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**Effect of residual doxycycline concentrations on resistance
selection and transfer in porcine commensal *Escherichia coli***

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35

25 **1. Abstract**

26 Pig feed may contain various levels of antimicrobial residues due to cross-contamination. A
27 previous study showed that a 3% carry-over level of doxycycline (DOX) in the feed results in
28 porcine faecal concentrations of approximately 4 mg/L.

29 This study aimed to determine the effect of residual DOX concentrations (1 and 4 mg/L) *in*
30 *vitro* on selection of DOX resistant porcine commensal *E. coli* and transfer of their resistance
31 plasmids.

32 Three different DOX resistant porcine commensal *E. coli* strains and their plasmids were
33 characterized. These strains were each brought in competition with a susceptible strain in a
34 medium containing 0, 1 and 4 mg/L of DOX. Resistant bacteria, susceptible bacteria and
35 transconjugants were enumerated after 24h and 48h.

36 The *tet(A)* carrying plasmids showed genetic backbones that are also present among human *E.*
37 *coli* isolates. Ratios of resistant to susceptible bacteria were significantly higher at 1 and 4 mg/L
38 DOX compared to the blank control, but no significant difference between 1 and 4 mg/L was
39 seen. Plasmid transfer frequencies were affected by 1 or 4 mg/L DOX in the medium for only
40 one of the resistance plasmids.

41 In conclusion, DOX concentrations of 1 and 4 mg/L can select for resistant *E. coli in vitro*.

42 Further research is needed to determine the effect of these concentrations in the complex
43 environment of the porcine intestinal microbiota.

44 **Keywords**

45 Doxycycline, residues, *E. coli*, resistance selection, plasmid transfer

46 **Abbreviations**

47 cfu, colony forming units; DOX, doxycycline; LB, Miller's LB broth; LC-MS/MS, liquid
48 chromatography – tandem mass spectrometry; MC, MacConkey n°3 agar; MIC, minimum

49 inhibitory concentration; RIF, rifampicin; R/S, ratio of resistant to susceptible bacteria; SD,
50 standard deviation

51 **2. Introduction**

52 Administration of antimicrobial drugs through feed medication is frequently applied in the pig
53 industry. These medicated feeds may cause cross-contamination of non-medicated feed at the
54 feed mill, during transport and/or at farm level [1]. In a previous *in vivo* study [2], the intestinal
55 and faecal concentrations of doxycycline (DOX), chlortetracycline and sulfadiazine-
56 trimethoprim, were determined in pigs. The animals were administered feed containing 3%
57 carry-over levels of these antimicrobials. These unintended concentrations possibly exert a
58 selective pressure on tetracycline resistant bacteria present in the intestinal microbiota and may
59 co-select for other resistance genes carried by these bacteria [3]. Such resistant bacteria can
60 spread to the human gut via different routes, where they could transfer their resistance genes to
61 human commensals and pathogens [4].

62 The present study aimed to investigate the selective pressure on DOX resistant *E. coli* of 4 mg/L
63 and 1 mg/L of DOX, corresponding with intestinal DOX concentrations caused by a cross-
64 contamination level of the feed of approximately 3% and 1%, respectively. *E. coli* field strains
65 isolated from pig faeces were selected as model bacteria for the pig microbiota.

66 **3. Materials and Methods**

67 ***3.1. Bacterial strains***

68 Three tetracycline resistant commensal *E. coli* strains (EC 682, EC 202, EC 292) isolated from
69 pig faeces were selected to be used as donor strains in competition experiments. These strains
70 were characterized using standard methods (Table 1).

71 From the same collection, three *E. coli* strains (EC 298, EC 400, EC 588) susceptible to all
72 antimicrobials tested, were selected as recipient strains and to compete with the donor strains.

73 As bacterial fitness plays a major role in competition between strains, bacterial growth curves
74 were set up for each strain separately, in three different conditions (0, 1 and 4 mg/L DOX),
75 using an automated microbiology growth curve analysis system (Figure S1).

76 ***3.2. Conjugation experiments and plasmid analysis***

77 The mobility of the DOX resistance conferring plasmids (DOX^R plasmids) in the selected donor
78 strains was first verified using standard conjugation experiments under blank conditions as
79 described before (recipient *E. coli* J5RIF) [5]. Co-transfer of resistance determinants was
80 verified according to EUCAST guidelines [6] and replicon typing of the DOX^R plasmids was
81 done by PCR [7]. The three susceptible strains were checked for presence of replicons of the
82 same type as the donor plasmids by PCR typing [7]. Subsequently, conjugation experiments [5]
83 with each donor strain were conducted to confirm if the three mobile plasmids could be
84 transferred to the selected susceptible strains.

85 Plasmid DNA (Qiagen plasmid midi kit, Antwerp, Belgium) was then sequenced (Illumina
86 HiSeq 2500 sequencing), assembled (CLC Genomics Workbench version 8.0), annotated
87 (Prokka [8], BLAST) and visualised (BRIG [9]). Nucleotide sequences were deposited in
88 EMBL-EBI [FMWN01000000 (pEC202), FNLQ01000000 (pEC292), FNLQ01000000
89 (pEC682)].

90 ***3.3. Competition experiments***

91 Two types of competition experiments were carried out, each type with two different
92 susceptible strains that matched the respective experiment design (see below). An experiment
93 always involved one recipient strain that was brought in competition with each of the three
94 donor strains separately, and was repeated four times. To be able to distinguish donor strains
95 from recipient strains (red colonies) in mixed cultures on MC, non-lactose fermenting mutants
96 (white colonies) of the donor strains were selected [10].

Experiment type 1. The three donor strains and one of the recipient strains (conjugating strain EC 400 or non-conjugating strain EC 298) were separately grown overnight in LB broth at 37°C on a horizontal shaker (IKA KS 260 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) and then mixed in a 1/1000 ratio (donor/recipient) in three different tubes with LB with a final volume of 30 mL and a final concentration of 0, 1 and 4 mg/L DOX, respectively. These cultures were incubated at 37°C during 48h on a horizontal shaker. After 24h and 48h of incubation, 1 mL was taken from each tube and appropriate dilutions were plated in duplicate on MC with and without 8 mg/L DOX and incubated overnight at 37°C. The donor strain was enumerated by counting white colonies on MC+DOX plates and the total number of recipient bacteria was counted on MC (red colonies).

