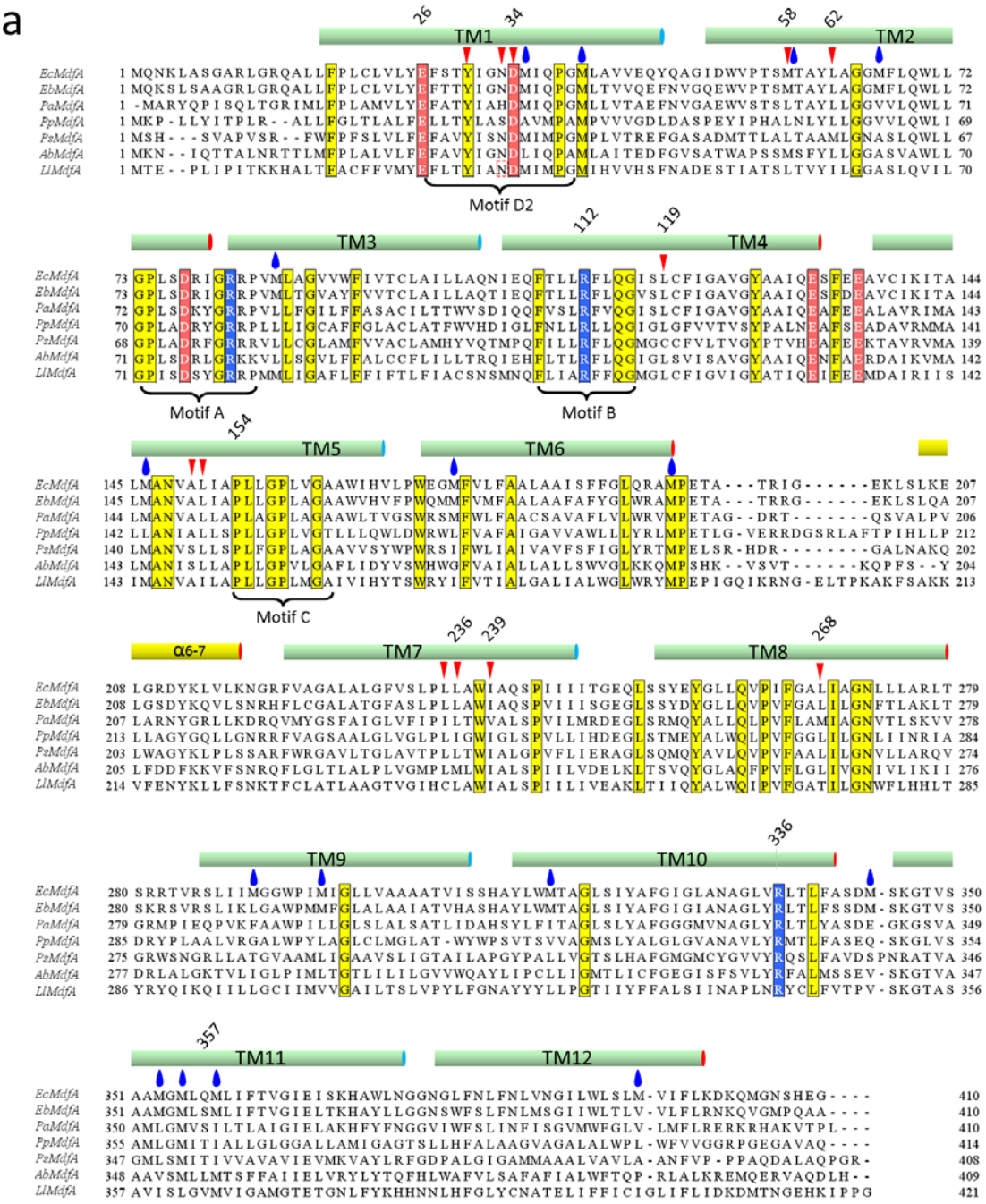
Figures

a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TM1
TM2
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EbMdfA 1 MQKSLSAAGRLGRQALLFPLCLVLYEFTTYIGNDMIQPGMLTVVQEFNVGQEWVPTSMTAYLAGGMFLQWLL 72
PaMdfA 1 -MARYQPISQLTGRIMLFPLAMVLYEFATYIAHDMIQPGMLLVTAEFNVGAEWVSTSLTAYLLGGVVLQWLL 71
PpMdfA 1 MKP-LLYITPLR--ALLFGLTLALFELLTYLASDAVMPAMPVVVGDLDASPEYIPHALNLYLLGGVVLQWLI 69
PsMdfA 1 MSH---SVAPVSR--FWFPFSLVLFEFAVYISNDMIMPGMPLVTREFGASADMTTLALTAAMLGNASLQWLL 67
AbMdfA 1 MKN--IQTTALNRTTLMFPLALVLFEFAVYIGNDLIQPAMLAITEDFGVSATWAPSSMSFYLLGGASVAWLL 70
LlMdfA 1 MTE--PLIPITKKHALTFACFFVMYEFLTYIANDLMIMPGMIHVVHSFNADESTIATSLTVYILGGASLQVIL 70
Motif D2
112
119
TM3
TM4
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EbMdfA 73 GPLSDRIGRRPVMLTGVAYFVVTCLAILLAQTIEQFTLLRFLQGVSLCFIGAVGYAAIQESFDEAVCIKITA 144
PaMdfA 72 GPLSDKYGRRPVLLFGILFFASACILTTWVSDIQQFVSLRFVQGISLCFIGAVGYAAIQEAFEEALAVRIMA 143
PpMdfA 70 GPLADRYGRRPLLLIGCAFFGLACLATFWVHDIGLFNLLRLLQGIGLGFVVTVSYPALNEAFSEADAVRMMA 141
PsMdfA 68 GPLADRFGRRRVLLCGLAMFVVACLAMHYVQTMPQFILLRFLQGMGCCFVLTVGYPTVHEAFEEKTAVRVMA 139
AbMdfA 71 GPLSDRLGRKKVLLSGVLFFALCCFLILLTRQIEHFLTLRFLQGIGLSVISAVGYAAIQEHNFAERDAIKVMA 142
LlMdfA 71 GPISDSYGRRPMMLIGAFLFFIFTLFIACSNSMNQFLIARFFQGMGLCFIGVIGYATIQEIFEEMDAIRIIS 142
Motif A
Motif B
154
TM5
TM6
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EbMdfA 145 LMANVALIAPLLGPLAGAAWVHVFPWQMMFVMFAALAAFAFYGLWRAMPETA---TRRG------EKLSLQA 207
PaMdfA 144 LMANVALLAPLAGPLAGAAWLTVGSWRSMFWLFAACSAVAFLVLWRVMPETAG--DRT-------QSVALPV 206
