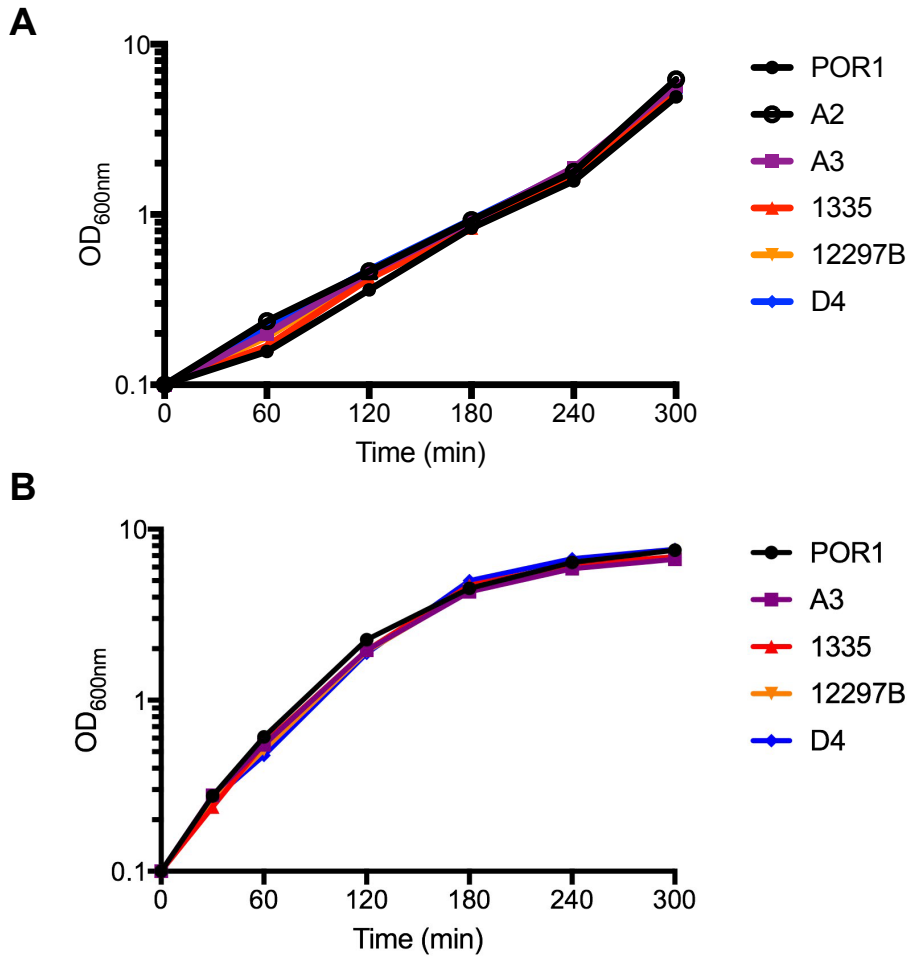
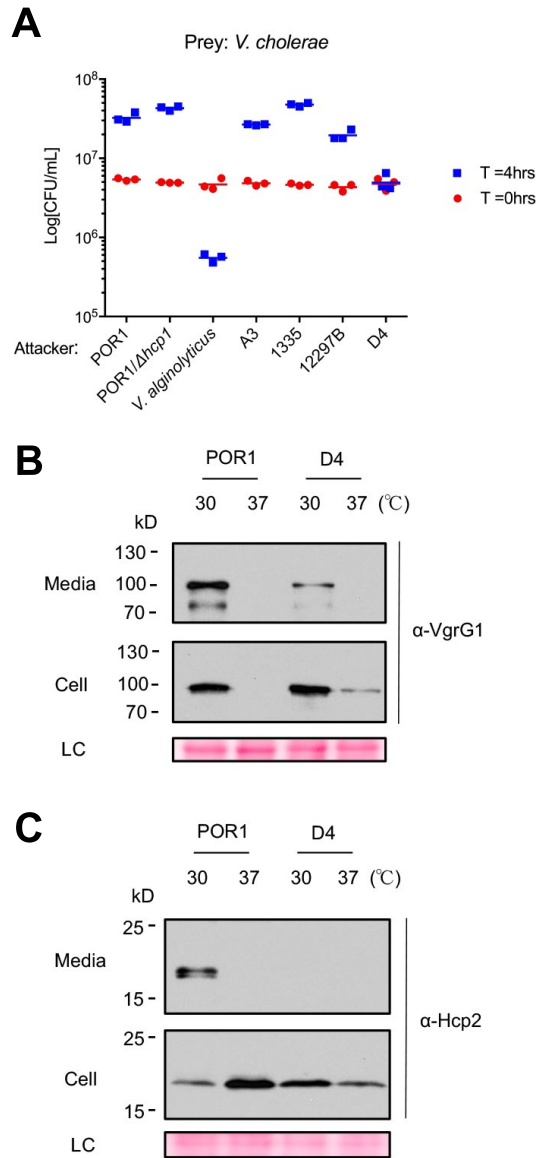


**Figure S1.** Eight different versions of T6SS1 site two in clinical and AHPND strains of *V. parahaemolyticus*. RIMD2210633 *vp1415-vp1420* are labeled in blue and corresponding genes in AHPND strains are labeled in blue when they are conserved (>90% identity) and other colors when they are not conserved. The same color indicates identical or highly conserved genes (>90% identity amino acid).



