomialis*.

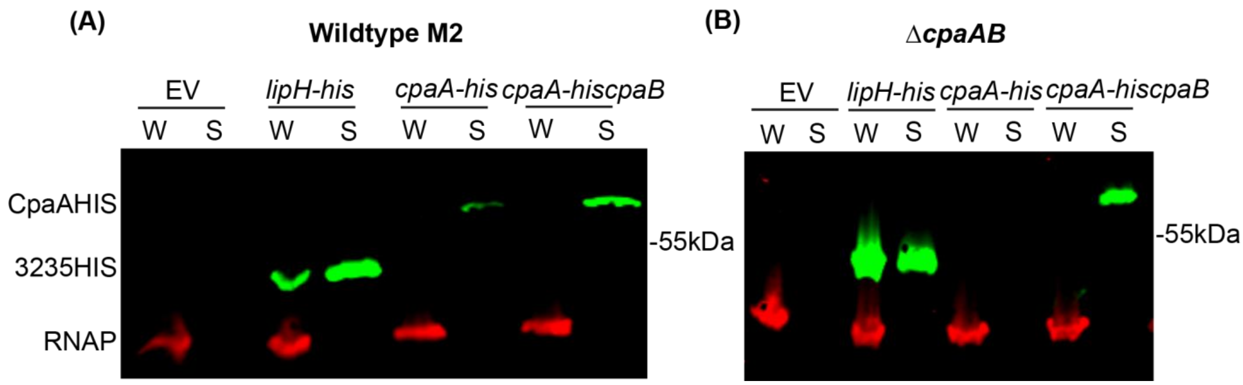

Figure S2- M2 CpaB is Required for Secretion of CpaA-His but Deletion of *cpaA* and *cpaB* does Not Affect Secretion of Type II Substrate LipH-His. Western blot analysis on whole cell (W) and cell free supernatant (S) fractions of (A) wildtype M2 or (B) Δ*cpaAB* M2 expressing empty vector, pWH-*lipH-his*, pWH-*cpaA-his*, or pWH-*cpaA-hiscpaB* probing for CpaA-His or LipH-His. All strains and fractions were probed for RNA polymerase as a cell lysis control. CpaA-His secretion was detected in strains carrying pWH-*cpaA-his* or pWH-*cpaA-hiscpaB*. CpaA-His secretion was only detected in cells expressing *cpaB*. LipH-His expression and secretion was detected in cells carrying pWH-*lipH-his*. CpaA and CpaB are not required for secretion of type II substrate LipH. CpaB is required for secretion of CpaA.

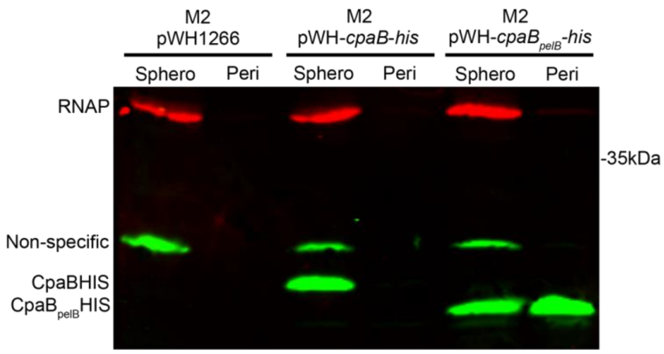

Figure S3-Replacement of the N-terminal Transmembrane Domain of CpaB with a Sec Secretion Signal Results in a Soluble Periplasmic Construct of CpaB. Western blot analysis on M2 cells fractionated into the periplasm (peri) or spheroplast (sphero) expressing empty vector, pWH-*cpaB-his* or pWH-*cpaB$_{pelB}$-his* probing for CpaB-His and RNA polymerase as a lysis control. CpaB-His is detected in the spheroplast fraction of the cells expressing full length CpaB. CpaB-His is detected in the spheroplast and periplasmic fractions of cells expressing the soluble *cpaB$_{pelB}$* construct where the N-terminal transmembrane domain has been replaced with a Sec secretion signal.

