Supplementary data

Experimental procedures:

Mutagenesis - Generation of inactive HtrA (*Hp*HtrA S221A, *Cj*HtrA S225A, *Ng*HtrA S246A, *Ep*HtrA S236A, *Sf*HtrA S236A) was performed by S→A mutations in the active center using a site-directed mutagenesis kit (Stratagene). Mutations of Q→A and A→Q in respective HtrA constructs (*Ng*HtrA Q263A, *Cj*HtrA A242Q, *Sf*HtrA A253Q) were performed accordingly (Tab. S1). Protein purification was performed as previously described (1).

Infection experiments - Cells were incubated with bacteria at a MOI 100 and routinely monitored by an inverse microscope (Olympus). To quantify adherent *C. jejuni*, cells were scraped into PBS and plated on agar plates. Colony forming units (CFU) were determined after 48 h. Invasion of *C. jejuni* into INT-407 cells was determined in a gentamicin protection assay (2). Transmigrating *C. jejuni* were quantified as previously described for *H. pylori* (3).

Mass-spectrometry - Proteins in negatively stained bands were excised from the gels and digested with the ProteoExtract All-In-One Trypsin Digestion Kit (Calbiochem, Gibbston, NJ). Resulting peptides were separated by capillary reversed phase high pressure liquid chromatography (rpHPLC) directly coupled to a Quadrupole-Time of Flight mass spectrometer (QTof Ultima Global, Waters, Milford, MA) and analyzed as described in (4). Obtained mass data were processed and analyzed with Protein Lynx Global Server version 2.2.5 (Waters, Milford, MA) using the SwissProt/TrEMBL database.

HtrA sequence alignment and structure modeling - HtrA amino acid sequences were aligned with ClustalW (EMBL-EBI) using default parameters. (*) indicates identical amino acids in all sequences, conserved amino acid substitutions are labeled with (:) and semi-conservative substitutions are marked with (.). Prediction of the signal peptide of *H. pylori* HtrA was performed using SignalP 4.0, the protease domain was predicted by MyHits Motive Scan and the PDZ1 and PDZ2 domains by SMART. HtrA protein sequences from *H. pylori* (gi|345645045), *C. jejuni* (gi|87249907), EPEC gi|215485324), *S. flexneri* (gi|30039963) and *N. gonorrhoeae* (gi|268598301) were retrieved from Pubmed. BLAST sequence alignments were used to retrieve the best structures available from the PDB database (5). PDB entry 3mh6 (6) showed the highest sequence identity to all sequences and was ranked first based on the calculated E-values. Identity to *S. flexneri* and EPEC was 99% (E-value: 0.0). *C. jejuni* shared 41% sequence identity (E-value: $2e^{-72}$) and *N. gonorrhoeae* exhibited the lowest sequence identity of 36% (E-value: $2e^{-88}$). The sequence of the resolved structure was aligned to all sequences using ClustalW.

Protease activity assay - Protease activity was quantified using a protease detection assay (Jena Bioscience) according to manufacturer's instructions with minor modifications. In brief, 0.75 µg recombinant HtrA wt or inactive mutant were incubated in 25 µl 50 mM Hepes (pH 7.5) together with 25 µl Incubation Buffer and 25 µl Substrate Solution for 16 h at 37°C. Subsequently, 50 µl were mixed with 150 µl Assay Buffer in a black, flat bottom 96 well plate (Greiner bio-one) and analyzed using a plate reader at 490 nm excitation and 520 nm emission wave lengths (Tecan infinite M200).

Western blotting - *Cj*HtrA was detected in Western blot analyses using a polyclonal antibodies specific for *C. jejuni* HtrA or *C. jejuni* MOMP. Where indicated Western blots of at least three independent experiments were quantified using the ChemiDoc XRS system (BioRad).