**Figure S2.** AHPND-causing *V. parahaemolyticus* strains exhibit similar growth rate to the RIMD2210633 clinical strain. Growth of indicated *V. parahaemolyticus* strains in LB (1% NaCl) at 23°C (**A**) or MLB (3% NaCl) at 30°C (**B**) based on OD<sub>600nm</sub> absorbance measurements. Data are mean ± SD, n=3. Data are representative of three independent experiments.



**Figure S3.** T6SSs of AHPND-causing *V. parahaemolyticus* strains are not active at 37°C. **A.** Viability counts of *V. cholerae* prey before (0h) and after (4h) co-culture with indicated *V. parahaemolyticus* or *V. alginolyticus* attacker strains on MLB (3% NaCl) solid media at 37°C. Data are representative of three independent experiments. **B.** Expression (Cell) and secretion (Media) of T6SS1 component VgrG1 by the indicated *V. parahaemolyticus* strains grown in MLB (3% NaCl) at 30°C and 37°C + 20 μM phenamil were analyzed by western blot using α-VgrG1 antibody. **C.** Expression (Cell) and secretion (Media) of T6SS2 component Hcp2 by the indicated *V. parahaemolyticus* strains grown in MLB (3% NaCl) at 30°C and 37°C + 20 μM phenamil were analyzed by western blot using α-Hcp2 antibody. LC: loading control.

A

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PirA
pdb|3X0T|A Vibrio parahaemolyticus M0605 10 DYSHDWTVEPNNGVTVDSKHTPIIEVGRSVDIENITGRG-ELTIQYWGAPFMAGGWK-VAKSHV
WP_045384433 Vibrio campbellii 8 DYSKDWAVEPNSKISVWD-KHTPIIEIGINVDIANITGRG-ELTIKREYGVPPMAGGWK-LAKEQK
WP_013050436 Shewanella violacea 16 RYTANKFKVAENDGYMYE-KNTPIIPEIGVNIITVINTGRE-EMEVHYEWAPPF--GGWQ-CASSTI
WP_042569345 Yersinia intermedia 36 RARVPTEVAENSSTEVLY-RSTPIIPESRNRVMIINDGAANVITAOYVYWSHSF-TSQWF-LYTSID
WP_013183676 Xenorhabdus nematophila 35 TNFRDITIEPHSSVEAIR-TDTPIIPESRPNVYVANSRGPASSVRAVYVYWSHSF-TSEWF-ESSSII
WP_041381204 Photorhabdus luminescens 29 RQGFVKVDNNSQEKFY-IATPIIPESRKNIVVINEGLADVITAKYVYWSHSF-TSEWF-EDNSVD
WP_036768447 Photorhabdus asymbiotica 33 VFLSDLTISPRSSVEFR-IDTPIIPESRRLRVVMTGLASSVTAKEYVYWSHSF-TSEWF-ESGSD
WP_025422633 Sodalis praecaptivus 39 VTEGEMRIEPRSLQATR-TDTPIIPESRGRFVYANAGKAKOVITAFVYWSHSF-TSEWF-EYSSTV
WP_053075293 Pectobacterium carotovorum 38 VGTSNYTVKAKSSIEIYR-VDTPIIPESTRSLIENIGRANEITATFYVYWSHSF-TSKWF-PEGLT
WP_071784500 Serratia fonticola 27 HPRGDVFGARSSLEIFR-TDTPIIPESTRLELVNIGRANEITATFYVYWSHSF-TNKWF-EEGSLT
WP_071661802 Candidatus Rickettsiella isopodorum 36 SEVYVNFIPNNSKDVNL-QNRGIIPGAHKQYRVNIGRDK-DIIAQIRWGTAV--QWGMVGMKQI
WP_059318262 Alcaligenes faecalis 31 LSGGTFVPHKSGVQAQK-YSTVEIPEIHKSYWIFNAGKANKIKAVFYVYWSHSI-TSSWF-EYSVSE
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WP_013050436 Shewanella violacea V-ARGETVNFTHPGNSFQHQRITVQNDGSSKGYLTIYPS-
WP_042569345 Yersinia intermedia I-PPDGKPVYIAHPSNAPVYQRIIAYKKESTAFGNCEY---
WP_013183676 Xenorhabdus nematophila V-NVGDskLVSPNSLQYKVVLIINNTSRKAYVTAEEK--
WP_041381204 Photorhabdus luminescens V-KAGEDVGLHSPGNSLQYKVVVYNDTKRAFVTGYNL--
WP_036768447 Photorhabdus asymbiotica V-KVGEskVLVAPSNPLQYKVVYHNNTKSAVAVTVREK--
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WP_053075293 Pectobacterium carotovorum I-ARGTDGIVMPNNSLQYKVVYHNNTKSAVAVTVREK--
WP_071784500 Serratia fonticola V-KRGDGLIKAPGNSFLQYKVVYHNNTKSAVAVTVREK--
WP_071661802 Candidatus Rickettsiella isopodorum V-KYERGLVNTPESSFLQYKVVYHNNTKSAVAVTVREK--
WP_059318262 Alcaligenes faecalis I-PPGETKIVRNEGRAYVCTCLQINETPSRSIVGASYAGV-
V-GIGEKKLQAPNSLQYKVVLEINETDQNAVTVSIVTAK-
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B

