SUPPLEMENTARY TABLES

Table S1. Strains and plasmid used and constructed in this study.

List of strains used, as well as the different single or multiple transduction, deletion and overexpression mutants performed to determine genes implicated in partial resistance to G2cps. Km: kanamycin; Cm: chloramphenicol; aTc: anhydrotetracycline.

Strains or plasmid	Characteristics – construction	Reference
E. coli strains		
TG1-c	F'[traD36 *proAB+ lacIq lacZΔM15] supE hsdΔ5 thi* Δ(lac-proAB) latt-*gfp cat* – Cm^R	Laboratory collection
CFT073 Δ*mchB*	Uropathogenic *E. coli* CFT073Δ*mchB*::Km. MchB microcin-defective; – Km^R	Laboratory collection
iai44[1]	*E. coli* Group A1 – human uropathogenic strain	(1)
Ec094[1]	*E. coli* Group D – commensal strain isolated from Pyrenean roe-deer	(2)
iai73[1]	*E. coli* Group B23 –human uropathogenic strain	(1)
H19[1]	*E. coli* Group B1 – human enterohemorrhagic strain	(3)
TG1 Δ*yjhB*::KmFRT	Transduction of Δ*yjhB*::KmFRT from mutant Keio JW5768	This study
TG1 Δ*yjgZ-yjhB*::Cm	Inactivation of the genomic region *yjgZ-yjhB* by the Cm cassette	This study
TG1 Δ*tar*::KmFRT	Transduction of Δ*tar*::KmFRT from mutant Keio JW1875	This study
TG1 Δ*tar*::FRT Δ*yjhB*::KmFRT	1) Km cassette removal from Δ*tar*::KmFRT 2) Transduction of Δ*yjhB*::KmFRT from mutant Keio JW5768 in Δ*tar*::FRT	This study
TG1 Δ*tar*::KmFRT Δ*yjgZ-yjhB*::Cm	Transduction of Δ*tar*::KmFRT from mutant Keio JW1875 in the *ΔyjgZ-yjhB*::Cm mutant	This study
TG1 *ptsH*::psc189Km	Transduction of *ptsH*::psc189Km from mutant D	This study
TG1 Δ*ydcF*::KmFRT	Transduction of Δ*ydcF*::KmFRT from mutant Keio JW1411	This study
TG1 *ptsH*::psc189 Δ*ydcF*::KmFRT	1) Km cassette removal from *ptsH*::psc189Km 2) Transduction of Δ*ydcF*::KmFRT from mutant Keio JW1411 in *ptsH*::psc189	This study
TG1 *pflB*::psc189Km	Transduction of *pflB*::psc189Km from mutant L	This study
TG1 Δ*pflB*::KmFRT	Transduction of Δ*pflB*::KmFRT from mutant Keio JW0886	This study
TG1 Δ*yggJ*::KmFRT	Transduction of Δ*yggJ*::KmFRT from mutant Keio JW2913	This study
TG1 Δ*pflB*::FRT Δ*yggJ*::KmFRT	1) Km cassette removal from Δ*pflB*::KmFRT 2) Transduction of Δ*yggJ*::KmFRT from mutant Keio JW2913 in Δ*pflB*::FRT	This study
TG1 Δ*dinG*::KmFRT	Transduction of Δ*dinG*::KmFRT from mutant Keio JW0784	This study
TG1 Δ*ycdG*::KmFRT	Transduction of Δ*ycdG*::KmFRT from mutant Keio JW5137	This study
TG1 Δ*dinG*::FRT Δ*ycdG*::KmFRT	1) Km cassette removal from Δ*dinG*::KmFRT 2) Transduction of Δ*ycdG*::KmFRT from mutant Keio JW5137 in Δ*dinG*::FRT	This study
TG1 Δ*ybiL*::Cm	Inactivation of gene *ybiL* by the Cm cassette	This study
TG1 Δ*araF*::KmFRT	Inactivation of gene *araF* by the KmFRT cassette	This study
TG1 Δ*yecI*::KmFRT	Inactivation of gene *yecI* by the KmFRT cassette	This study
TG1 Δ*araF-yecI*::Cm	Inactivation of the genomic region *araF-yecI* by the **C**m cassette	This study
TG1 *ybiL*::psc189Km Δ*araF-yecI*::Cm	Transduction of *ybiL*::psc189Km from mutant E in the Δ*araF-yecI*::Cm mutant	This study
TG1 KmRExTet-*araF*	Insertion of the aTc-inducible KmRExTet cassette upstream from *araF*	This study
TG1 KmRExTet-*yecI*	Insertion of the aTc-inducible KmRExTet cassette upstream from *yecI*	This study
TG1 Δ*phnD*::KmFRT	Transduction of Δ*phnD*::KmFRT from mutant Keio JW4066	This study
TG1 Δ*yeeW-yoeF*::Cm	Inactivation of genomic region *yeeW-yoe**F*** by the Cm cassette	This study