Statistical analysis - All experiments were repeated 3–4 times. All data were evaluated using student t-test. P values = $***p \leq 0.001$, $**p \leq 0.01$ and $*p \leq 0.05$ were considered statistically significant.

Table S1: Primer pairs used in this study.

HtrA[a]	primer[b]	SA-Mutagenesis primer[b]	AQ/QA Mutagenesis primer
Hp	f: 5´-AAG GAT CCG GCA ATA TCC AAA TCC AGA GCA TG-3´ r: 5´-AAG AAT TCG ACC CAC CCC TAT CAT TTC ACC-3´	f: 5‘-GCT TCC ATC AAT CCT GGA AAT GCT GGC GGC GCT TTA ATT GAT AGC-3’ r: 5’- GCT ATC AAT TAA AGC GCC GCC AGC ATT TCC AGG ATT GAT GGA AGC-3’	
Ng	f: 5’-GGA TCC GGC AGC TTT TTC GGT GCG GAC AA-3’ r: 5’-GAA TTC TTA TTG CAG GTT TAA TGC GAT GAA CAG CGT G-3’	f: 5’-AAT CCG GGC AAT GCC GGC GGC CCG CTG-3’ r: 5’-CAG CGG GCC GCC GGC ATT GCC CGG ATT-3’	f : 5’-TC GGC ATC AAT TCG GCA ATA TAC AGC CGC AG-3’ r : 5’-CT GCG GCT GTA TAT TGC CGA ATT GAT GCC GA-3‘
Cj	f: 5’-GGA TCC GCA AGT ATT AAT TTT AAC G-3’ r: 5’-CCC GGG TTA TTT AAG CAC AAG-3’	f: 5’-CAA TCC AGG AAA TGC AGG TGG AGC TTT GG-3’ r: 5’-CCA AAG CTC CAC CTG CAT TTC CTG GAT TG-3‘	f: 5’-GTA GGT ATT AAT TCA CAA ATT CTT TCT CGT GGT-‘3 r: 5’-ACC ACG AGA AAG AAT TTG TGA ATT AAT ACC TAC-‘3
Ep	f: 5’-GGA TCC GCT GAG ACT TCT TCA-3’ r: 5’-CCC GGG TTA CTG CAT TAA CAG-3’	f: 5’-CAA CCG GGG TAA CGC AGG TGG TGC GTT G-3’ r: 5’-CAA CGC ACC ACC TGC GTT ACC CCG GTT G-3’	
Sf	f: 5’-GGA TCC GCT GAG ACT TCT TCA-3’ r: 5’-CCC GGG TTA CTG CAT TAA CAG-3’	f: 5’-CAA CCG GGG TAA CGC AGG TGG TGC GTT G-3’ r: 5’-CAA CGC ACC ACC TGC GTT ACC CCG GTT G-3’	f: 5’-GGT ATC AAC ACC CAG ATC CTC GCA CCG-‘3 r: 5’-CGG TGC GAG GAT CTG GGT GTT GAT ACC-‘3

[a] Abbreviation used: *Hp, H. pylori; Ng, N. gonorrhoeae, Cj, C. jejuni, Ep, EPEC, Sf, S. flexneri*
[b] note that HtrA from *EPEC* and *S. flexneri* exhibits a high identity and differs only by five amino acids in the protein sequence

Table S2: Identified proteins in zymograms.

band	accession no.	protein name	MW [Da]	no. of matched peptides	sequence coverage [%]
1	n.d.[a]	n.d.	n.d.	n.d.	n.d.
2	C6UEL8_ECOBR	ATPase and specificity subunit of ClpA ClpP ATP dependent serine protease chaperone	78718	6	13.9437
3	E1ITP8_ECOLX	HtrA	42558	5	23.0958
4	C6UEL8_ECOBR	ATPase and specificity subunit of ClpA ClpP ATP dependent serine protease chaperone	78718	7	15.6338
5	E1ITP8_ECOLX	HtrA	42558	8	31.941
6	A1W0L1_CAMJJ	HtrA	50984	16	37.7119