Experiment type 2 was designed to be able to enumerate transconjugants and was conducted with the three donor strains and conjugating recipient strains EC 400 and EC 588. In this way, the transfer frequency of the plasmids, the total resistant bacteria (donor + transconjugants) and the total susceptible bacteria (total recipients – transconjugants) could be calculated. Enumeration of transconjugants was made possible by using RIF resistant mutants (EC 400RIF and EC 588RIF) of the conjugating recipient strains. The mutants were selected by consecutive plating on MC plates containing 0 to 200 mg/L RIF. Preparation of the competition cultures was done as in experiment type 1. Appropriate tenfold dilutions were now plated on MC with DOX (8 mg/L) to count the donor strain, with RIF (80 mg/L) to count the recipient (total count) and with DOX (8 mg/L) + RIF (80 mg/L) to count transconjugants (DOX resistant recipient). The transfer frequency of the plasmids was calculated by dividing the number of transconjugants (cfu/mL) by the total number of recipients (cfu/mL). The stability of DOX in LB at both 1 and 4 mg/L during 48h at 37°C was verified using liquid chromatography – tandem mass spectrometry (LC-MS/MS) [11].

3.4. Statistical analysis

After determination of homogeneity of the results, a linear mixed model was used to assess the effect of medium, resistant donor strain, susceptible recipient strain and time on the resistant/susceptible ratio (R/S) and transfer frequency (SPSS 23, IBM, Chicago, IL, U.S.) [12]

4. Results

4.1. Characterization of bacterial strains

The characteristics of the lactose negative mutants of DOX resistant porcine *E. coli* strains EC 682, EC 202 and EC 292 are shown in Table 1. The MIC's of DOX for susceptible strains EC 298, EC 400, EC 400RIF, EC 588 and EC 588RIF were 1, 2, 0.5, 2 and 1 mg/L respectively. The growth curves and growth rates of all strains are given in Figure S1. All susceptible strains showed a lower growth rate at 1 and 4 mg/L DOX than at 0 mg/L DOX. Growth rates of resistant strains EC 202 and EC 292 were hardly affected by 1 or 4 mg/L DOX, while 4 mg/L DOX reduced the growth rate of EC 682 by 0.0037 min⁻¹ (Figure S1).

4.2. Genetic mapping of *tet(A)*-carrying plasmids

Replicon typing showed that plasmids pEC682, pEC202 and pEC292 belonged to the IncII, IncFII, and the IncFII-FIB group, respectively. Plasmid sequencing of pEC682 revealed that the *tet(A)* gene was flanked by *tetR* and located on a transposon (Tn7). BLAST analysis showed that this plasmid shared nucleotide sequence identity with other plasmids previously isolated from chicken caeca, human feces and pig feces (Figure S2).

Comparison of nucleotide sequences of pEC202 and pEC292 revealed that their direct environment of *tet(A)* was identical (flanked by *tetR* and on a transposon) and that they had the same backbone which was also shared with a plasmid from a previously described human *E. coli* (Figure S3).

All susceptible strains, except for EC 298 that carried an IncFIB plasmid, were negative for plasmids of the same Inc group as plasmids pEC682, pEC202 and pEC292.

4.3. Residual DOX concentrations (1 and 4 mg/L) can select for DOX resistant E. coli

Figure 1 shows the ratios between DOX resistant strains EC 682, EC 202 and EC 292 and susceptible strains EC 298, EC 400, EC 400RIF and EC 588RIF during 48h of competition in LB with different concentrations of DOX. EC 400 and its RIF resistant mutant EC400RIF were included as different strains because of their difference in growth rate and MIC for DOX. The selective effect on the resistant strain in the competition experiments is represented by the resistant/susceptible ratio (R/S).

For every strain combination, statistical analysis revealed a significant higher R/S at 1 and 4 mg/L DOX when compared to the blank LB. In contrast, no significant difference in R/S was found between 1 and 4 mg/L DOX.

The R/S in DOX media for susceptible strain EC 298 was slightly lower after 48h of incubation than after 24h, whereas the opposite occurred for the other susceptible strains.

Enumeration data of donor, recipient and transconjugant strains and data used for statistical analyses of R/S and transfer frequencies are given in Table S3 (EC 298), Table S4 (EC 400), Table S5 (EC 400RIF) and Table S6 (EC 588RIF).

The transfer frequencies of plasmids pEC682, pEC202 and pEC292 to recipient strains EC 400RIF and EC 588RIF under the experimental conditions are given in Table S1. The transfer frequency of pEC682 and pEC202 did not increase in LB with 1 or 4 mg/L DOX compared to the blank control. However, the transfer frequency of pEC292 was significantly higher at 1 mg/L DOX than at 0 ($p=0.009$) and 4 mg/L DOX ($p=0.001$) when EC 400RIF was used as recipient and significantly higher at 1 and 4 mg/L (both $p=0.02$) than in blank LB in case of recipient strain EC 588RIF.

LC-MS/MS analysis showed that the recovery of DOX in LB at concentrations of 1 and 4 mg/L ranged from 65% to 76% of the initial concentration after 48h of incubation at 37°C (Table S2).

5. Discussion

Characterization of the *tet(A)* carrying plasmids indicated that all three plasmids had well conserved backbones that circulate between *E. coli* strains from different hosts such as human, poultry and pigs (Figure S2 and S3). In all three characterized resistance plasmids, *tet(A)* was located on a Tn7 transposon, which has the ability to transfer from one plasmid to another plasmid or the chromosome in the same or another cell and therefore can quickly spread resistance genes among bacteria [13].

None of the investigated conjugative plasmids were able to transfer to susceptible strain EC 298 which could be explained by surface exclusion or the presence of a restriction system [14] and in case of EC 292, the presence of plasmids of the same Inc group [7].