TG1 *phnD*::psc189Km Δ*yeeW-yoeF*::Cm	Transduction of *phnD*::psc189Km from mutant M in Δ*yeeW-yoeF*::Cm mutant	This study
TG1 Δ*yegO*::Cm	Inactivation of gene *yegO* by the Cm cassette	This study
TG1 *phnD*::psc189Km Δ*yegO*::Cm	Transduction of *phnD*::psc189Km from mutant P in the Δ*yegO*::Cm mutant	This study
Plasmids		
Tnpsc189	*oriT* Π-dependent *ori* R6K *mariner*-based transposon TnSC189. Km^R and Ap^R	(4)
pCP20	Plasmid carrying the *flp* recombinase gene; 30ºC replication; CmR and AmpR	(1)
pKOBEGA	Arabinose-inducible λ-red recombinase expression plasmid (oriR101, repA101ts, P_{ara}B-*gam-bet-exo*, AmpR)	(5)

[1] Anti-biofilm activity of culture supernatants against WT TG1-c was resistant to proteinase K and heat treatments, suggesting a polysaccharidic compound (data not shown).

1. Escobar-Paramo, P., K. Grenet, A. Le Menac'h, L. Rode, E. Salgado, C. Amorin, S. Gouriou, B. Picard, M. C. Rahimy, A. Andremont, E. Denamur, and R. Ruimy. 2004. Large-scale population structure of human commensal Escherichia coli isolates. Applied and environmental microbiology 70:5698-700.
2. Escobar-Paramo, P., A. Le Menac'h, T. Le Gall, C. Amorin, S. Gouriou, B. Picard, D. Skurnik, and E. Denamur. 2006. Identification of forces shaping the commensal Escherichia coli genetic structure by comparing animal and human isolates. Environ Microbiol 8:1975-84.
3. Willshaw, G., H. Smith, S. Scotland, and B. Rowe. 1985. Cloning of genes determining the production of vero cytotoxin by Escherichia coli. Microbiology 131:3047.
4. Chiang, S. L., and E. J. Rubin. 2002. Construction of a mariner-based transposon for epitope-tagging and genomic targeting. Gene 296:179-85.
5. Chaveroche, M. K., J. M. Ghigo, and C. d'Enfert. 2000. A rapid method for efficient gene replacement in the filamentous fungus Aspergillus nidulans. Nucleic Acids Res 28:E97.

Table S2. Primers used in this study.

List of primers used to determine transposon (Tn) and Mu insertion points within the genome of mutants partially resistant to G2cps, for gene inactivation and overexpression and for verification of mutations performed.

