**SUPPLEMENTARY FILE FOR**

**Breaking antimicrobial resistance by disrupting extracytoplasmic protein folding**

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**This file includes:**

 Supplementary Tables 1 to 5

 Supplementary references

**Supplementary Table 1.** Antibiotic resistance profiles of the clinical isolates tested in this study. The table shows MIC values (µg/mL) for a range of commonly used antibiotics. Values highlighted in pink indicate resistance, as defined by the EUCAST clinical breakpoint guidelines, whilst values highlighted in light blue indicate antibiotics for which there is no EUCAST clinical breakpoint. The remaining values (white cells) indicate sensitivity to the tested antibiotic compound. Cells shaded in grey indicated antibiotic compounds that were not tested for particular clinical isolates. Strains shaded in yellow are multidrug resistant. The following abbreviations are used: AC, amoxicillin; XM, cefuroxime; TZ, ceftazidime; IP, imipenem; AT, aztreonam; PT, piperacillin/tazobactam; GM, gentamicin; CO, colistin; CI, ciprofloxacin; NF, nitrofurantoin; TR, trimethoprim.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain** | **AC** | **XM** | **TZ** | **IP** | **AT** | **PT** | **GM** | **CO** | **CI** | **NF** | **TR** |
| *E. coli* BM16(*bla*TEM-1b *bla*KPC-2) | **>256** | **>256** | **192** | **12** | **>256** | **>64** | 8 | 1 | **>32** | **>512** | **>32** |
| *E. coli* LIL-1(*bla*TEM-1 *bla*OXA-9 *bla*KPC-2) | **>256** | **>256** | **8** | **3** | **192** | **>64** | 1.5 | 2 | **>32** | 6 | **>32** |
| *E. coli* CNR1790(*bla*TEM-15 *mcr-1*) | **>256** | **>256** | **32** | 0.5 | **16** | <2 | 1 | **4** | **>32** | 16 | **>32** |
| *E. coli* CNR20140385 (*bla*OXA-48 *mcr-1*) | **>256** | **96** | 1 | 0.25 | 0.38 | **32** | 2 | **4** | **>32** | 8 | **>32** |
| *E. coli* WI2(*bla*OXA-48 *bla*KPC-28 *mcr-1*) | **>256** | **>256** | **>256** | 1.5 | **32** | **>64** | 1.5 | **4** | 0.016 | 6 | 0.25 |
| *E. coli* 1073944(*mcr-1*) | **>256** | 3 | 0.19 | 0.19 | 0.19 | **64** | **>256** | **16** | - | - | - |
| *E. coli* 41489(*mcr-1*) | **>256** | **>256** | 1 | 0.19 | 4 | 4 | 1.5 | **16** | - | - | - |
| *E. coli*(*mcr-1*) | **>256** | 6 | 0.19 | 0.38 | 0.094 | **64** | **16** | **16** | - | - | - |
| *E. coli* 1256822(*mcr-1.5*) | **>256** | 8 | 0.125 | 0.064 | 0.094 | **32** | **96** | **8** | - | - | - |
| *E. coli* 27841(*bla*CTX-M-55 *mcr-3.2*) | **>256** | **>256** | **16** | 0.19 | **64** | <2 | **32** | **3** | **>32** | 8 | **>32** |
| *E. coli* 1144230(*bla*CMY-2 *mcr-5*) | **>256** | **96** | **12** | 0.5 | **6** | 8 | 1.5 | **4** | 0.025 | 48 | 1 |
| *K. pneumoniae* ST234(*bla*SHV-27 *bla*KPC-2) | **>256** | **>256** | **48** | **16** | **128** | **>64** | 0.38 | 2 | 0.047 | **96** | 1 |
| *C. freundii* BM19(*bla*KPC-2) | **>256** | **>256** | **128** | 4 | **64** | **>64** | **24** | 2 | **>32** | 8 | **>32** |
| *E. cloacae* DUB(*bla*FRI-1) | **>256** | **>256** | **16** | **12** | **>256** | **>64** | 1.5 | **>4** | 0.016 | 48 | 0.75 |
| *P. aeruginosa* PA43417(*bla*OXA-198) | **>256** | **>256** | 2 | **>32** | 6 | **32** | **16** | 1 | **>32** | **>256** | **>32** |
| *P. aeruginosa* PAe191(*bla*OXA-19) | **>256** | **>256** | **>256** | 2 | **>256** | **>256** | **>256** | 2 | **>32** | **>256** | **-** |