Competition between the resistant and susceptible strains at different residual DOX concentrations revealed one main observation: selection of the DOX resistant strain was in all cases significantly higher in LB with 1 and 4 mg/L of DOX compared to the blank control. Surprisingly, no significant difference in selective effect was found between the two concentrations of DOX. This could partially be explained by the growth curve analysis of the susceptible strains (Figure S1). First, growth rates of the susceptible strains showed minor differences when comparing 1 and 4 mg/L of DOX. Second, all strains except EC 400RIF reached the same maximum OD value in both 1 and 4 mg/L of DOX. On the other hand, the rather high standard deviation (SD) values for the mean R/S (Table S3-S6), indicate that more replicates of the experiments might reveal more significant differences in selective effect between 1 and 4 mg/L of DOX.

Not only the presence of DOX in the growth medium affected the competition results, also strain fitness played its role. First, the three resistant strains showed similar growth rates. As a

result, no significant differences in R/S between these strains in competition with any of the susceptible strains were found. In contrast, the susceptible strains did show significant differences in R/S, except when comparing EC 400 and EC 588RIF. These differences are probably mainly caused by the (sometimes small) differences in growth rates (fitness) between the susceptible strains (Figure S1). The differences in fitness between EC 400 and EC 400RIF and between EC 588 and EC 588RIF could possibly be assigned to the RIF mutation [15]. The fitness cost caused by the RIF mutation may also have affected the results of the standard MIC test with fixed incubation time, that showed different MIC values for the original strains and their RIF mutant, respectively.

Susceptible strain EC 298 in particular showed an interesting behaviour: the selective effect on any of the resistant strains in competition with EC 298 decreased with time. This observation is supported by the growth curve of EC 298 (Figure S1) and might be assigned to *de novo* resistance development, adaptive resistance or the potential presence of a resistant determinant, that was not expressed in the initial standard conditions [3, 16].

Only the transfer frequency of pEC292 was increased due to the presence of 1 or 4 mg/L DOX. The lower transfer frequency to recipient EC 400RIF at 4 mg/L than at 1 mg/L could possibly be explained by the lower fitness of EC 400RIF at 4 mg/L than 1 mg/L DOX. Indeed, our experimental design implicates that the transfer frequency is likely a combined result of both plasmid transfer and enrichment of transconjugants [17]. Hence, EC 400RIF transconjugants were probably enriched significantly less at 4 mg/L of DOX than at 1 mg/L.

Interestingly, DOX appeared to be rather unstable under the experimental conditions: after 48h of incubation the recovery ranged between 65-76% (Table S2). This partial recovery was not translated into lower selective effects at 48h compared to 24h of incubation. In contrast, resistant/susceptible ratios were found to be significantly higher after 48h incubation time than 24h incubation time in all cases except for the experiments with EC 298.

To our knowledge no previous studies on the selective pressure of residual concentrations of DOX are available in literature.

In conclusion, this study showed that residual concentrations of DOX (1 and 4 mg/L) have the potential to enrich tetracycline resistant commensal *E. coli in vitro*. Consequently, cross-contamination of pig feed could be an important contributor to the spread of bacterial resistance [3], as important resistance genes in human medicine could be co-transferred together with the tetracycline resistance genes. However, further research is needed to fully understand the long term effect of cross-contaminated feed in the complex environment of the intestinal microbiota.

6. Acknowledgements

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7. Funding

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8. Declarations of interest

Conflicts of interest: none

9. Contributors

LP, PB, FH and AS contributed to the conception and design of the study. LP performed the experiments. LP, SC, GR, HI, ED, JD, MH and AS contributed to the analysis and interpretation of the data. LP and AS drafted the manuscript. All authors revised the manuscript critically and approved the final manuscript.

10. Ethical approval

Not required.

11. References

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12. Figure captions

Figure 1: Results of competition experiments between doxycycline resistant strains EC682, EC202 and EC292 and susceptible recipient strains EC400 (blue), EC400RIF (gray), EC298 (yellow) and EC588RIF (green). The bars represent the ratio of logarithmic transformed counts of resistant bacteria (R, cfu/ml) to logarithmic transformed counts of susceptible bacteria (S, cfu/mL), as indicated on the y-axis. In case of susceptible strains EC 400 and EC 298, R = total counts of donor (cfu/mL) and S = total counts of recipient (cfu/mL). In case of susceptible strains EC 400RIF and EC 588RIF, R = total counts of donor + tranconjugants (cfu/mL) and S = total counts of recipient – transconjugants (cfu/mL). Different concentrations of DOX in the medium (DOX 0 = 0 mg/L; DOX 1 = 1 mg/L; DOX 4 = 4 mg/L) and sampling time points (24h, 48h) are indicated on the x-axis. Significant differences are denoted in Additional files S1, S2, S3 and S4. Note: Statistical analysis was done using the log transformed ratios $\log(R/S)$ but the graph shows $\log(R)/\log(S)$ to obtain a better image.

Table 1. Characteristics of the doxycycline resistant *E. coli* strains (lactose negative mutants) that were used as donor strains in the competition experiments.

Strain n ^o †	Phenotypic resistance profile* (Prevalence)	Co-transferred resistance ^β	MIC DOX [‡] (mg/L)	Tetracycline resistance gene**	Inc-group of <i>tet</i> (A) carrying plasmid (>100kb)
EC 682	AMP-SMX-STR-TET-TMP (6.5%)	AMP-SMX-STR-TET-TMP	16	<i>tet</i> (A)	IncII
EC 202	AMP-CHL-STR-TET (0.3%)	CHL-STR-TET	32	<i>tet</i> (A)	IncFII
EC 292	CHL-SMX-TET-TMP (1%)	CHL-SMX-TET-TMP	32	<i>tet</i> (A)	IncFII-FIB

AMP, ampicillin; SMX, sulphonamides; STR, streptomycin; TET, tetracycline (and doxycycline); TMP, trimethoprim; CHL, chloramphenicol.