[a] n.d., not determined

Figure legends

Fig. S1: Identification and activity of proteins exhibiting proteinolytical activities. (A) For preparative analyses, bacteria were lysed and analyzed by zymography. Negatively stained protease bands no. 1-6 were excised, and analyzed by mass-spectrometry. **(B)** Quantification of recombinant HtrA activity using fluorophore-labeled casein. 0.75 µg recombinant active (●) and inactive (●) HtrA were incubated with equal amounts of fluorophore-labeled substrate followed by the detection of fluorescence. Data are presented as fold induction compared to background fluorescence (○, CTL) with standard errors from three independent experiments with three replications. Asterisks indicate statistically significant differences between HtrA wildtype compared to the corresponding inactive HtrA (***, $p \leq 0.001$). (C) Quantification of full length E-cadherin amounts was performed from five independent experiments and is presented as the relative cleavage activity by wildtype HtrA (●) and inactive HtrA (●) compared to the uncleaved E-cadherin control (○, CTL). Asterisks indicate statistical significance (**, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant).

Fig. S2: Generation of an *htrA*-deletion mutant in *C. jejuni*. (A) Lysates of *C. jejuni* wildtype (*Cj*wt) and the isogenic *htrA* knock-out mutant (*Cj*Δ*htrA*) were analyzed by casein zymography (left panel) and Coomassie-stained SDS-PAGE (right panel). **(B)** *Cj*wt and *Cj*Δ*htrA* were grown in liquid brain heart infusion (BHI) medium. Aliquots of supernatants and pelleted bacteria were analyzed for HtrA secretion (lanes 1-2) or HtrA expression (lanes 3-4) by Western blot using an anti-*Cj* HtrA antibody. To demonstrate secretion by an equal number of vital *C. jejuni*, the major outer membrane protein (MOMP) was detected (lower panel). **(C)** Equal growth of *Cj* wt (●) and *Cj* Δ*htrA* (●) in liquid BHI was quantified by determination of the $O.D._{600}$.

Fig. S3: Alignment of HtrA sequences from *H. pylori*, *C. jejuni*, EPEC, *S. flexneri* and *N. gonorrhoeae*. **(A)** Signal peptide (blue), protease domain (pink), catalytic triad (red), PDZ1 (orange and PDZ2 domain (green) of *H. pylori* HtrA are indicated. **(B)** Alignment tree of all different HtrA sequences based on pairwise sequence alignment using BLAST und build using the neighbor joining technique. **(C)** Quantification of Western blots obtained from three independent experiments displayed the relative cleavage activity of HtrAs (●) and HHI-treated HtrAs (●) compared to the E-cadherin control (○, CTL). Asterisks indicate statistical significances (*, $p \leq 0.05$; **, $p \leq 0.01$; ns = not significant).