**Supplementary Table 2.** Bacterial strains used in this study. All listed isolates are clinical strains except for *Escherichia coli* 27841 (ST744), which is an environmental strain. For clinical and environmental isolates, the multi-locus sequence types (ST) are given in parenthesis, where available.

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Source** |
| ***Escherichia coli*** |
| DH5α | F– *end*A1 *gln*V44 *thi*-1 *rec*A1 *rel*A1 *gyr*A96 *deo*R *nup*G *pur*B20 φ80d*lacZ*∆M15 ∆(*lac*ZYA*-arg*F*)*U169 *hsd*R17(rK–mK+) λ– | ([1](#_ENREF_1)) |
| CC118λpir | *ara*D Δ(*ara*, *leu*) Δ*lac*Z74 *pho*A20 *gal*K *thi*-1 *rsp*E *rpo*B *arg*E *rec*A1 λ*pir* | ([2](#_ENREF_2)) |
| HB101 | supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 | ([3](#_ENREF_3)) |
| MC1000 | *ara*D139 ∆(*ara, leu*)7697 ∆*lac*X74 *gal*U *gal*K *str*A | ([4](#_ENREF_4)) |
| MC1000 *dsbA* | *dsbA::aphA*, KanR | ([5](#_ENREF_5)) |
| MC1000 *dsbA* *att*Tn*7*::P*tac-dsbA* | *dsbA::aphA* *att*Tn*7*::*dsbA*, KanR | This study |
| MG1655 | K-12 F– λ*–* *ilv*G– *rfb*-50 *rph*-1 | ([6](#_ENREF_6)) |
| MG1655 *dsbA* | *dsbA::aphA*, KanR | This study |
| MG1655 *dsbA att*Tn*7*::P*tac-dsbA* | *dsbA::aphA* *att*Tn*7*::*dsbA*, KanR | This study |
| MG1655 *acrA* | *acrA* | This study |
| MG1655 *tolC* | *tolC* | This study |
| MG1655 *degP* | *degP::strAB*, StrR | This study |
| **Clinical / environmental isolates** |
| *Escherichia coli* BM16 | *bla*TEM-1b *bla*KPC-*2* | ([7](#_ENREF_7)) |
| *Escherichia coli* LIL-1 | *bla*TEM-1 *bla*OXA-9 *bla*KPC-2 | ([7](#_ENREF_7)) |
| *Escherichia coli* CNR1790 | *bla*TEM-15 *mcr-1* | ([8](#_ENREF_8)) |
| *Escherichia coli* CNR20140385 | *bla*OXA-48 *mcr-1* | ([8](#_ENREF_8)) |
| *Escherichia coli* WI2 (ST1288) | *bla*OXA-48 *bla*KPC-28 *mcr-1* | ([9](#_ENREF_9)) |
| *Escherichia coli* 1073944 (ST117) | *mcr-1* | ([10](#_ENREF_10)) |
| *Escherichia coli* 41489 | *mcr-1* | ([8](#_ENREF_8)) |
| *Escherichia coli* | *mcr-1* | ([8](#_ENREF_8)) |
| *Escherichia coli* 1256822 (ST48) | *mcr-1.5* | ([10](#_ENREF_10)) |
| *Escherichia coli* 27841 (ST744) | *bla*CTX-M-55 *mcr-3.2* | ([11](#_ENREF_11)) |
| *Escherichia coli* 1144230 (ST641) | *bla*CMY-2 *mcr-5* | ([10](#_ENREF_10)) |
| *Klebsiella pneumoniae* (ST234) | *bla*SHV-27 *bla*KPC-2 | ([12](#_ENREF_12)) |
| *Citrobacter freundii* BM19 | *bla*KPC-2 | ([7](#_ENREF_7)) |
| *Enterobacter cloacae* DUB | *bla*FRI-1 | ([13](#_ENREF_13)) |
| *Pseudomonas aeruginosa* PA43417 | *bla*OXA-198 | ([14](#_ENREF_14)) |
| *Pseudomonas aeruginosa* PA43417 *dsbA1* | *dsbA1 bla*OXA-198 | This study |
| *Pseudomonas aeruginosa* PAe191 | *bla*OXA-19 | ([15](#_ENREF_15)) |
| *Pseudomonas aeruginosa* PAe191 *dsbA1* | *dsbA1 bla*OXA-19 | This study |