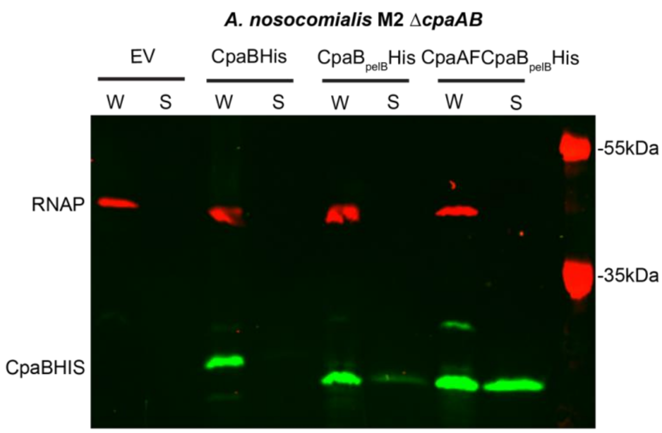

Figure S4-CpaA is Required for Efficient Secretion of Soluble Periplasmic CpaB. Western blot analysis on whole cell (W) and cell free supernatant (S) fractions of Δ*cpaAB* M2 expressing empty vector, pWH-*cpaB-his,* pWH-*cpaB$_{pelB}$-his* or pWH-*cpaB$_{pelB}$-his* and pBAV-*cpaA-flag* was probed for CpaB-His. All fractions were probed for RNA polymerase as a lysis control. CpaB-His was detected in all cells carrying pWH-*cpaB-his* or pWH-*cpaB$_{pelB}$-his*. A low level of CpaB-His secretion was detected in the absence of CpaA in cells carrying pWH-*cpaB$_{pelB}$-his.* Cells carrying pBAV-*cpaA-flag* efficiently secreted CpaB-His when compared to cells devoid of CpaA.

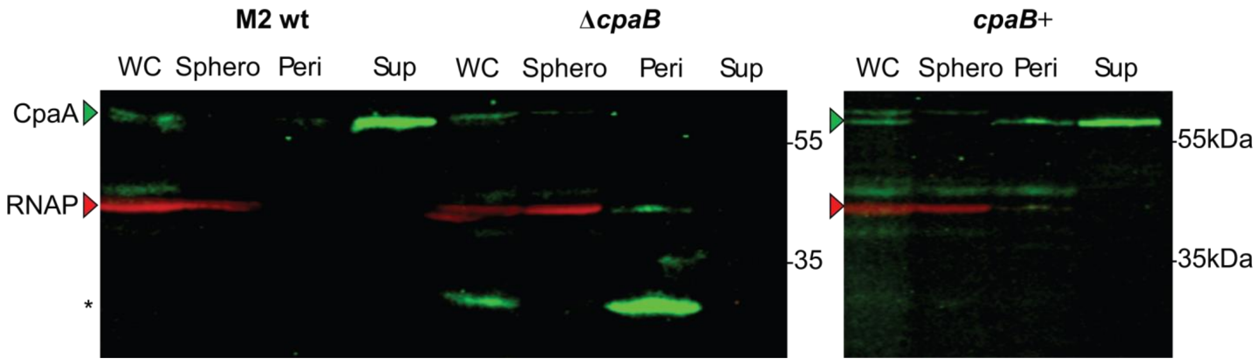

Figure S5-CpaA is Unstable and Degraded in the Periplasm in the Absence of CpaB. Whole cells (WC), spheroplasts (sphero), periplasmic (peri) and supernatant (sup) preparations of *A. nosocomialis* M2 wildtype (wt), Δ*cpaB* mutant, and *cpaB+* complemented strains were analyzed by Western blot using anti-CpaA polyclonal antibodies. RNA polymerase was used as a lysis control. The asterisk indicates the proteolytic fragment derived from CpaA.