† Selected from a collection that was obtained between 2011 to 2013 for an antimicrobial resistance monitoring program of the Belgian Federal Agency for the Safety of the Food Chain [18].

* Determined with Sensititre micro broth dilution (EUVSEC plate, TREK Diagnostic Systems, West Sussex, UK) according to EURL-AR [19] guidelines. The prevalences of the phenotypic resistance profiles in the 2011-2013 collection of porcine *E. coli* strains (total of 569 strains) are indicated between brackets.

^β Determined by disk diffusion (EUCAST guidelines [6]) in triplicate on each J5RIF transconjugant. Transconjugants were obtained as follows: Conjugation experiments with as acceptor a rifampicin (RIF) resistant *E. coli* lab strain (J5RIF) [5] were performed overnight in LB at 37°C with a donor/recipient ratio of 1:5. Transconjugants were grown selectively on MacConkey n°3 agar (MC, Oxoid Ltd, Basingstoke, UK) with 8 mg/L DOX and 80 mg/L RIF (both from Sigma-Aldrich, Bornem, Belgium).

[‡] Determined by the broth microdilution method according to the CLSI document M07-A10.

** Presence of common tetracycline resistance genes *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(G) was verified by PCR assays [20].

Figure S1. Growth curves of non-lactose fermenting mutants of doxycycline resistant *E. coli* EC 682, EC 202 and EC 292, susceptible *E. coli* EC 298, EC 400 and EC 588 and rifampicin resistant mutants EC 400RIF and EC 588RIF.

In each well of a 96-well plate, 100 μ L of an overnight bacterial culture in Miller's LB broth (LB) of approximately 10^5 colony forming units (cfu)/mL was added to 100 μ L of LB with a final concentration of 0, 1 or 4 mg/L DOX, respectively. The plate was incubated at 37°C in an automated microbiology growth curve analysis system (Infinite 200Pro, Tecan Group Ltd., Männedorf, Switzerland) during 18h and every 5 min OD₆₀₀ was measured.

Each graph compares the growth of a strain in blank LB broth and LB with 1 and 4 mg/L doxycycline, incubated at 37°C during 18h (mean values of triplicates). The y-axis represents ln transformed OD₆₀₀ values and the x-axis shows the incubation time (hours). The growth rates (μ , min⁻¹) were calculated manually according to Hall *et al.*¹ and are given in the figure legends. The growth curves of the resistant strains EC 682, EC 202 and EC 292 represent the lactose negative mutants of these strains. Growth curves of the original lactose fermenting strains were also performed but are not shown. These growth curves did not differ from the growth curves of the lactose negative mutants.

1. Hall BG, Acar H, Nandipati A et al. Growth Rates Made Easy. *Molecular Biology and Evolution* 2013; **31**: 232-8.

[illegible]

Relevant genes are indicated with an arrow.

Plasmid sequencing of pEC682 revealed that the *tet(A)* gene (Figure 1) was flanked by *tetR* and located on a transposon (Tn7), together with mercury resistance genes. The co-transfer of four other resistance determinants together with the tetracycline resistance (Table 1) was confirmed by the presence of the corresponding resistance genes on pEC682: the *bla*_{TEM-1} gene encoding resistance to narrow-spectrum β -lactamases was located on a second transposon (Tn3) close to an integron class 1 that carried genes conferring resistance to sulphonamides (*sul2*), streptomycin (*strA* and *strB*) and trimethoprim (*dhfr1*) and a multidrug efflux pump coding gene (*emrE*). BLAST analysis showed that this plasmid shared nucleotide sequence identity with other plasmids previously isolated from chicken caeca (pE17.16, accession number CP008733) [1], human feces (FHI4, accession number LM995927) and pig feces (PND11 107, accession number HQ114281) [2].

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Figure S3

Figure S3: Partial nucleotide sequences of plasmid pEC292 (yellow) and pEC202 (light blue) compared with plasmid pH1038-142 of human origin (purple). Relevant genes are indicated with an arrow. Note: the gene denoted by 'bcr' encodes for a drug transporter of the *bcr/CfiA* subfamily conferring resistance to chloramphenicol.

The *tet(A)* gene of pEC292 was flanked by *tetR* and located on a Tn7 transposon that also carried genes conferring resistance to streptomycin, chloramphenicol and trimethoprim (*ant1_1* and *ant1_2*, drug transporter of *bcr/CfiA* subfamily, *dfrA*, respectively). This explains the co-transfer of these resistances (Table 1). Additionally, the transposon carried metal resistance coding genes and a multidrug efflux pump encoding gene. For plasmid pEC202, the direct environment of *tet(A)* was identical to that of pEC292. Both pEC202 and pEC292 shared their backbone sequence with a plasmid from a previously described human *E. coli* isolate (accession number KJ484634) [1].

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Table S1. Transfer frequency of resistance plasmids to susceptible *E. coli* EC 400RIF and EC 588RIF

	EC 400RIF		EC 588RIF	
	24h	48h	24h	48h
Blank	1,58E-05	1,85E-03	1,57E-06	6,88E-05
1 mg/L DOX	1,69E-05	1,30E-03	3,36E-06	1,19E-04
4 mg/L DOX	1,01E-05	9,98E-04	7,35E-07	7,46E-04
Blank	1,04E-05	1,73E-04	1,59E-05	3,43E-04
1 mg/L DOX	8,27E-05	6,95E-04	6,08E-06	9,68E-04
4 mg/L DOX	3,82E-05	9,17E-05	1,85E-07	1,51E-05
Blank	3,21E-05	5,20E-04	5,04E-07	6,36E-06
1 mg/L DOX	1,78E-04	2,22E-03	2,83E-05	9,24E-04
4 mg/L DOX	5,88E-05	7,79E-05	1,97E-05	1,53E-02

Mean values of 4 replicates. Transfer frequency was defined as the number of transconjugants (cfu/mL) divided by the total number of recipients (cfu/mL) and was determined in three types of LB medium: Blank, 1 mg/L DOX and 4 mg/L DOX. Enumerations were performed after 24h and 48h of competition between donor and recipient strain.