**Supplementary Table 3.** Plasmids used in this study.

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Source** |
| pDM1 | pDM1 vector (GenBank MN128719), p15A *ori*, P*tac* promoter, MCS, TetR | Lab stock |
| pDM1-*bla*L2-1 | *bla*L2-1 cloned into pDM1, TetR | This study |
| pDM1-*bla*GES-1 | *bla*GES-1 cloned into pDM1, TetR | This study |
| pDM1-*bla*GES-2 | *bla*GES-2 cloned into pDM1, TetR | This study |
| pDM1-*bla*GES-11 | *bla*GES-11 cloned into pDM1, TetR | This study |
| pDM1-*bla*SHV-27 | *bla*SHV-27 cloned into pDM1, TetR | This study |
| pDM1-*bla*OXA-4 | *bla*OXA-4 cloned into pDM1, TetR | This study |
| pDM1-*bla*OXA-10 | *bla*OXA-10 cloned into pDM1, TetR | This study |
| pDM1-*bla*OXA-198 | *bla*OXA-198 cloned into pDM1, TetR | This study |
| pDM1-*bla*FRI-1 | *bla*FRI-1 cloned into pDM1, TetR | This study |
| pDM1-*bla*L1-1 | *bla*L1-1 cloned into pDM1, TetR | This study |
| pDM1-*bla*KPC-2 | *bla*KPC-2 cloned into pDM1, TetR | This study |
| pDM1-*bla*KPC-3 | *bla*KPC-3 cloned into pDM1, TetR | This study |
| pDM1-*bla*SME-1 | *bla*SME-1 cloned into pDM1, TetR | This study |
| pDM1-*mcr-1* | *mcr-1* cloned into pDM1, TetR | This study |
| pDM1-*mcr-3* | *mcr-3* cloned into pDM1, TetR | This study |
| pDM1-*mcr-4* | *mcr-4* cloned into pDM1, TetR | This study |
| pDM1-*mcr-5* | *mcr-5* cloned into pDM1, TetR | This study |
| pDM1-*mcr-8* | *mcr-8* cloned into pDM1, TetR | This study |
| pDM1-*bla*L2-1-StrepII | *bla*L2-1 encoding L2-1 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*GES-1-StrepII | *bla*GES-1 encoding GES-1 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-StrepII-*bla*OXA-4 | *bla*OXA-4 encoding OXA-4 with an N-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*OXA-10-StrepII | *bla*OXA-10 encoding OXA-10 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*OXA-198-StrepII | *bla*OXA-198 encoding OXA-198 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*FRI-1-StrepII | *bla*FRI-1 encoding FRI-1 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*L1-1-StrepII | *bla*L1-1 encoding L1-1 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*bla*KPC-3-StrepII | *bla*KPC-3 encoding KPC-3 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*mcr-1*-StrepII | *bla*MCR-1 encoding MCR-1 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*mcr-3*-StrepII | *bla*MCR-3 encoding MCR-3 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*mcr-4*-StrepII | *bla*MCR-4 encoding MCR-4 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*mcr-5*-StrepII | *bla*MCR-5 encoding MCR-5 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pDM1-*mcr-8*-StrepII | *bla*MCR-8 encoding MCR-8 with a C-terminal StrepII tag cloned into pDM1, TetR | This study |
| pGRG25 | Encodes a Tn*7* transposon and *tnsABCD* under the control of P*araB*, thermosensitive pSC101 *ori*, AmpR | ([16](#_ENREF_16)) |
| pGRG25-P*tac::dsbA* | P*tac::dsbA* fragment cloned within the Tn*7* of pGRG25; when inserted into the chromosome and the plasmid cured, the strain expresses DsbAupon IPTG induction, AmpR | This study |
| pSLTS | Thermosensitive pSC101*ori,* P*araB* for λ-Red, P*tetR* for I-SceI, AmpR | ([17](#_ENREF_17)) |
| pUltraGFP-GM | Constitutive sfGFP expression from a strong Biofab promoter, p15A *ori*, GentR | ([18](#_ENREF_18)) |
| pKD4 | Conditional oriRγ *ori*, (template for the *aphA* cassette), AmpR | ([19](#_ENREF_19)) |
| pCB112 | Inducible *lacZ* expression under the control of the Plac promoter, pBR322 *ori*, CamR | ([20](#_ENREF_20)) |
| pKNG101 | Gene replacement suicide vector, *ori*R6K, *oriT*RK2, *sacB*, (template for the *strAB* cassette), StrR | ([21](#_ENREF_21)) |
| pKNG101-*dsbA1* | PCR fragment containing the regions upstream and downstream *P. aeruginosa dsbA1* cloned in pKNG101; when inserted into the chromosome the strain is a merodiploid for *dsbA1* mutant, StrR | This study |
| pRK600 | Helper plasmid, ColE1 ori, mobRK2, traRK2, CamR | ([22](#_ENREF_22)) |
| pMA-T *mcr-3* | GeneArt® cloning vector containing *mcr-3*, ColE1 *ori*, (template for *mcr-3*), AmpR | This study |
| pMK-T *mcr-8* | GeneArt® cloning vector containing *mcr-8*, ColE1 *ori*, (template for *mcr-8*), KanR | This study |