Primers	Sequence
Determination of Tn and Mu insertion points	
Mu1470.500-5	5'-GTTACTTTTCAAAATTTAAAC-3'
Mu1470.500-3	5'-CGCAGATAATCTGCAATCAG-3'
ARBN1	5'-GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT-3'
ARBN6	5'-GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC-3'
ARBbis	5'-GGCCACGCGTCGACTAGTAC-3'
pscIR2Km	5'-CTGACCGCTTCCTCGTGCTTTACGG-3'
pscIR2Kmbis	5'-TTCTGAGCGGGACTCTGGGGTACG-3'
MuR200-5	5'-AATTTAATCAGTATCGCTAC-3'
MuL200-3	5'-GTTTTGAACGTTTTTTGAAG-3'
MuR100-5	5'-ATGTAATGAATAAAAAGCAG-3'
MuL100-3	5'-CTAAAATTTGCACTACAGGC-3'
Gene inactivation	
yjgZ.500-5	5'-CACTGGTACTGCAAGAGGGGGCCT-3'
yjgZ.Cm.L3	5'-CTGCGAAGTGATCTTCCGTCACAGGAAGGGCTGAATCTCTCCC TGAAAC-3'
yjhB.Cm.L5	5'-GATGAGTGGCAGGGCGGGGCGTAATGGCTAAATGATTGGAGG CTTTAT-3'
yjhB.500-3	5'-CATAATATGCCCGGCCATAAAGGT-3'
ybiL.500-5	5'-GATCTGGAGGATAAACTGGCA-3'
ybiL.Cm.L3	5'-CTGCGAAGTGATCTTCCGTCACAGGTTTGCAGGTGACTTTTTCT TATAT-3'
ybiL.Cm.L5	5'-GATGAGTGGCAGGGCGGGGCGTAATTCAGATGTGGGGCGCAG GCC-3'
ybiL.500-3	5'-TGTGGATGGCTGCGTACTGCG-3'
araF.500-5	5'-CGGTTATTACACCATTTCAAAAAC-3'
araF.KmFRT.L3	5'-GAAGCAGCTCCAGCCTACACAGGTTCTCTCCAGCTTTAGTGTC GT-3'
araF.KmFRT.L5	5'-GAACTTCGGAATAGGAACTAAAGAAGAACTGGAGAAAAAAGG TT-3'
araF.500-3	5'-TCCGGCACGAGATGCAGTTCCTGGT-3'
yecI.500-5	5'-GAACACGTATAATGAGAGCCATC-3'
yecI.KmFRT.L3	5'-GAAGCAGCTCCAGCCTACACAACCTAATATCCTTATATCCAGA AG-3'
yecI.KmFRT.L5	5'-GAACTTCGGAATAGGAACTAACATCATCGGCGCTAATGCATTG CG-3'
yecI.500-3	5'-GGCTTTCAAAATGCTATCAGGAG-3'
araF.Cm.L5-L3	5'-CTGCGAAGTGATCTTCCGTCACAGGAGAAGAACTGGAGAAAA AAGGTT-3'
yecI.Cm.L5	5'-GATGAGTGGCAGGGCGGGGCGTAACATCATCGGCGCTAATGC ATTGCG-3'
yeeW.500-5	5'-CTGTATTACCCGGGCAGGCGG-3'
yeeW.Cm.L3	5'-CTGCGAAGTGATCTTCCGTCACAGGTTCGCCTCCGGATACTTA CCCAGG-3'
yoeF.Cm.L3	5'-CTGCGAAGTGATCTTCCGTCACAGGGGAACGTAGTTCTCACCA ATAAAT-3'
yoeF.500-5	5'-GGCAAGAGAAGATGTCCTTACCCT-3'
yegO.500-5	5'-CATGATGATCGACTTCGCGCTGGCT-3'
yegO.Cm.L3	5'-CTGCGAAGTGATCTTCCGTCACAGGTTACGCCTCCTCTTCATG ACGG-3'
yegO.Cm.L5	5'-GATGAGTGGCAGGGCGGGGCGTAAATGACAGATCTTCCCGAC AGCACCC-3'
yegO.500-3	5'-CCGCCAACGCCCTGTAACGCGC-3'