Table S2: Stability of DOX in LB broth (30 mL) with or without inoculation of strain EC 682 during 48 hours of incubation at 37°C on a horizontal shaker.

Initial DOX concentration (mg/L)	Medium	Percentage of initial concentration after 23h	Percentage of initial concentration after 48h
	LB	88	70
	LB + EC 682	82	65
	LB	86	76
	LB + EC12 682	87	71

Table S3. Competition experiment (type 1) between susceptible *E. coli* EC 298 and three tetracycline resistant *E. coli* strains (EC 682, EC 202, EC 292)

Number of Resistant Bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	6,00E+06	2,01E+07	3,51E+07	1,46E+07	1,89E+07	1,06E+07	nd	1,62E+07	3,26E+07	3,35E+07	2,74E+07	7,95E+06
	1 mg/L DOX	1,19E+08	3,44E+08	1,62E+08	1,71E+08	1,99E+08	8,62E+07	6,20E+07	3,21E+08	1,01E+08	2,28E+08	1,78E+08	1,03E+08
	4 mg/L DOX	5,45E+07	1,40E+08	1,19E+08	1,28E+08	1,10E+08	3,30E+07	2,19E+07	6,45E+07	4,50E+07	2,13E+07	3,82E+07	1,80E+07
	Blank	4,50E+06	8,84E+07	3,71E+07	2,40E+07	3,85E+07	3,10E+07	1,05E+07	4,49E+07	7,06E+07	5,53E+07	4,53E+07	2,21E+07
	1 mg/L DOX	7,15E+07	2,25E+08	8,75E+07	1,27E+08	1,28E+08	5,95E+07	1,93E+07	2,03E+07	1,19E+08	2,56E+07	4,59E+07	4,20E+07
	4 mg/L DOX	3,65E+07	2,15E+08	1,48E+08	1,35E+08	1,34E+08	6,36E+07	1,72E+07	8,25E+06	1,40E+07	1,66E+07	1,40E+07	3,53E+06
	Blank	1,05E+07	8,61E+07	1,57E+07	4,24E+07	3,87E+07	2,99E+07	9,73E+07	8,38E+07	7,76E+07	4,63E+07	7,62E+07	1,87E+07
	1 mg/L DOX	8,16E+07	3,09E+08	8,90E+07	8,80E+07	1,42E+08	9,63E+07	2,54E+08	1,97E+08	1,65E+08	1,08E+08	1,81E+08	5,26E+07
	4 mg/L DOX	6,11E+07	2,86E+08	7,65E+07	1,15E+08	1,34E+08	8,94E+07	3,89E+07	3,05E+07	4,80E+07	1,32E+08	6,24E+07	4,07E+07
Number of Susceptible Bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	4,50E+08	8,15E+08	4,17E+08	5,35E+08	5,54E+08	1,57E+08	3,20E+08	1,33E+09	6,60E+08	5,35E+08	7,10E+08	3,75E+08
	1 mg/L DOX	1,98E+08	6,55E+07	8,50E+07	1,62E+08	1,28E+08	5,42E+07	1,15E+08	2,77E+08	7,80E+07	1,13E+08	1,46E+08	7,70E+07
	4 mg/L DOX	6,50E+07	3,10E+07	2,55E+07	3,40E+07	3,89E+07	1,54E+07	2,85E+07	2,27E+08	2,86E+07	4,62E+07	8,24E+07	8,35E+07
	Blank	3,71E+08	6,20E+08	6,25E+08	2,45E+08	4,65E+08	1,64E+08	6,50E+08	9,30E+08	9,40E+08	8,50E+08	8,43E+08	1,16E+08
	1 mg/L DOX	1,36E+07	3,50E+07	2,90E+06	1,63E+07	1,69E+07	1,16E+07	5,65E+06	4,15E+06	2,45E+07	3,83E+06	9,53E+06	8,67E+06
	4 mg/L DOX	5,60E+06	1,60E+07	6,85E+06	3,00E+06	7,86E+06	4,90E+06	4,30E+06	8,75E+06	4,20E+06	2,15E+06	4,85E+06	2,41E+06
	Blank	3,65E+08	4,50E+08	3,55E+08	3,45E+08	3,79E+08	4,18E+07	4,55E+08	5,00E+08	4,35E+08	6,30E+08	5,05E+08	7,59E+07
	1 mg/L DOX	1,37E+08	1,08E+08	9,40E+07	1,19E+08	1,14E+08	1,55E+07	1,50E+08	1,58E+08	7,90E+07	1,09E+08	1,24E+08	3,18E+07
	4 mg/L DOX	5,75E+07	8,75E+07	7,35E+07	6,25E+07	7,03E+07	1,15E+07	2,31E+08	6,70E+07	7,95E+07	5,70E+07	1,09E+08	7,09E+07
LOG10 (Resistant/Susceptible)		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	-1,88	-1,61	-1,07	-1,57	-1,53	0,29	-3,10	-1,91	-1,31	-1,20	-1,88	0,75
	1 mg/L DOX*	-0,22	0,72	0,28	0,02	0,20	0,35	-0,27	0,06	0,11	0,31	0,05	0,21
	4 mg/L DOX*	-0,08	0,65	0,67	0,58	0,46	0,31	-0,11	-0,55	0,20	-0,34	-0,20	0,28
	Blank	-1,92	-0,85	-1,23	-1,01	-1,25	0,41	-1,79	-1,32	-1,12	-1,19	-1,35	0,26
	1 mg/L DOX*	0,72	0,81	1,48	0,89	0,97	0,30	0,53	0,69	0,68	0,82	0,68	0,10
	4 mg/L DOX*	0,81	1,13	1,33	1,65	1,23	0,31	0,60	-0,03	0,52	0,89	0,50	0,33
	Blank	-1,54	-0,72	-1,35	-0,91	-1,13	0,33	-0,67	-0,78	-0,75	-1,13	-0,83	0,18
	1 mg/L DOX*	-0,22	0,46	-0,02	-0,13	0,02	0,26	0,23	0,09	0,32	0,00	0,16	0,12
	4 mg/L DOX*	0,03	0,51	0,02	0,26	0,21	0,20	-0,77	-0,34	-0,22	0,36	-0,24	0,41

