Environ Health Perspect

DOI: 10.1289/EHP6635

Supplemental Material

The 'SELection End points in Communities of bacTeria' (SELECT) Method: A Novel Experimental Assay to Facilitate Risk Assessment of Selection for Antimicrobial Resistance in the Environment

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Figure S4. SELECT predicted no effect concentrations for resistance ($PNEC^R$s, logged) were determined for four antibiotics, using wastewater samples from two different time points (2016 and 2018), two different wastewater treatment plants (WWTPs 'A' serving circa. 43,000, and 'B' serving circa. 77,000) and two different wastewater types (influent and effluent). Bars represent the test concentrations directly above and below the no observed effect concentrations used to determine the $PNEC^R$.

Figure S5. Logged SELECT predicted no effect concentrations for resistance ($PNEC^R$s) were determined for four antibiotics using wastewater treatment plant (WWTP) A influent (2018) samples using Iso-sensitest broth or artificial sewage, at 20 °C or 37 °C. Bars represent the test concentrations directly above and below the no observed effect concentrations used to determine the $PNEC^R$.

References

Additional File- Excel Document

Table S1. All primers and gBlocks used in this study for targeting 16S rRNA, *CTX-M* group, *ermF, qnrS* and *intI1* genes.

Gene target	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product size (bp)	gBlock sequence and length (bp)	References
16S rRNA	CGGTGAATACGTTCYCGG	GGWTACCTTGTTACGACT	142	ACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG - 144	(Suzuki et al. 2000) (Murray et al. 2018)
CTX-M group	ATGTGCAGYACCAGTAARGTKATGGC	ATCACKCGGRTCGCCXGGRAT	~300	GATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT	(Birkett et al. 2007) (Murray et al. 2018)

				CCGGGCGATCCGCGTGATA - 338	
ermF	TCTGGGAGGTTCCATTGTCCT	ACTTTCAGGACCTACCTCATAGA		TCTGATGCCCGAAATGTTCAAGTTGTCGGTTGTGATTTTAGGAATTTTGCAGTTCCGAATTTCCTTTCAAAGTGGTGTCAAATATTCTTATGGCATTACTTCCGATATTTTCAAAATCTGATGTTTGAGAGTCTTGGAAATTTTCTGGGAGGTTCCATTGTCCTTCAATTAGAACCTACACAAAAGTTATTTTCGAGGAAGCTTTACAATCCATATACCGTTTTCTATCATACTTTTTTTGATTTGAAACTTGTCTATGAGGTAGGTCCTGAAAGTTTCTTGCCACCGCCA-294	(Stanton et al. 2020)
qnrS	CGACGTGCTAACTTGCGTGA	GGCATTGTTGGAAACTTGCA		TTCGACGTGCTAACTTGCGTGATACGACATTCGTCAACTGCAAGTTCATTGAACAGGGTGATATCGAAGGCTGCCACTTTGATGTCGCAGATCTTCGTGATGCAAGTTTCCAACAATGCCAACTT - 125	(Colomer-Lluch et al. 2014) (Stanton et al. 2020)

intI1	GCCTTGAT GTTACCCG AGAG	GATCGGT CGAATGC GTGT		GGCCTTGATGTTACCC GAGAGCTTGGCACCC AGCCTGCGCGAGCAG CTGTCGCGTGCACGG GCATGGTGGCTGAAG GACCAGGCCGAGGG CCGCAGCGGCGTTG CGCTTCCCGACGCC CTTGAGCGGAAGTA TCCGCGCGCCGGG CATTCCTGGCCGTG GTTCTGGGTTTTTG CGCAGCACACGCA TTCGACCGATCC - 198	(Barraud et al. 2010) (Stanton et al. 2020)

For Excel Tables S1, S2, S3 & S4 see attached excel sheet.