References

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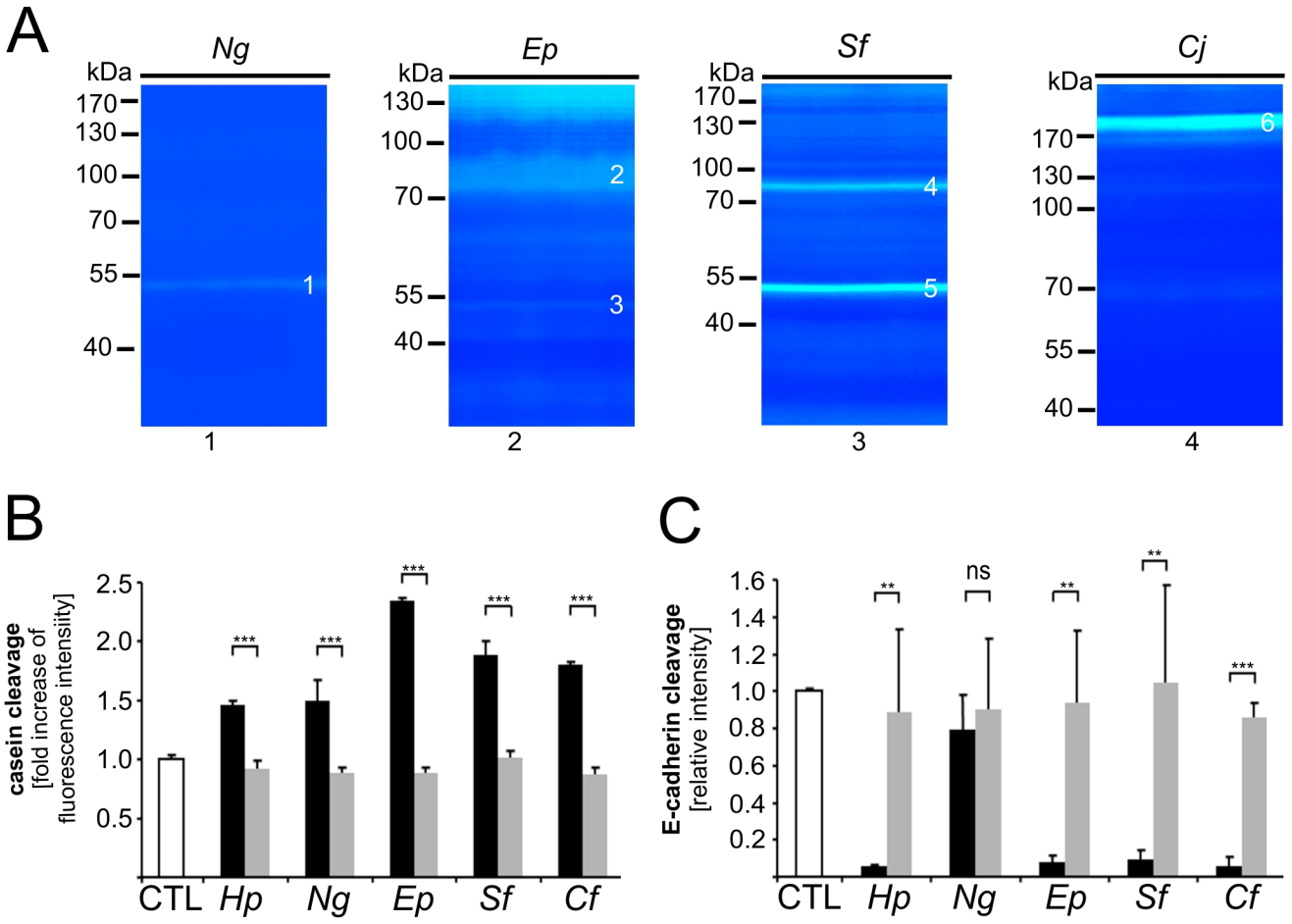
Figure S1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Cj
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40
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2