The table shows the results (+ mean values and SD of quadruplicates 1- to 4-) of enumerations (after 24h and 48h) of resistant and susceptible bacteria and the results that were used for statistical analysis, namely LOG10 (Resistant/Susceptible). DOX concentrations that resulted in significant higher LOG10 (Resistant/Susceptible) values and thus have a significant higher selective effect than blank medium are denoted by an *

Table S4. Competition experiment (type 1) between susceptible *E. coli* EC 400 and three tetracycline resistant *E. coli* strains (EC 682, EC 202, EC 292)

Number of Resistant Bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	4,17E+05	1,25E+06	1,35E+06	1,48E+05	7,91E+05	5,19E+05	9,17E+05	8,33E+03	1,50E+05	2,50E+05	3,31E+05	3,49E+05
	1 mg/L DOX	3,17E+07	2,33E+08	3,17E+07	1,25E+07	7,73E+07	9,04E+07	1,83E+08	3,13E+08	2,85E+08	1,85E+08	2,41E+08	5,86E+07
	4 mg/L DOX	1,41E+08	1,03E+08	6,75E+07	1,12E+08	1,06E+08	2,61E+07	2,21E+08	9,75E+07	9,58E+07	1,28E+08	1,35E+08	5,09E+07
	Blank	1,42E+06	1,50E+06	4,08E+05	6,42E+05	9,92E+05	4,75E+05	2,87E+06	3,25E+06	1,98E+06	8,17E+06	4,07E+06	2,41E+06
	1 mg/L DOX	2,75E+07	1,88E+08	2,08E+07	2,42E+07	6,50E+07	7,08E+07	9,50E+07	2,41E+08	1,49E+08	2,03E+08	1,72E+08	5,52E+07
	4 mg/L DOX	1,64E+08	1,44E+08	1,82E+08	2,53E+08	1,86E+08	4,12E+07	1,28E+08	5,67E+07	4,42E+07	3,75E+07	6,67E+07	3,63E+07
	Blank	2,00E+06	6,00E+06	1,34E+06	1,54E+06	2,72E+06	1,91E+06	6,98E+06	1,38E+07	6,50E+06	1,39E+07	1,03E+07	3,55E+06
	1 mg/L DOX	2,33E+07	1,63E+08	4,00E+07	1,50E+07	6,04E+07	6,01E+07	1,43E+08	3,79E+08	1,81E+08	2,03E+08	2,26E+08	9,09E+07
	4 mg/L DOX	1,91E+08	2,23E+08	2,22E+08	2,75E+08	2,28E+08	3,02E+07	3,06E+08	2,20E+08	1,83E+08	1,88E+08	2,24E+08	4,94E+07
Number of Susceptible Bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	1,04E+09	9,75E+08	9,58E+08	1,24E+09	1,05E+09	1,13E+08	1,20E+09	1,31E+09	1,52E+09	1,63E+09	1,41E+09	1,70E+08
	1 mg/L DOX	2,81E+08	2,72E+08	5,92E+07	6,67E+06	1,55E+08	1,23E+08	6,58E+07	8,17E+07	2,58E+07	6,33E+07	5,92E+07	2,05E+07
	4 mg/L DOX	1,58E+08	9,92E+07	1,92E+07	2,50E+07	7,52E+07	5,70E+07	1,83E+08	8,42E+07	3,50E+07	5,75E+07	9,00E+07	5,66E+07
	Blank	1,21E+09	1,38E+09	1,12E+09	1,24E+09	1,24E+09	9,26E+07	1,04E+09	1,42E+09	1,42E+09	1,42E+09	1,32E+09	1,62E+08
	1 mg/L DOX	2,35E+08	2,69E+08	1,92E+07	2,50E+07	1,37E+08	1,16E+08	4,00E+07	5,33E+07	2,50E+07	4,42E+07	4,06E+07	1,02E+07
	4 mg/L DOX	1,49E+08	1,18E+08	3,33E+07	4,33E+07	8,58E+07	4,89E+07	8,08E+07	4,75E+07	4,83E+07	4,50E+07	5,54E+07	1,47E+07
	Blank	1,21E+09	1,76E+09	1,13E+09	1,10E+09	1,30E+09	2,67E+08	1,56E+09	1,93E+09	1,50E+09	1,46E+09	1,61E+09	1,89E+08
	1 mg/L DOX	2,21E+08	2,93E+08	6,67E+06	3,25E+07	1,38E+08	1,22E+08	7,42E+07	6,42E+07	6,08E+07	5,08E+07	6,25E+07	8,33E+06
	4 mg/L DOX	1,38E+08	1,48E+08	7,25E+07	6,83E+07	1,07E+08	3,67E+07	1,33E+08	1,54E+08	8,67E+07	1,13E+08	1,22E+08	2,50E+07
LOG10 (Resistant/Susceptible)		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	-3,40	-2,89	-2,85	-3,93	-3,27	0,44	-3,12	-5,20	-4,00	-3,82	-3,59	0,75
	1 mg/L DOX*	-0,95	-0,07	-0,27	0,27	-0,25	0,45	0,44	0,58	1,04	0,47	0,71	0,24
	4 mg/L DOX*	-0,05	0,02	0,55	0,65	0,29	0,31	0,08	0,06	0,44	0,35	0,26	0,16
	Blank	-2,93	-2,96	-3,44	-3,29	-3,15	0,21	-2,56	-2,64	-2,85	-2,24	-2,52	0,22
	1 mg/L DOX*	-0,93	-0,16	0,04	-0,01	-0,27	0,39	0,38	0,65	0,78	0,66	0,64	0,15
	4 mg/L DOX*	0,04	0,09	0,74	0,77	0,41	0,34	0,20	0,08	-0,04	-0,08	0,05	0,11
	Blank	-2,78	-2,47	-2,93	-2,85	-2,76	0,18	-2,35	-2,15	-2,36	-2,02	-2,20	0,14
	1 mg/L DOX*	-0,98	-0,25	0,78	-0,34	-0,20	0,63	0,28	0,77	0,47	0,60	0,57	0,18
	4 mg/L DOX*	0,14	0,18	0,49	0,60	0,35	0,20	0,36	0,15	0,32	0,22	0,27	0,08