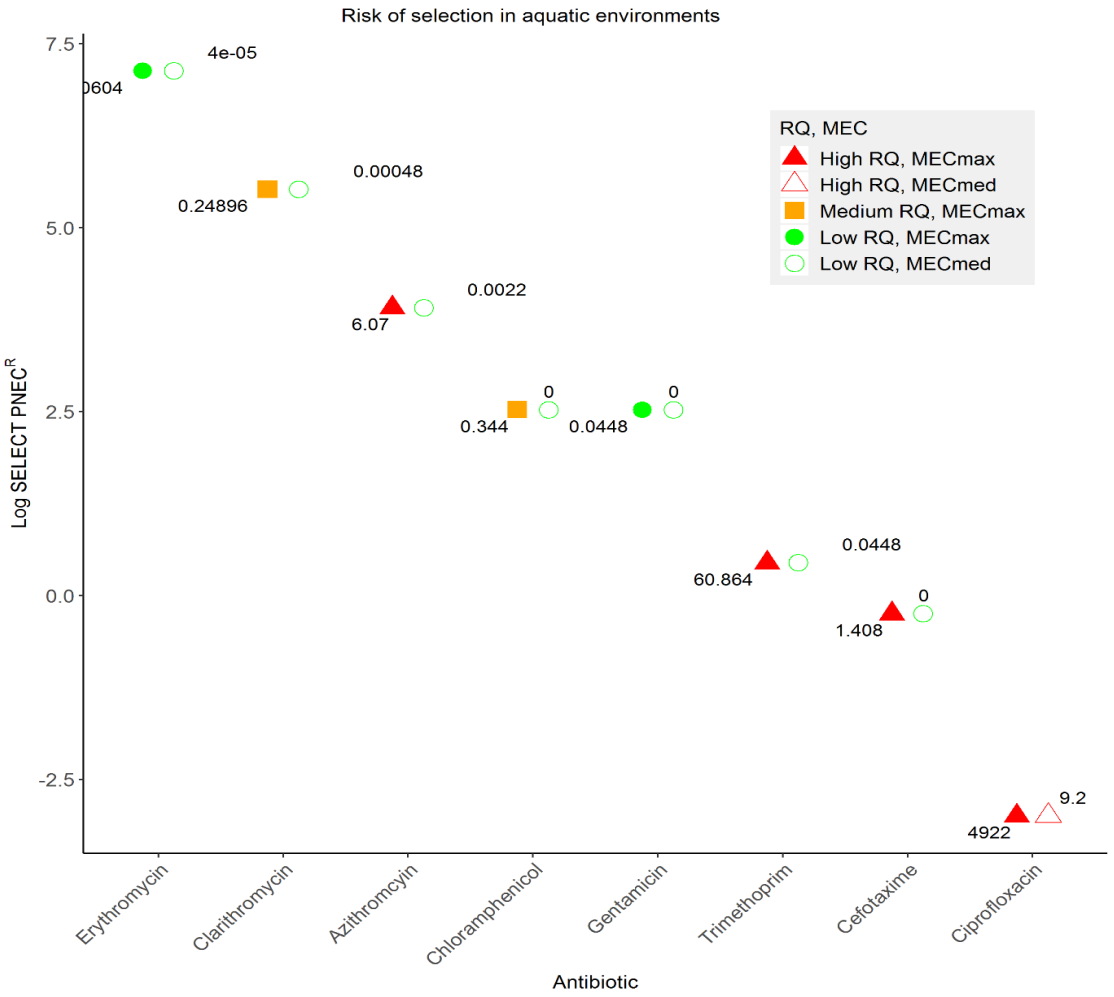

Figure S1. Risk Quotients (RQs) = lowest determined SELECT predicted no effect concentration for resistance ($PNEC^R$)/maximum measured environmental concentration (MEC_{max}) or median measured environmental concentration (MEC_{med}). MEC_{med} values include the non-detects. Measured environmental concentrations were extracted for all antibiotics except gentamicin from the UmweltBundesamt (2019) pharmaceuticals in the environment database. For gentamicin, predicted environmental concentration data were used. Risk was broadly classified in a traffic light colour system. Red, bold triangle = High risk (RQ >1), using MEC_{max}; red, empty triangle = high risk (RQ >1), using MEC_{med}; orange, bold square = medium risk (RQ > 0.1 and <1), using MEC_{max}; green, bold circle = low risk (RQ < 0.1), using MEC_{max}; green, empty circle = low risk (RQ < 0.1), using MEC_{med}. Exact RQ values are reported.