Gene overexpression	
araF.KmRexTet.L-3	5’-GAGAATCCAAGCACTAGTAACCACTCGTTTTGTGTAGGGCAAA AACG-3’
araF.KmRexTet.L-5	5’-CACATCAGCAGGACGCACTGACCGACACTAAAGCTGGAGAGA ACCATG-3’
araF.KmRexTet.500-3	5’-GTACGGCGGCGGGCGGTATCCAGT-3’
yecI.KmRexTet.L-3	5’-GAGAATCCAAGCACTAGTAACCACGAAGTGGAGAGGTGCAAG ATAAAT-3’
yecI.KmRexTet.L-5	5’-CACATCAGCAGGACGCACTGACCGATGGATATAAGGATATTA GGTATGG-3’
yecI.KmRexTet.500-3	5’-TGACACGACATTCAGAACATG-3’
Verification of genetic modifications	
yjhB.ext5	5’-ATACTGCAAAGGAAAACAGCTA-3’
yjhB.ext3	5’-CGTGCATATTGAACCCCATTGA-3’
tar.ext5	5’-CACGGTAGTTATCGTCCTGAAT-3’
tar.ext3	5’-TTGCAATCCCCTGACGTGCTC-3’
ptsH.ext3	5’-CTCTTCTGGAGCAGCTGTTG-3’
ptsH.ext5	5’-ACTTCTTGCTGGAACATTGTATTTC-3’
ydcF.ext5	5’-GAAGACGTGAAAGAGATCCGT-3’
ydcF.ext3	5’-GCGTTCAATAGCAGGCAACGCT-3’
pflB.ext5	5’-CCTGATGGTATGTCTGGCA-3’
pflB.ext3	5’-GAAGCGTTCATAAAGTGGC-3’
yggJ.ext5	5’-ATGGCGATCGCGGCAACTTTAT-3’
yggJ.ext3	5’-CTTGTTAACGATCAGCGTCCCTTTCT-3’
pflB.ATG+75-3	5’-CTGAATGAAGTCACGGACGTT-3’
dinG.ext5	5’-TCTTTGACCGACTCCCAGTTTT-3’
dinG.ext3	5’-GCACGCGGGTTGGATCTTCGCT-3’
ycdG.ext5	5’-TCTGCAGTGTGACCGATACGCC-3’
ycdG.ext3	5’-TTTGCAGAAGACCGCGAAAGAG-3’
ybiL.ext5	5’-AACTGGCGCATCAAACCTAAGC-3’
ybiL.ext3	5’-AACAGCGTGGCAGAAAGGTCAG-3’
araF.ext5	5’-TTTCCACATCTTTGAGTTGC-3’
araF.ext3	5’-GGCAGCTGGCCGAGATAG-3’
yecI.ext5	5’-ACGTCATTCATTTCGTCATT-3’
yecI.ext3	5’-GATGTGACTATCGCTGAAG-3’
phnD.ext5	5’-GGTGCATTTTGCCCATCAG-3’
phnD.ext3	5’-GGTTTTCGGCGCTCATCAG-3’
yeeW.ext5	5’-ATCTGCATATCAGGAAAATCTT-3’
yoeF.ext5	5’-ACAGCACTGTTGTCCTGTATTAAG-3’
yegO.ext5	5’-GTTGCTGATTGCTGGTAGCGAACT-3’
yegO.ext3	5’-CCATATATTGCTCGCGCGGTACG-3’