**Supplementary Table 4.** Oligonucleotide primers used in this study. The “Brief description” column provides basic information on the primer design (restriction enzyme used for cloning, encoded protein or gene replaced by antibiotic resistance cassette, forward or reverse orientation of the primer (F or R); QC stands for QuickChange primers and SQ stands for sequencing primers).

|  |  |  |
| --- | --- | --- |
| **Number** | **Brief description** | **Sequence (5ˊ-3ˊ)** |
| P1 | SacI.L2.F | ctggagctcctcgcccgtcgccgatt |
| P2 | XmaI.L2.R | ctgcccgggtcatccgatcaaccggtcggca |
| P3 | SacI.GES.F | ctggagctccgcttcattcacgcac |
| P4 | XmaI.GES.R | ctgcccgggctatttgtccgtgctcaggatg |
| P5 | SacI.SHV.F | ctggagctccgttatattcgcctgtg |
| P6 | XmaI.SHV.R | ctgcccgggttagcgttgccagtgctcga |
| P7 | SacI.OXA-4.F | ctggagctcaaaaacacaatacatataacttcgc |
| P8 | KpnI.OXA-4.R | cagggtaccttataaatttagtgtgtttagaatggtg |
| P9 | SacI.OXA-10.F | ctggagctcaaaacatttgccgcatatgtaattatcgc |
| P10 | KpnI.OXA-10.R | cagggtaccttagccaccaatgatgccctc |
| P11 | NdeI.OXA-198.F | actgcatatgcataaacacatgagtaagctcttc |
| P12 | KpnI.OXA-198.R | ctgggtaccttattcgatgatcccctttgctt |
| P13 | SacI.FRI-1.F | ctggagctctttttttttaaaaaaggtgcaagtac |
| P14 | XmaI.FRI-1.R | ctgcccgggttatttataacttccataaactgcctttatagc |
| P15 | SacI.L1.F | ctggagctccgttctaccctgctcgc |
| P16 | XhoI.L1.R | actgagctctcagcgggccccggccgt |
| P17 | SacI.KPC.F | ctggagctctcactgtatcgccgtc |
| P18 | KpnI.KPC.R | ctgccatggttactgcccgttgacgccca |
| P19 | SacI.SME-1.F | ctggagctctcaaacaaagtaaattttaaaacgg |
| P20 | XmaI.SME-1.R | ctgcccgggttaatcaattgcctgaattgcaatacg |
| P21 | SacI.MCR-1.F | ctggagctcatgcagcatacttctgtgtggtac |
| P22 | XmaI.MCR-1.R | ctgcccgggtcagcggatgaatgcggtgc |
| P23 | NdeI.MCR-3.F | ctgatacatatgccttcccttataaaaataaaaattgttccg |
| P24 | XmaI.MCR-3.R | cagcccgggttattgaacattacgacattgactgaaaatatctag |
| P25 | SacI.MCR-4.F | ctggagctccgtgctgacgagatttaaaaccc |
| P26 | XmaI.MCR-4.R | ctgcccgggttaaccgcggcagcgggcaaaaatatc |
| P27 | SacI.MCR-5.F | ctggagctccggttgtctgcatttatcac |
| P28 | XmaI.MCR-5.R | ctgcccgggtcattgtggttgtccttttctg |
| P29 | SacI.MCR-8.F | ctggagctcttcaagtatcttttatctttcaaact aacc |
| P30 | XmaI.MCR-8.R | ctgcccgggctaaccattcccatctgttttctc |
| P31 | QC.GES5-GES1.F | aaagagccggagatgggcgacaacacacctg |
| P32 | QC.GES5-GES1.R | caggtgtgttgtcgcccatctccggctcttt |
| P33 | QC.KPC2-KPC3.F | ctaacaaggatgacaagtacagcgaggccgtcatc |
| P34 | QC.KPC2-KPC3.R | gatgacggcctcgctgtacttgtcatccttgttag |
| P35 | XmaI.StrepII.L2.R | ctgcccgggttatttttcaaattgcggatggctccaagcgctccctccgatcaaccggtcggca |