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4
5
6
B
casein cleavage
[fold increase of fluorescence intensity]
2.5
2.0
1.5
1.0
0.5
***
CTL
Hp
Ng
Ep
Sf
Cf
C
E-cadherin cleavage
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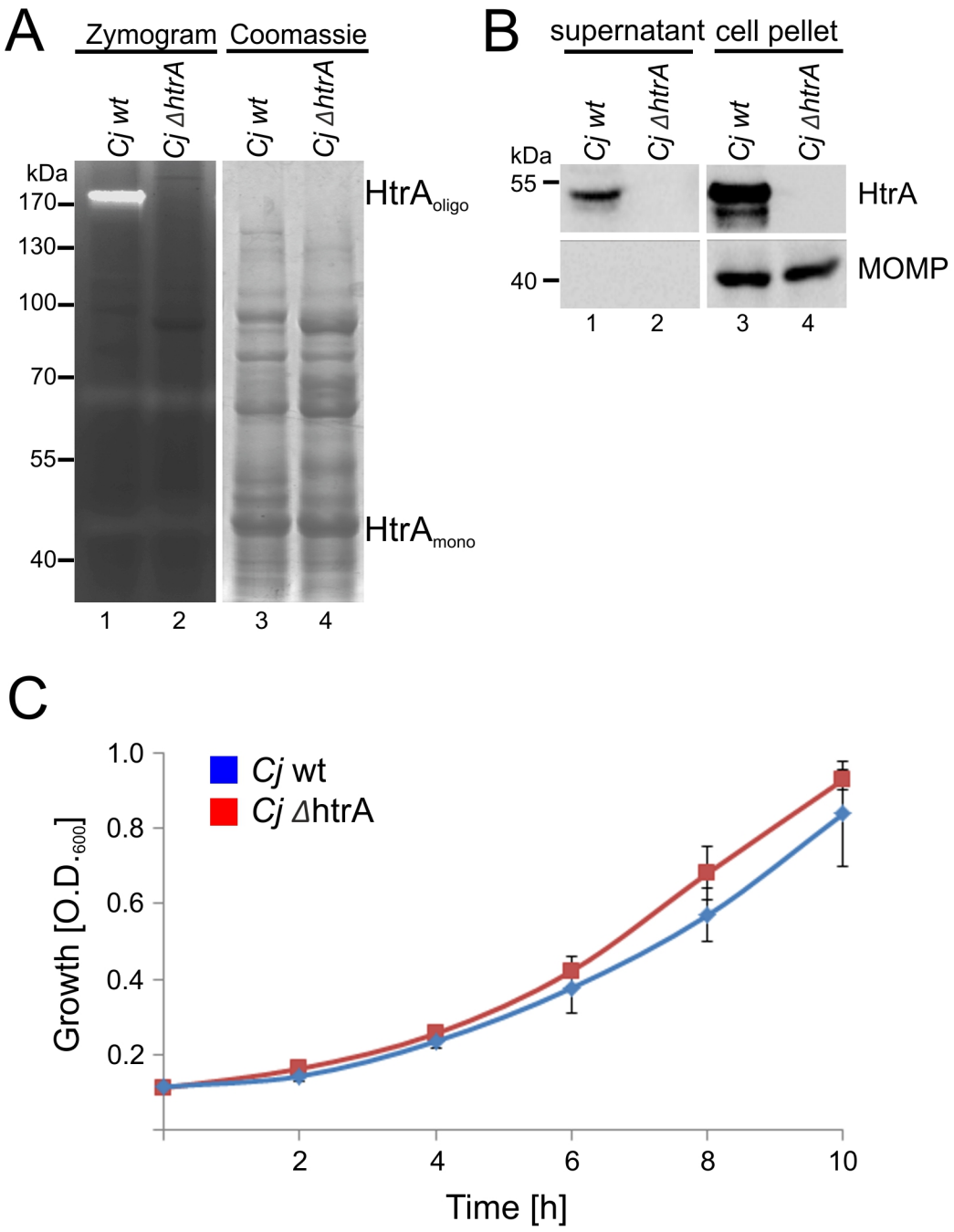
0.2
**
ns
***