SUPPLEMENTARY FIGURES

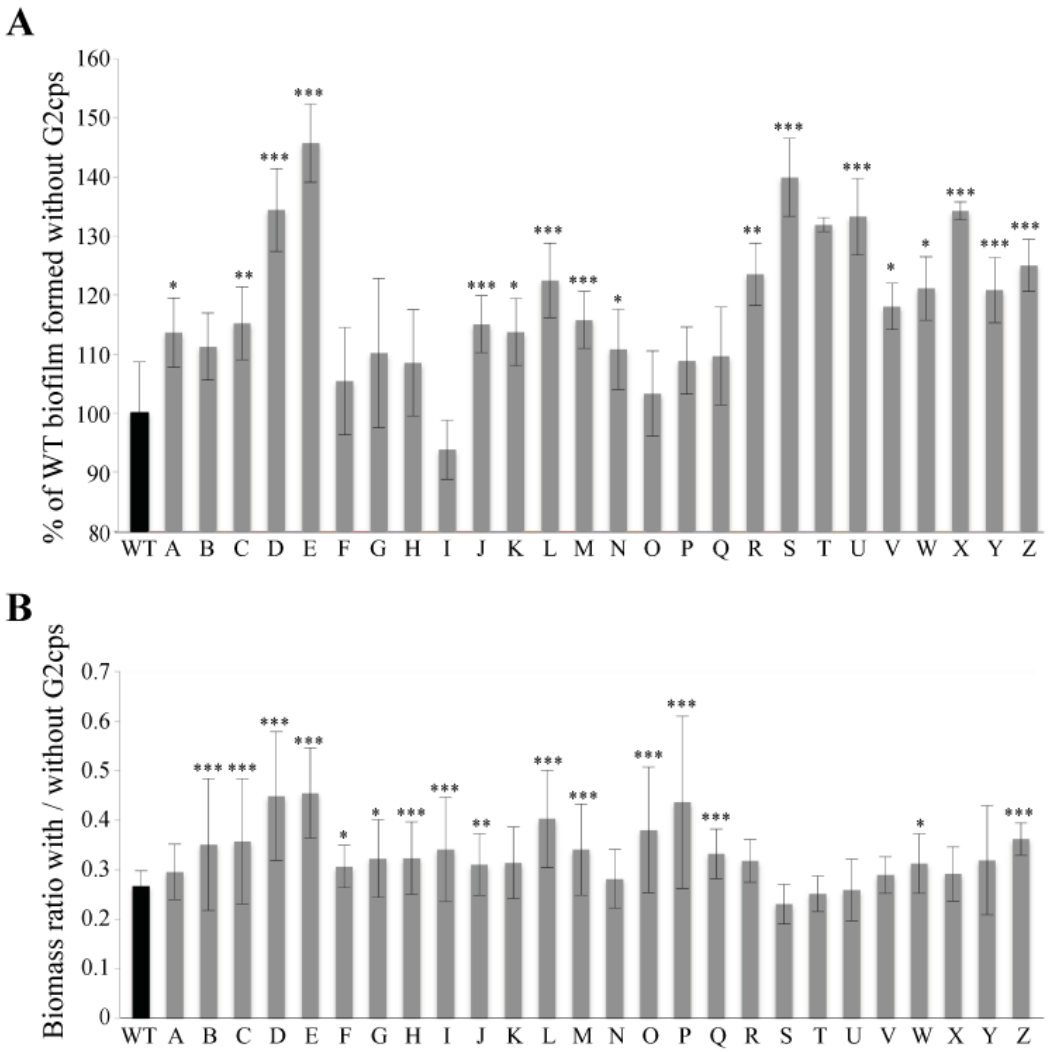

Fig. S1. Determination of mutants partially resistant to G2cps.

(A) Quantification of biofilm formed by WT TG1-c and the 26 mutants partially resistant to G2cps without G2cps. Biofilm quantities were standardized and p-values were determined according to biofilm formed by WT (black) (value: 100). **(B)** WT and mutant biofilm ratio determined after division of the biomass formed in the presence of G2cps by the biomass formed without G2cps. The higher the biofilm ratio, the more resistant the mutant is. p-values were determined compared to WT biofilm ratio (black).