The table shows the results (+ mean values and SD of quadruplicates 1- to 4-) of enumerations (after 24h and 48h of incubation) of resistant and susceptible bacteria and the results that were used for statistical analysis, namely LOG10 (Resistant/Susceptible). DOX concentrations that resulted in significant higher LOG10 (Resistant/Susceptible) values and thus have a significant higher selective effect than blank medium are denoted by an *

Table S5. Competition experiment (type 2) between susceptible *E. coli* EC 400RIF and three tetracycline resistant *E. coli* strains (EC 682, EC 202, EC 292)

Number of Donor Bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	2,13E+07	1,45E+07	2,63E+07	2,03E+07	2,06E+07	4,17E+06	5,69E+07	1,70E+08	1,58E+07	6,87E+06	6,24E+07	6,49E+07
	1 mg/L DOX	9,50E+07	1,11E+08	1,09E+08	3,12E+08	1,57E+08	8,96E+07	4,08E+08	3,75E+08	2,28E+08	2,24E+08	3,09E+08	8,34E+07
	4 mg/L DOX	2,70E+08	2,84E+08	3,41E+08	2,08E+08	2,76E+08	4,74E+07	3,84E+08	4,58E+08	3,40E+08	1,72E+08	3,38E+08	1,05E+08
	Blank	2,50E+07	1,01E+07	3,42E+06	1,39E+08	4,44E+07	5,53E+07	1,79E+08	2,54E+08	5,24E+07	2,11E+08	1,74E+08	7,51E+07
	1 mg/L DOX	9,00E+07	7,50E+07	9,00E+07	2,77E+08	1,33E+08	8,32E+07	4,16E+08	3,05E+08	1,99E+08	2,21E+08	2,85E+08	8,53E+07
	4 mg/L DOX	4,41E+08	5,18E+08	3,08E+08	3,43E+08	4,03E+08	8,23E+07	1,98E+08	2,11E+08	4,50E+08	1,67E+08	2,56E+08	1,13E+08
	Blank	2,83E+08	7,50E+07	5,17E+06	1,96E+08	1,40E+08	1,07E+08	9,50E+07	9,13E+07	3,61E+07	3,54E+08	1,44E+08	1,23E+08
	1 mg/L DOX	1,73E+08	1,29E+08	1,44E+08	3,18E+08	1,91E+08	7,49E+07	5,13E+08	4,80E+08	2,93E+08	3,21E+08	4,01E+08	9,60E+07
	4 mg/L DOX	4,86E+08	5,48E+08	5,06E+08	3,44E+08	4,71E+08	7,65E+07	6,86E+08	6,04E+08	6,63E+08	2,34E+08	5,47E+08	1,83E+08
Total number of recipient (EC 400RIF)		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	1,68E+08	6,75E+07	1,88E+08	1,61E+08	1,46E+08	4,63E+07	1,09E+08	2,46E+06	2,19E+08	2,45E+07	8,86E+07	8,49E+07
	1 mg/L DOX	5,59E+06	3,20E+06	6,22E+06	1,40E+06	4,10E+06	1,92E+06	2,53E+05	5,40E+04	1,29E+06	4,00E+03	4,01E+05	5,23E+05
	4 mg/L DOX	8,50E+05	1,25E+05	2,45E+07	8,57E+05	6,59E+06	1,04E+07	1,39E+05	1,57E+04	3,38E+04	2,73E+03	4,77E+04	5,37E+04
	Blank	1,88E+08	7,61E+07	1,95E+08	1,99E+08	1,65E+08	5,12E+07	1,20E+08	3,90E+06	1,28E+08	4,85E+07	7,51E+07	5,14E+07
	1 mg/L DOX	4,59E+06	1,94E+06	4,55E+06	2,53E+06	3,40E+06	1,19E+06	2,03E+05	1,11E+05	3,21E+05	3,47E+04	1,67E+05	1,07E+05
	4 mg/L DOX	2,96E+06	2,91E+06	2,83E+06	1,13E+07	4,99E+06	3,62E+06	1,96E+06	3,83E+05	1,97E+06	1,85E+06	1,54E+06	6,69E+05
	Blank	1,44E+08	7,38E+07	2,04E+08	1,95E+08	1,54E+08	5,17E+07	1,28E+08	9,76E+07	2,93E+08	1,79E+08	1,75E+08	7,45E+07
	1 mg/L DOX	1,68E+06	1,24E+06	2,98E+06	1,96E+07	6,37E+06	7,66E+06	2,37E+06	2,33E+05	1,89E+06	4,19E+06	2,17E+06	1,41E+06
	4 mg/L DOX	5,53E+06	3,33E+06	3,15E+06	1,62E+07	7,05E+06	5,37E+06	1,06E+07	3,61E+06	9,57E+06	1,43E+07	9,53E+06	3,85E+06
Number of Transconjugants		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	1,99E+03	2,03E+03	1,14E+03	2,47E+03	1,91E+03	4,79E+02	1,70E+04	1,72E+04	2,18E+04	3,88E+03	1,50E+04	6,68E+03
	1 mg/L DOX	5,00E+01	0,00E+00	2,90E+02	1,67E+01	8,92E+01	1,17E+02	8,75E+01	3,75E+01	8,33E+00	1,67E+01	3,75E+01	3,08E+01
	4 mg/L DOX	2,50E+01	0,00E+00	3,00E+01	8,33E+00	1,58E+01	1,22E+01	6,25E+01	0,00E+00	1,67E+01	8,33E+00	2,19E+01	2,42E+01
	Blank	2,60E+03	7,50E+02	1,00E+03	2,52E+03	1,72E+03	8,47E+02	5,19E+03	2,01E+03	1,07E+04	2,32E+03	5,05E+03	3,48E+03
	1 mg/L DOX	2,25E+02	5,00E+01	8,80E+02	1,58E+02	3,28E+02	3,25E+02	3,38E+02	2,50E+01	2,25E+02	6,67E+00	1,49E+02	1,39E+02
	4 mg/L DOX	8,75E+01	1,25E+01	1,69E+02	6,67E+02	2,34E+02	2,56E+02	4,00E+02	1,25E+01	0,00E+00	2,40E+02	1,63E+02	1,67E+02
	Blank	6,13E+03	7,50E+02	1,03E+02	1,46E+04	5,39E+03	5,81E+03	1,18E+05	5,98E+04	1,08E+05	3,14E+04	7,94E+04	3,55E+04
	1 mg/L DOX	1,38E+02	1,88E+02	8,80E+02	3,56E+03	1,19E+03	1,40E+03	5,23E+03	1,26E+03	1,48E+03	1,89E+03	2,47E+03	1,61E+03
	4 mg/L DOX	1,38E+02	3,75E+01	9,50E+01	2,73E+03	7,51E+02	1,15E+03	6,25E+02	8,75E+01	6,67E+01	3,17E+03	9,88E+02	1,28E+03