Figure S2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Zymogram
Coomassie
Cj wt
Cj ΔhtrA
Cj wt
Cj ΔhtrA
kDa
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130
100
70
55
40
HtrA oligo
HtrA mono
1
2
3
4
B
supernatant
cell pellet
Cj wt
Cj ΔhtrA
Cj wt
Cj ΔhtrA
kDa
55
40
HtrA
MOMP
1
2
3
4
C
Cj wt
Cj ΔhtrA
Growth [O.D.600]
1.0
0.8
0.6
0.4
0.2
2
4
6
8
10
Time [h]

Figure S3

A

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Helicobacter    -MKKTLFISLALALSLNAGNIQIQ------------SMPKVKERVSVPSKDDTIYSYHDS 47
Campylobacter   -MKK-IFLSLSLASALFAASINFN------------ESTATANRVN-PAAGNAVLSYHDS 45
EPEC            -MKK---TTLALSALALSLGLALS------------PLSATAAETSSATTAQQMPSLAPM 44
Shigella        -MKK---NTLALSALALSLGLALS------------PLSATAAETSSATTAQQMPSLAPM 44
Neisseria       MFKKYQYFALAALCAALLAGCEKAGSFFGADKKEASFVERIEHTKDDGSVSMLLPDFAQL 60
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Helicobacter    IKDSIKAVVNISTE---KKIKNNFIGG---GVFNDP---------FFQQFFG-DLGGMIP 91
Campylobacter   IKDAKKSVVNISTS---KTITRANRPSPLDDFFNDP---------YFKQFFDFDFPQRKG 93
EPEC            LEKVMPSVVSINVEGS-TTVNTPRMPRNFQQFFGDDSPFCQEGSPFQSSPFCQGGLGGNG 103
Shigella        LEKVMPSVVSINVEGS-TTVNTPRMPRNFQQFFGDDSPFCQEGSPFQSSPFCQGGQGGNG 103
Neisseria       VQSEGPAVVNIQAAPAPRTQNGSGNAETDSDPLADSDP-----FYEFFKRLVPNMPEIPQ 115
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Helicobacter    K--ERMERALGSGVIISKD-GYIVTNNHVIDGADKIKVTIPGSNKEYSATLVGTDSESDL 148
Campylobacter   KNDKEVVSSLGSGVIISKD-GYIVTNNHVVDDADTITVNLPGSDIEYKAKLIGKDPKTDL 152
EPEC            GGQQQKFMALGSGVIIDADKGYVVTNNHVVDNATVIKVQLS-DGRKFDAKMVGKDPRSDI 162
Shigella        GGQQQKFMALGSGVIIDADKGYVVTNNHVVDNATVIKVQLS-DGRKFDAKMVGKDPRSDI 162
Neisseria       EEADDGGLNFGSGFIISKN-GYILTNTHVVAGMGSIKVLLN-DKREYTAKLIGSDVQSDV 173
                   .     :***.**. : **::**.**: .   *.* :  .  :: *.::*.* .:*:

Helicobacter    AVIRITKD-NLPTIKFSDSNDISVGDLVFAIGNPFGVGESVTQGIVSALNKSGIGINSYE 207
Campylobacter   AVIKIEAN-NLSAITFTNSDDLMEGDVVFALGNPFGVGFSVTSGIISALNKDNIGLNQYE 211
EPEC            ALIQIQNPKNLTAIKMADSDALRVGDYTVAIGNPFGLGETVTSGIVSALGRSGLNAENYE 222
Shigella        ALIQIQNPKKLTAIKMADSDALRVGDYTVAIGNPFGLGETVTSGIVSALGRSGLNAENYE 222
Neisseria       ALLKIDATEELPVVKIGNPKNLKPGEWVAAIGAPFGFDNSVTAGIVSAKGRS-LPNESYT 232
                *:::*    :*.:.: :.. :  *: . *:* ***.. :** **:** .:. :  :.*

Helicobacter    NFIQTDASINPGNSGGALIDSRGGLVGINTAIISKTGGNHGIGFAIPSNMVKDTVTQLIK 267
Campylobacter   NFIQTDASINPGNSGGALVDSRGYLVGINSAILSRGGGNNGIGFAIPSNMVKDIAKKLIE 271
EPEC            NFIQTDAAINRGNSGGALVNLNGELIGINTAILAPDGGNIGIGFAIPSNMVKNLTSQMVE 282
Shigella        NFIQTDAAINRGNSGGALVNLNGELIGINTAILAPDGGNIGIGFAIPSNMVKNLTSQMVE 282
Neisseria       PFIQTDVAINPGNSGGPLFNLKGQVVGINSQIYSRSGGFMGISFAIPIDVAMNVAEQLKN 292
                 *****.:** *****.*.: .* ::***: * :  **  **.**** ::. : . :: :