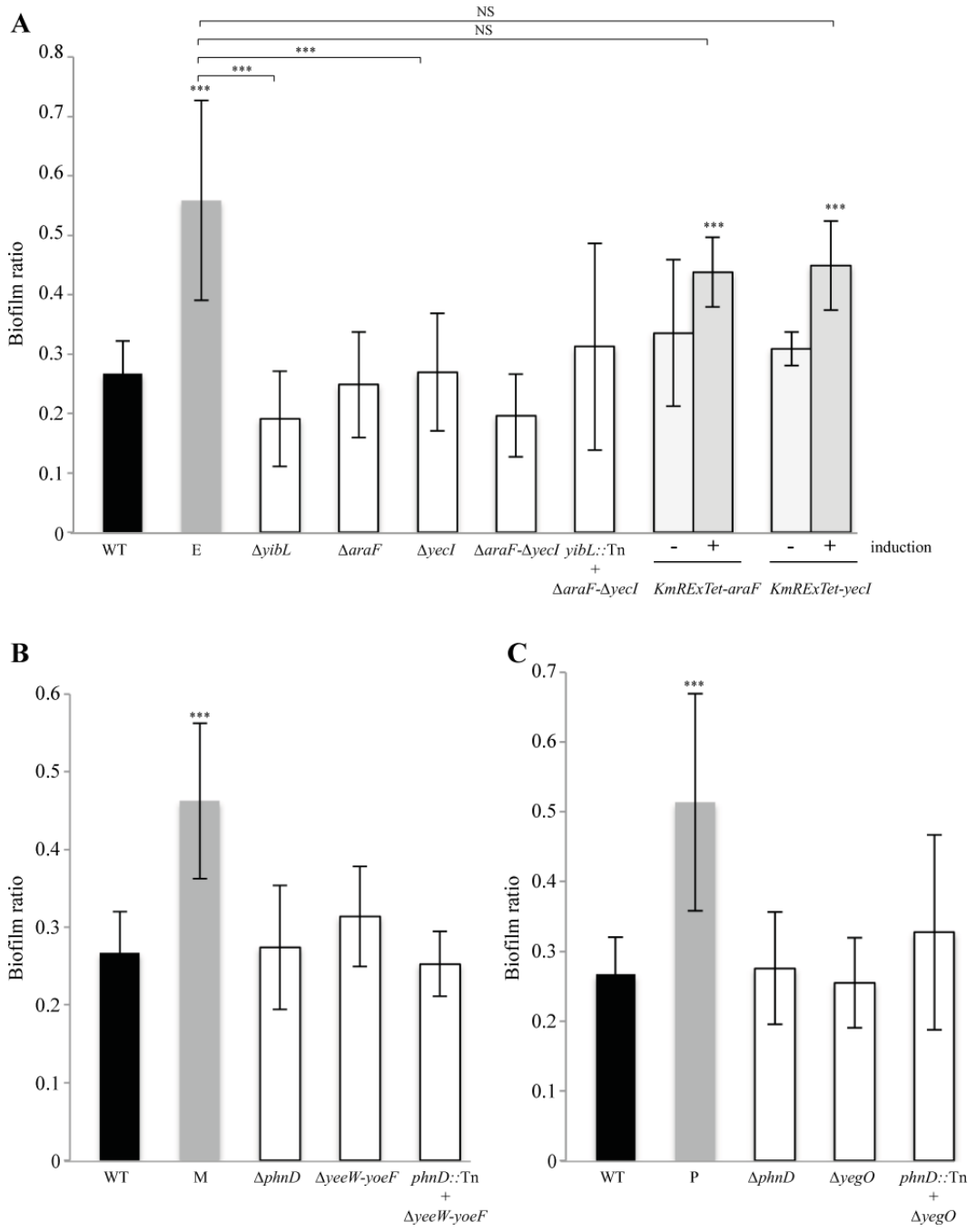

Fig. S2. Determination of genes implicated in partial resistance to G2cps.

Biofilm ratio of WT TG1-c, mutants E, M and P partially resistant to G2cps and either single or multiple deletion and transduction mutants, as well as inducible overexpression mutants, carried out according to the different insertion points of both the transposon (Tn) and Mu within the genome of mutants E **(A)**, M **(B)** and P **(C)**. p-values were determined compared to both the WT biofilm ratio (black) and E, M or P mutants (gray).