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PirB
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WP_045384430 Vibrio campbellii 39 YEVPNYAKRAMVSFGLSNIPYAGGFLSTLWNIWFNPPTNPEPDIEINWEQLDRDIQDLVDESIIIDAI
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WP_042569344 Yersinia intermedia 28 FVHSSAILQAVLKGISFIPYVGYDLSIIIFGFWD----QERDWEQELGRVQOILIEENLVKAI
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WP_011148522 Photorhabdus luminescens 37 YEKDMSIILDAIIGGIFIPGPGSAISFLGLGFVQ----QTNWTEQILQVQEQMIQEQANLTKI
WP_065823474 Photorhabdus asymbiotica 26 YEKDMSIILDAIIGGIFIPGPGSAISFLGLGFVQ----QTNWTEQILQVQEQMIQEQANLTKI
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WP_071661803 Candidatus Rickettsiella isopodorum 33 EVDYVNAALRSVLIQKISIKIPGVGELALLDIFVQ----KKNVWQOILHEVENMIETSKLTI
WP_059318263 Alcaligenes faecalis 26 GDVYSAILFSSVMFGLQIPVYVGRALSTIVGLFVWPK----NEKDVWQKSLDELEIDNQLIAT
pore formation
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WP_013050437 Shewanella violacea NGILLDSKIKEMRDKIQDINLTIERF-EYADAKDDYMNLVNTFLIIGLEENFKREIEGDEWAIAYAILPLST
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pore formation
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WP_059318263 Alcaligenes faecalis TVMLKVSVYVYVHKSQEMGLTAREVEEMQDIDRLVDRSSA---YCHALYDTEYDEIINTSDYITDND
pore formation
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WP_065823474 Photorhabdus asymbiotica -NERFNKIASVKGVIY-VRIGGAFVGGLEIIFENGNYQOQ--QATNEHEI-VNLKGNLKTLEWVNG
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WP_024531413 Serratia fonticola -NGKRTKIRHITGCI-TRIGGAFVGGLEIIFPDGRHQGLG--SITSETAS-ISLDGRNITSLVWNG
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WP_059318263 Alcaligenes faecalis -NGQNRNKIKKVTGFI-IRIGGAFVGGIKVTFANDASYTLG--TTVTEVNS-IELNSVITSLVWNG
pore formation
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WP_013050437 Shewanella violacea TVAVDQVAFHSLGDEFIKIGED-SRREKTRQL-EGEFCVAGMFADEKSD--KIAAFVSYEMLHPNM
WP_042569344 Yersinia intermedia TYAINYIKFTLTDGRTMSVGEQ-SGDTQL-LGF-DNBTIAAIFVDESSD--KISCVSVCIPQYQE--
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WP_059318263 Alcaligenes faecalis --AIDEAKFTLSDGRTFRLQGR-YSTNRYKFALE-EGEYISGFIASDRSLVGOAANIVSYHMIADK
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**Figure S4.** Multiple protein sequence alignment of **(A)** PirA and **(B)** PirB and their homologues. PDB or accession number of each protein is listed before the species names. Sequence alignments were limited to sequence ranges that correspond to the PirA and PirB structural domains, noting the starting residue numbers to the left of the alignments. Residue positions are highlighted with colors according to conservation: yellow for hydrophobic (including also S, T, W and Y), gray for small, dark yellow for aromatic, or black for invariant polar. The helix pair (a4, a5) that includes a conserved hydrophobic central helix (a5) corresponding to a region critical for pore formation in the structurally related cry toxins is marked by \* above the PirB alignment.