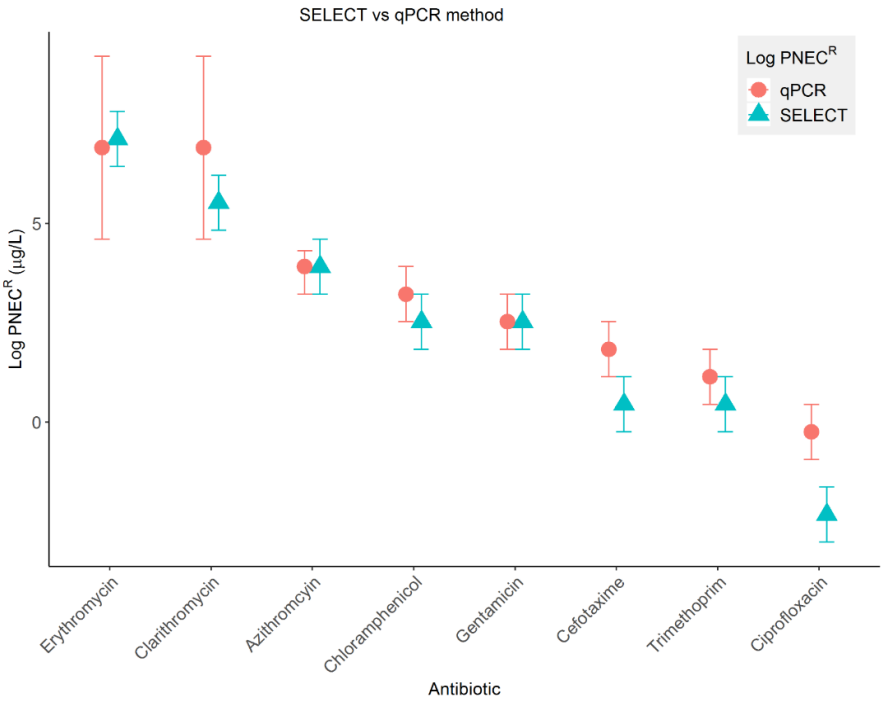

Figure S2. Predicted no effect concentrations for resistance (PNEC^{R}s,logged) determined using the SELECT (blue triangle) and qPCR methods (pink circle). Error bars represent the test concentrations directly above and directly below the NOECs used to calculate the PNEC^{R}s. All SELECT PNEC^{R}s were determined by taking the no observed effect concentrations and applying an assessment factor of 10. For all qPCR PNEC^{R}s, the *intI1* gene target is presented. QPCR PNEC^{R}s for azithromycin, clarithromycin, erythromycin and ciprofloxacin were taken from Stanton *et al.* (2020).

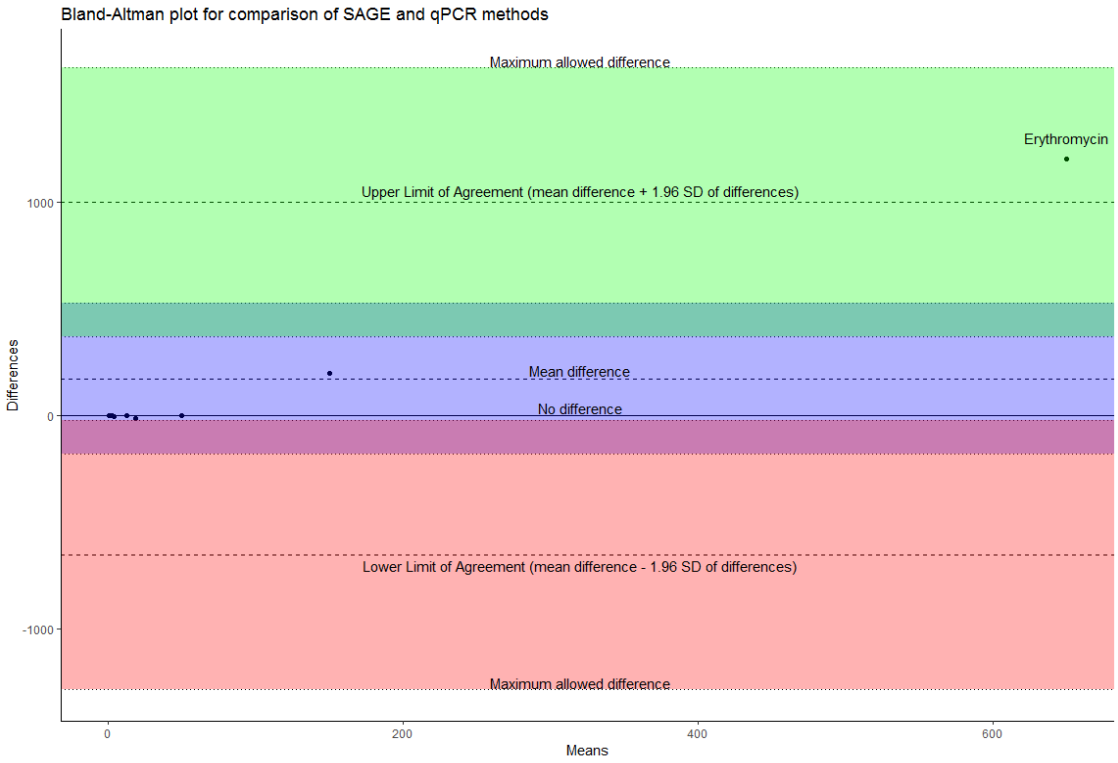

Figure S3. Bland-Altman analysis to compare the SELECT and qPCR-derived $PNEC^R$s. QPCR $PNEC^R$s for azithromycin, clarithromycin, erythromycin and ciprofloxacin were taken from Stanton *et al.* (2020).