PpMdfA 142 LLANIALLSPLLGPLVGTLLLQWLDWRWLFVAFAIGAVVAWLLLYRLMPETLG-VERRDGSRLAFTPIHLLP 212
PsMdfA 140 LMANVSLLSPLFGPLAGAAVVSYWPWRSIFWLIAIVAVFSFIGLYRTMPELSR-HDR--------GALNAKQ 202
AbMdfA 143 LMANISLLAPLLGPVLGAFLIDYVSWHWGFVAIALLALLSWVGLKKQMPSHK--VSVT------KQPFS--Y 204
LlMdfA 143 IMANVAILAPLLGPLMGAIVIHYTSWRYIFVTIALGALIALWGLLWRYMPEPIGQIKRNG-ELTPKAKFSAKK 213
Motif C
236
239
268
α6-7
TM7
TM8
EcMdfA 208 LGRDYKLVLKNGRFVAGALALGFVSLPLLAWIAQSPIIIITGEQLSSYEYGLLQVPIFGALIAGNLLLARLT 279
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PsMdfA 203 LWAGYKLPLSSARFWRGAVLTGLAVTPLLTWIALGPVFLIERAGLSQMQYAVLQVPVFAALILGNVLLARQV 274
AbMdfA 205 LFDDFKKVFSNRQFLGLTLALPLVGMPLMLWIALSPIILVDELKLTSVQYGLAQFPVFLGLIVGNIVLIKII 276
LlMdfA 214 VFENYKLLFSNKTFCLATLAAGTVGIHCLAWIALSPIILIVEAKLTIIQYALWQIPVFGATILGNWFLHHLT 285
336
TM9
TM10
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AbMdfA 277 DRLALGKTVLIGLPIMLTGTLILILGVVWQAYLIPCLLIGMTLICFGEGISFSVLYRFALMSSEV-SKGTVA 347
LlMdfA 286 YRYQIKQIILLGCIIMVVGAILTSLVPYLFGNAYYYLLPGTIIYFFALSIINAPLNRYCLFVTPV-SKGTAS 356
357
TM11
TM12
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PaMdfA 350 AMLGMVSILTLAIGIELAKHFYFNGGVIWFSLINFISGVMWFGLV-LMFLRERKRHAKVTPL--- 410
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PsMdfA 347 GMLSMITIVVAVAVIEVMKVAYLRFGDPALGIGAMMAAALVAVLA-ANFVP-PPAQDALAQPGR- 408
AbMdfA 348 AAVSMLLMTSFFAIIELVRYLYTQFHLWAFVLSAFAFIALWFTQP-RLALKREMQERVAQDLH-- 409
LlMdfA 357 AVISLGVMVIGAMGTETGNLFYKHHNNLHFGLYCNATELIFFICIGLIFLIDKDMTNGEHKIPPG 421