Helicobacter    TGKIERGYLGVGLQDLSGDLQNSYDN--KEGAVVISVEKDSPAKKAGILVWDLITEVNGK 325
Campylobacter   KGKIDRGFLGVTILALQGDTKKAYKN--QEGALITDVQKGSSADEAGLKRGDLVTKVNNK 329
EPEC            YGQVKRGELGIMGTELNSDLAKAMKVDAQRGAFVSQVLPNSSAAKAGIKAGDVITSLNGK 342
Shigella        YGQVKRGELGIMGTELNSELAKAMKVDAQRGAFVSQVLPNSSAAKAGIKAGDVITSLNGK 342
Neisseria       TGKVQRGQLGVIIQEVSYGLAQSFGLDKASGALIAKILPGSPAERAGLQAGDIVLSLDGG 352
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Campylobacter   VIKSPIDLKNYIGTLEIGQKISLSYERDGENKQASFILKGEKENPK----GVQS---DLI 382
EPEC            PISSFAALRAQVGTMPVGSKLTLGLLRDGKQVNVNLELQQSSQNQVD-----SS---TIF 394
Shigella        PISSFAALRAQVGTMPVGSKLTLGLLRDGKQVNVNLELQQSSQNQVD-----SS---SIF 394
Neisseria       EIRSSGDLPVMVGAITPGKEVSLGVWRKGEEITIKAKLGNAAEHTGASSKTDEAPYTEQQ 412
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Campylobacter   DGLSLRNLDPRLKDRLQIPKDVNGVLVDSVKEKSKGKNSGFQEGDIIIGVGQSEIKNLKD 442
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Neisseria       SGTFSVESAGITLQTHTDS---SGKHLVVVRVSDAAERAGLRRGDEILAVGQVPVNDEAG 469
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Helicobacter    FNHALEKYKGKPKRFLVLDLNQGYRIILVK--- 475
Campylobacter   LEQALKQVN-KKEFTKVWVYRNGFATLLVLK-- 472
EPEC            LRKVLDSKP----SVLALNIQRGDSTIYLLMQ- 474
Shigella        LRKVLDSKP----SVLALNIQRGDSTIYLLMQ- 474
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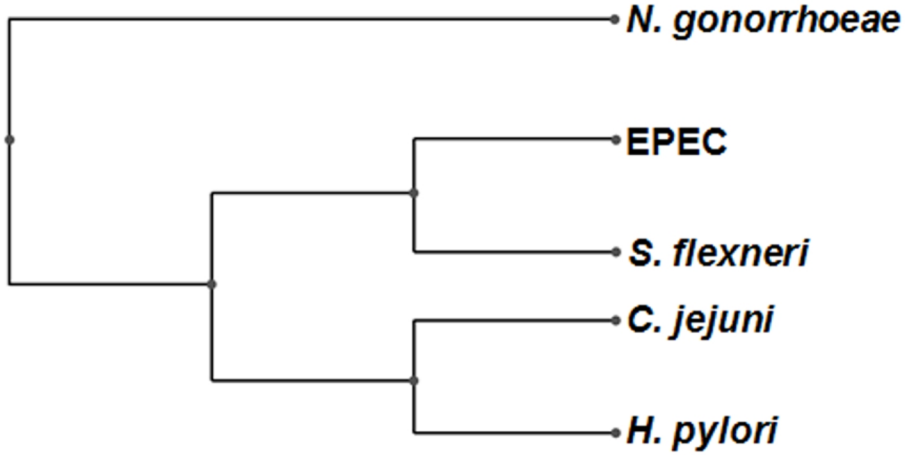

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