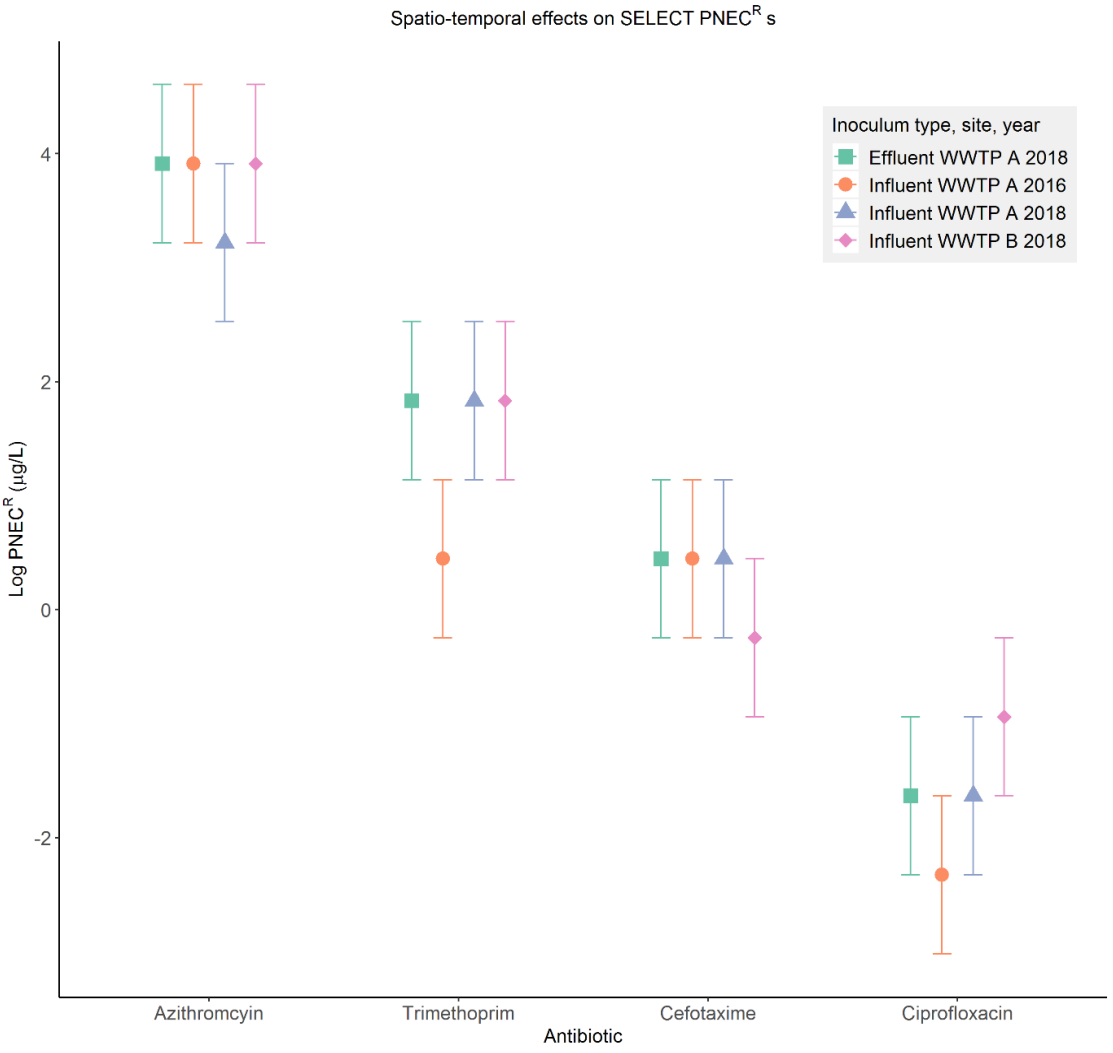

Figure S4. SELECT predicted no effect concentrations for resistance (PNEC^{R}s, logged) were determined for four antibiotics, using wastewater samples from two different time points (2016 and 2018), two different wastewater treatment plants (WWTPs 'A' serving circa. 43,000, and 'B' serving circa. 77,000) and two different wastewater types (influent and effluent). Bars represent the test concentrations directly above and below the no observed effect concentrations used to determine the PNEC^R.