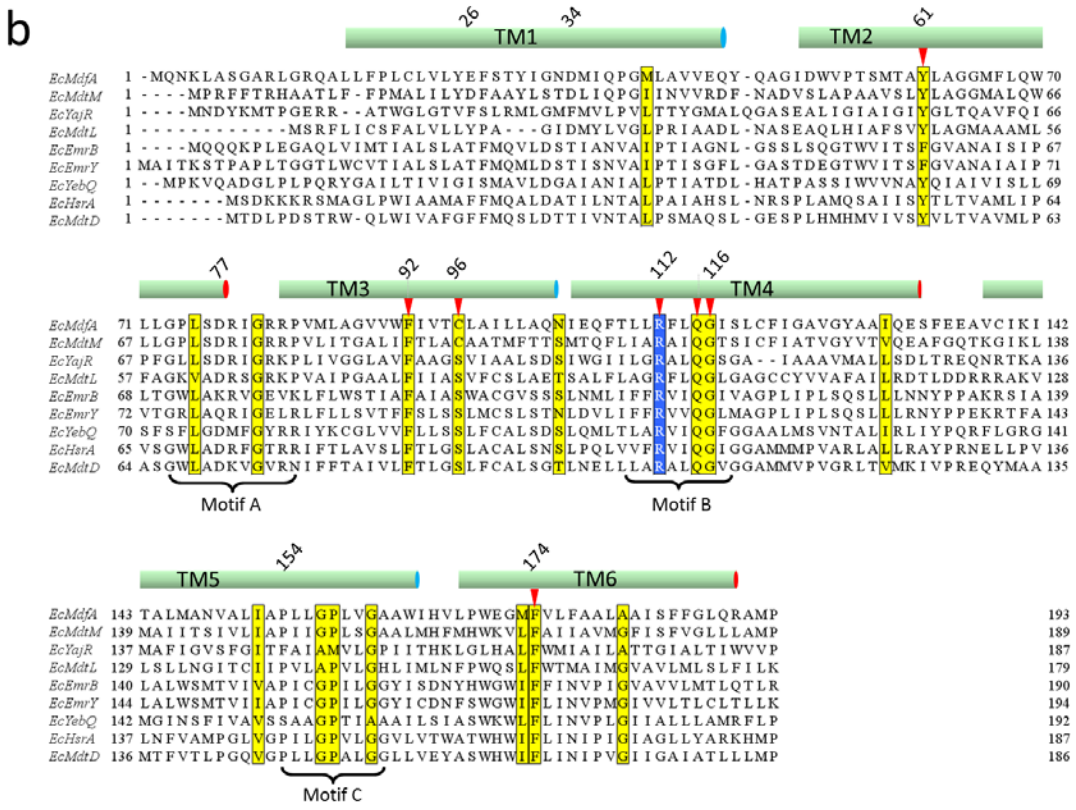

Figure S1. Analysis of amino acid sequences of MdfA

(a) Sequence alignment of MdfA proteins from *E. coli* and six other common pathogenic bacteria. EcMdfA is from *E. coli* (UniProt ID: C6EIU1); EbMdfA, *Enterobacteriaceae bacterium* (E5YMB4); PaMdfA, *Pantoea ananatis* (G9ARR9); PpMdfA, *Pseudomonas putida* (L0FMS0); PsMdfA, *Pandoraea sp.* (R7WXW3); AbMdfA, *Acinetobacter baumannii* (B0VQ72); and LlMdfA, *Legionella longbeachae* (D1REP8). Secondary structures of ecMdfA are marked above the alignment. Selected residues of ecMdfA are labelled as follows: red triangles indicate residues that are involved in the binding of chloramphenicol in the crystal structure; blue drops mark the 16 positions of Se-Met residues used in the initial phasing. *(b)* Sequence alignment of all motif-B containing, MFS antiporters from *E. coli*. MdfA (UniProt ID: C6EIU1); MdtM (Q8XB84); YajR (C6EL42); MdtL (C6EG93); EmrB (C6EJU0); EmrY (C6ELA1); YebQ (C6EC01); HsrA (C6UDA2); and MdtD (C6EAM8). The alignment of their C-domains is poor, and only the N-

domain part of the alignment is shown. Red triangles indicate residues that are involved in motif-B formation in the crystal structure. Sequences were aligned with the program ClustalX [14] and formatted with Jalview [15].