| P36 | XmaI.StrepII.GES.R | ctgcccgggctatttttcaaattgcggatggctccaagcgctccctttgtccgtgctcaggatgag |
| P37 | OXA-4.body.F | tcaacagatatctctactgttgca |
| P38 | OXA-4.StrepII.R | tgcaacagtagagatatctgttgatttttcaaattgcggatggctccaagcgctccctgcactggcgctgctgta |
| P39 | KpnI.StrepII.OXA-10.R | cagggtaccttatttttcaaattgcggatggctccaagcgctcccgccaccaatgatgccctcacttg |
| P40 | KpnI.StrepII.OXA-198.R | ctgggtaccttatttttcaaattgcggatggctccaagcgctcccttcgatgatcccctttgcttg |
| P41 | XmaI.StrepII.FRI-1.R | ctgcccgggttatttttcaaattgcggatggctccaagcgctccctttataacttccataaactgcctttatagc |
| P42 | KpnI.StrepII.L1.R | gggggtacctcatttttcaaattgcggatggctccaagcgctcccgcgggccccggccgtttccttggccaactgc |
| P43 | KpnI.StrepII.KPC.R | ctgccatggttatttttcaaattgcggatggctccaagcgctcccctgcccgttgacgcccaatc |
| P44 | XmaI.StrepII.MCR-1.R | cagcccgggttatttttcaaattgcggatggctccaagcgctcccgcggatgaatgcggtgcggt |
| P45 | XmaI.StrepII.MCR-3.R | cagcccgggttatttttcaaattgcggatggctccaagcgctcccttgaacattacgacattgactgaaaatatctag |
| P46 | XmaI.StrepII.MCR-4.R | ctgcccgggctatttttcaaattgcggatggctccaagcgctcccaccgcggcagcgggcaaaaatatc |
| P47 | XmaI.StrepII.MCR-5.R | ctgcccgggctatttttcaaattgcggatggctccaagcgctcccttgtggttgtccttttctgca |
| P48 | XmaI.StrepII.MCR-8.R | ctgcccgggctatttttcaaattgcggatggctccaagcgctcccaccattcccatctgttttctctcttac |
| P49 | NotI.Ptac.EcDsbA.F | ctggcggccgctgacaattaatcatcggctcgtataatgtgtggaattgtgactagtcgaggtccaggacctcggatcgctaagataggatgattgtatgaaaaagatttggctggc |
| P50 | XhoI.EcDsbA.R | ctgctcgagttattttttctcggacagatatttc |
| P51 | EcdsbA::aphA.F | atgaaaaagatttggctggcgctggctggtttagttttagcgtttagcgcgtgtaggctggagctgcttc |
| P52 | EcdsbA::aphA.R | ttattttttctcggacagatatttcactgtatcagcatactgctgaacaagggaattagccatggtccat |
| P53 | EcacrA::aphA.F | atgaacaaaaacagagggtttacgcctctggcggtcgttctggtgtaggctggagctgcttc |
| P54 | EcacrA::aphA.R | ttaagacttggactgttcaggctgagcaccgcttgcggcttggggaattagccatggtccat |
| P55 | EctolC::aphA.F | ttttacagtttgatcgcgctaaatactgcttcaccacaaggaatgcaagtgtaggctggagctgcttc |
| P56 | EctolC::aphA.R | tcgtcgtcatcagttacggaaagggttatgatgggaattagccatggtcc |
| P57 | EcdegP::strAB.F | atgaaaaaaaccacattagcactgagtgcactggctctgagtttaggtttggaactgcacattcgggatatttctc |
| P58 | EcdegP::strAB.R | ttactgcattaacaggtagatggtgctgtcgccgcgctgaatgttgagtgccaggccggatctagatatctagtatga |
| P59 | SQ.dsbA1.Paeruginosa.F | tacctgctcaagcagatgcatg |
| P60 | SQ.dsbA1.Paeruginosa.R | ggtgttcatgtcgcccatca |
| P61 | XbaI.dsbA1.F | ggttcctctagagcctacttcgccagccagaa |
| P62 | dsbA1.body.R | ctacttcttgttacgcatcgttcactc |
| P63 | dsbA1.body.F | atgcgtaacaagaagtaggcaaggtga |
| P64 | BamHI.dsbA1.R | aattaaggatcctcatcactaccaccagcgcg |