Supplemental Table 1
Plasmids and bacterial strains used in this study

Description	Source
Plasmids	
pBAVMCS	(50)
pBAV-*cpaA*-*flag*	This study
pET28a	Novagen
pET28a-*cpaB*-*his*	This study
pETDUET	Novagen
pETDUET-*cpaA*-*his*-*CpaB*	This study
pFLP2	(51)
pGEM-*cpaA*::kansacB	This study
pGEM-*cpaAB*::kansacB	This study
pRSM4063	(44)
pRSM4063-*pcpaA*	This study
pWH1266	(52)
pWH-*cpaA*-*his*	(28)
pWH-*cpaA*-*his*-*cpaB*	(28)
pWH-*lipH*-*his*	(28)
pWH-*cpaA*-*flag*-*cpaB*	This study
pWH-*cpaA*-*flag*-*cpaB*-*his*	This study
pWH-*cpaB*-*his*	This study
pWH-*cpaB*-*pelB*-*his*	This study
Strains	
A. nosocomialis M2	(38, 53)
A. nosocomialis M2 Δ*gspD*::frt	(28)
A. nosocomialis M2 Δ*cpaB*::frt	(28)
A. nosocomialis M2 Δ*cpaA*::frt	This study
A. nosocomialis M2 Δ*cpaAB*::frt	This study
A. nosocomialis M2 Δ*cpaA::*frt (*cpaAB*+)	This study
E. coli Rosetta 2	Novagen
A. baumannii RUH134	(54)
A. baylyi ADPI	(55)
A. baumannii SDF	(56)
A. baumannii AYE	(56)
A. baumannii AB5075	(57)
A. baumannii AB04	(58)
A. baumannii AB004	(29)
A. baumannii AB005	(29)
A. baumannii AB006	(29)
A. baumannii AB007	(29)
A. baumannii AB008	(29)
A. baumannii AB009	(29)
A. baumannii AB010	(29)
A. baumannii AB011	(29)
A. baumannii AB012	(29)
A. baumannii AB013	(29)

A. baumannii AB014	(29)
A. baumannii AB033	(29)
A. baumannii AB034	(29)
A. baumannii AB028	(29)
A. pittii 31205	(59)
A. calcoaceticus 27693	(59)
A. pittii 28207	(59)
A. pittii 30005	(59)
A. pittii 31975	(59)
A. pittii 31357	(59)
A. pittii 33632	(59)
A. pittii 32493	(59)
A. nosocomialis 26959	(59)
A. nosocomialis 33904	(59)