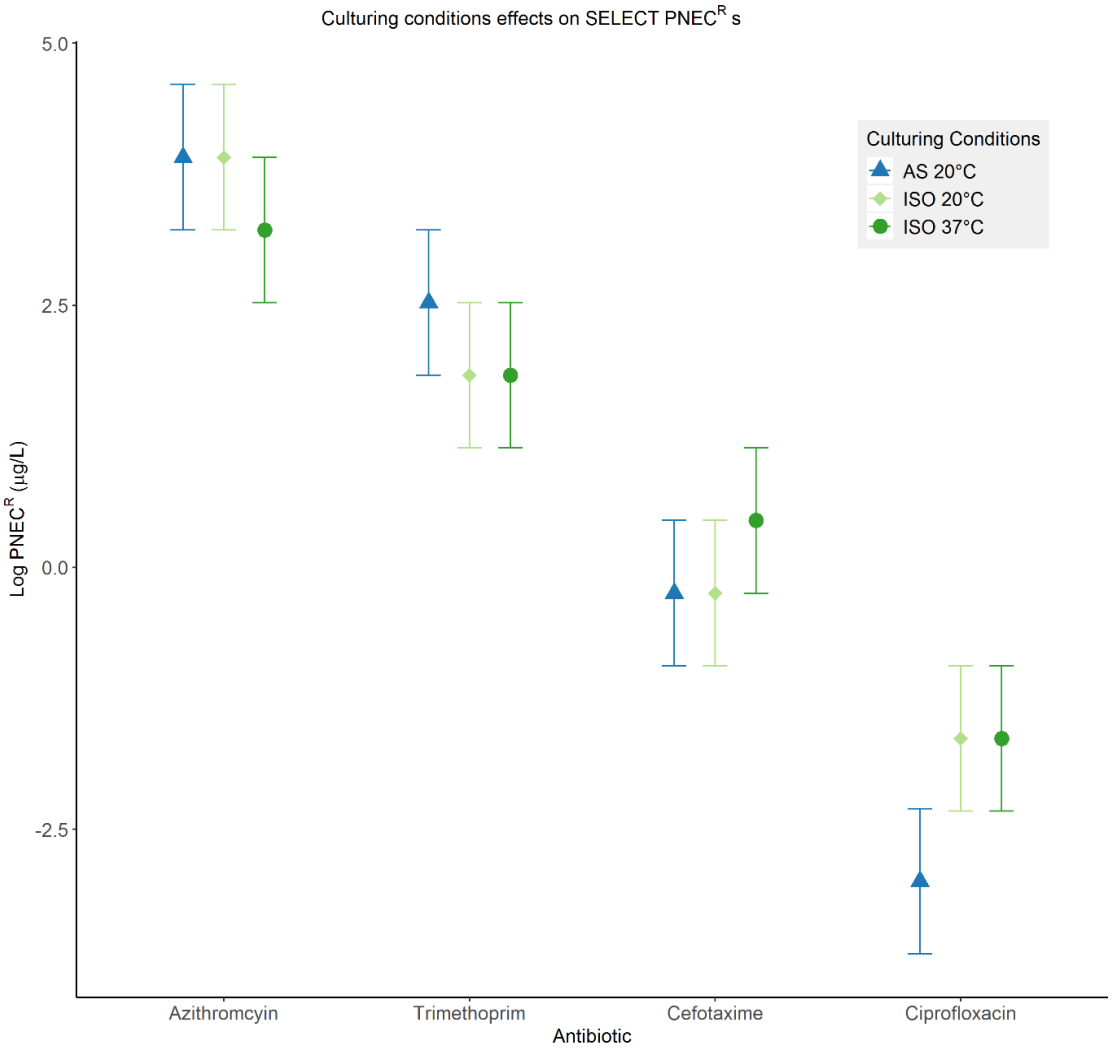

Figure S5. Logged SELECT predicted no effect concentrations for resistance ($PNEC^R$s) were determined for four antibiotics using wastewater treatment plant (WWTP) A influent (2018) samples using Iso-sensitest broth or artificial sewage, at 20 °C or 37 °C. Bars represent the test concentrations directly above and below the no observed effect concentrations used to determine the $PNEC^R$.

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