**Supplementary Table 5.** Sources of genomic DNA used for amplification of β-lactamase and MCR genes in this study.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Gene(s)** | **Source** |
| *Stenotrophomonas maltophilia* ATCC 13637 | *bla*L2-1 *bla*L1-1 | ATCC |
| *Pseudomonas aeruginosa* GW-1 | *bla*GES-2 | ([23](#_ENREF_23)) |
| *Enterobacter cloacae* CHE-2 | *bla*GES-5 | ([24](#_ENREF_24)) |
| *Acinetobacter baumannii* K45 | *bla*GES-11 | ([25](#_ENREF_25)) |
| *Klebsiella pneumoniae* ST234 | *bla*SHV-27 *bla*KPC-2 | ([12](#_ENREF_12)) |
| *Pseudomonas aeruginosa* SOF1 | *bla*OXA-4 | ([26](#_ENREF_26)) |
| *Pseudomonas aeruginosa* PU21 | *bla*OXA-10 | ([27](#_ENREF_27)) |
| *Pseudomonas aeruginosa* PA41437 | *bla*OXA-198 | ([14](#_ENREF_14)) |
| *Enterobacter cloacae* DUB | *bla*FRI-1 | ([13](#_ENREF_13)) |
| *Serratia marcescens* | *bla*SME-1 | ([12](#_ENREF_12)) |
| *Escherichia coli* CNR1790 | *mcr-1* | ([8](#_ENREF_8)) |
| *Shewanella bicestrii* JAB-1 | *mcr-4* | ([28](#_ENREF_28)) |
| *Escherichia coli* 1144230 | *mcr-5* | ([10](#_ENREF_10)) |

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