Supplemental Table 2

Primers used in this study

Description	**Sequence**
petDuetfwinfuse	$GA_2T_2CGAGCTCG_2(CG)_2C_2TGCAG$
petDuetrevinfuse	$GCTGC_3ATG_2(TA)_2TCTC_2T_2C$
CpaBcytorevKpnI	$(AT)_2G_2TAC_3TATG_2ATGATC(ATG)_2TA_2CTC_2$
CpaBcytofwNdeI	$(AT)_2C(AT)_2GATGCAGCA_3GT_2CT_2CTGCT_3A_2CG$
CpaAcytofwduet15bp	$(TA)_2C_2ATG_3CAGCATG_2CA_2C_2GTACTGT(CA)_2A_3TCAG$
CpaAcytoHisrevduet15bp	$GC_2GAGCTCGA_2T_2CT_2AGTG_2TGATGATG_2TGATG_2T_2A(TA)_2(GA)_2A_2T_6GTGC$
5100fwbamhI	$(AT)_2G_2ATC_2ATG_2TGA_2T_4A_3T_2A_5CATCAC$
5100flagrevpstI	$(AT)_2CTGCAGT_2ACT_2ATCGTCGTCATC_2T_2GTA_2TCGT_2ATATAGAGA_3T_6GTG$
Kmsacb2rev	$TGTAG_2CTG_2AGCTGCT_2CG$
Kmsacb1fw	$AT_2C_2G_4ATC_2GTCGAC_2$
3510015bppgem2rev	$(CG)_2A_2T_2CACTAGTCGC_2T_2GCAG_2T_2G(CA)_2GACT_2CAC$
3510015bpkmsacB2fw	$CAGCTC_2AGC_2TACATA_2T(CT)_2AT_5AGCTGTA_3TA_2TG$
5510015bpkmsacBrev	$ACG_2ATC_4G_2A_2T(CA)_2GTAGC_2TCT_4AT_3GT_5AG$
5510015bppgem1fw	$C_2G_2C_2GC_2ATG_2CGACGC_2A_2TGAT_2GATCTG_2A_2T_2G$
6cpaABpromrev	$AGTAGC_2TCT_4AT_3GT_5AGAT_4CG$
5CpaAFLAGrev	$T_2ACT_2ATC(GTC)_2ATC_2T_2GTA_2TCGT(TA)_3(GA)_2A_2T_6GTGC$
3cpaApromfwpwh1	$(ACT_2)_2CTGACA_2CGATCGCTA(TC)_2AGC_2AT_3GT_3CT_2C_2TG$
2Pwhrev1	$T_2GTCAGA_2GTA_2GT_2G_2C_2G$
1Pwhfw2	$GAG_2AC_2GA_2G_2AGCTA_2C_2GC$
9CpaBnoTMpelB15bppromfw	$ATA_4GAG_2CTACTCGATCGATGA_3TAC_2(TGC)_2CGAC_2(GCT)_3G_2TC(TGC)_2TC_2TCGCTGC_3AGC_2G_2CGATG_2C_2ATGT(CA)_2GCA_3GT_2CT_2CTGCT_3A_2CG$
8CpaBHisrevpwh2	$GCTC_2T_2CG_2(TC_2)_2GATCGCTA_2(TGA)_2(TGG)_3TG(ATG)_2TG_2ATGATC(ATG)_2TA_2CTC_2TG$
7CpaBfwpromoverhang	$ATA_4GAG_2CTACT_2CTCTAT_5AGCTGTA_3TA_2TGA_2G$
11CpaBfw15bpcpaAF	$(GAC)_2GATA_2GTA_2T_2(CT)_2AT_5AGCTGTA_3TA_2TGA_2G$
cpaBrevNT15bppwh2	$GCTC_2T_2CG_2(TCC)_2TATG_2ATGATC(ATG)_2TA_2CTC_2$
cpaABKOdw15bppgemrev4	$(CG)_2A_2T_2CACTAGTA_2GCTA_2CTCATGTA_2TGT_2GA_3TG$
cpaABKOdw15bpkmsacBfw3	$CAGCTC_2AGC_2TACATAGAG_2A_2TA_3TGAT_4G_2TAG_3$
cpaABKOup15bppgemfw2	$C_2G_2C_2GC_2ATG_2CGTCTACAGTAG_2TG_2CG_2TG$
CpaABkoup15bpkmsacBrev1	$ACG_2ATC_4G_2A_2T_2A_2GCAC_3A_2CTA(TG)_2CATAGC$
kpnIcpaABrevlocus	$(AT)_2G_2TAC_2AC_2T(AC)_2CA(CG)_2T_2C_2TGTA_3TA_3TA_2GC$
pstIcpaABfwlocus	$(AT)_2CTGCAGCTA(TC)_2AGC_2AT_3GT_3CT2C_2TGT_2A_2CTG$
Pet28ahisfw	$CAC_2(ACC)_4ACT(GA)_2T$
Pet28auprev	$CATG_2(TA)_2(TC)_2(CTT)_2A_3GT$
CytocpaBrev	$(GTG)_5TG_2ATGATC(ATG)_2TA_2CTC_2TG$
CytoCpaBfw	$AG_2AGA(TA)_2C_2AT(GCA)_2A_2GT_2CT_2CTGCT_3A_2CG$