LOG10 (Resistant/Susceptible)*		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	-0,90	-0,67	-0,85	-0,90	-0,83	0,09	-0,28	1,84	-1,14	-0,55	-0,03	1,13
	1 mg/L DOX°	1,23	1,54	1,24	2,35	1,59	0,45	3,21	3,84	2,25	4,75	3,51	0,91
	4 mg/L DOX°	2,50	3,36	1,14	2,38	2,35	0,79	3,44	4,47	4,00	4,80	4,18	0,51
	Blank	-0,88	-0,88	-1,76	-0,16	-0,92	0,57	0,17	1,81	-0,39	0,64	0,56	0,81
	1 mg/L DOX°	1,29	1,59	1,30	2,04	1,55	0,30	3,31	3,44	2,79	3,80	3,34	0,36
	4 mg/L DOX°	2,17	2,25	2,04	1,48	1,99	0,30	2,00	2,74	2,36	1,95	2,27	0,32
	Blank	0,29	0,01	-1,60	0,00	-0,32	0,74	-0,13	-0,03	-0,91	0,30	-0,19	0,44
	1 mg/L DOX°	2,01	2,02	1,68	1,21	1,73	0,33	2,34	3,32	2,19	1,88	2,43	0,54
	4 mg/L DOX°	1,94	2,22	2,21	1,33	1,92	0,36	1,81	2,22	1,84	1,21	1,77	0,36
Plasmid transfer frequency		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	-0,90	-0,67	-0,85	-0,90	-0,83	0,09	-0,28	1,84	-1,14	-0,55	-0,03	1,13
	1 mg/L DOX	1,23	1,54	1,24	2,35	1,59	0,45	3,21	3,84	2,25	4,75	3,51	0,91
	4 mg/L DOX	2,50	3,36	1,14	2,38	2,35	0,79	3,44	4,47	4,00	4,80	4,18	0,51
	Blank	-0,88	-0,88	-1,76	-0,16	-0,92	0,57	0,17	1,81	-0,39	0,64	0,56	0,81
	1 mg/L DOX	1,29	1,59	1,30	2,04	1,55	0,30	3,31	3,44	2,79	3,80	3,34	0,36
	4 mg/L DOX	2,17	2,25	2,04	1,48	1,99	0,30	2,00	2,74	2,36	1,95	2,27	0,32
	Blank	0,29	0,01	-1,60	0,00	-0,32	0,74	-0,13	-0,03	-0,91	0,30	-0,19	0,44
	1 mg/L DOX°	2,01	2,02	1,68	1,21	1,73	0,33	2,34	3,32	2,19	1,88	2,43	0,54
	4 mg/L DOX	1,94	2,22	2,21	1,33	1,92	0,36	1,81	2,22	1,84	1,21	1,77	0,36

The table shows the results (+ mean values and SD of quadruplicates 1- to 4-) of enumerations (after 24h and 48h of incubation) of resistant bacteria, susceptible bacteria and transconjugants and the results that were used for statistical analysis, namely LOG10 (Resistant/Susceptible). DOX concentrations that resulted in significant higher LOG10 (Resistant/Susceptible) values and thus have a significant higher selective effect than blank medium are denoted by an °. DOX concentrations that resulted in significantly higher transfer frequencies are also indicated with an °

*Resistant = donor+ transconjugants ; Susceptible = recipient-transconjugants

Table S6. Competition experiment (type 2) between susceptible *E. coli* EC 588RIF and three tetracycline resistant *E. coli* strains (EC 682, EC 202, EC 292)

Number of Donor bacteria		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	1,00E+05	7,45E+03	1,51E+05	2,79E+05	1,34E+05	9,79E+04	1,17E+05	4,45E+04		5,65E+06	1,94E+06	2,63E+06
	1 mg/L DOX	3,76E+07	4,05E+07	3,96E+07	4,38E+07	4,04E+07	2,24E+06	1,13E+08	8,30E+07	5,03E+08	1,36E+08	2,09E+08	1,71E+08
	4 mg/L DOX	1,44E+07	4,95E+07	3,80E+07	4,18E+07	3,59E+07	1,31E+07	1,05E+08	4,60E+07	2,05E+08	1,53E+08	1,27E+08	5,86E+07
	Blank	5,00E+03	3,58E+04	5,25E+03	1,35E+05	4,53E+04	5,33E+04	1,30E+04	1,65E+04	6,87E+05	4,78E+04	1,91E+05	2,86E+05
	1 mg/L DOX	1,47E+07	2,86E+07	9,65E+06	5,40E+07	2,67E+07	1,72E+07	3,01E+07	2,22E+07	1,19E+08	5,75E+07	5,72E+07	3,80E+07
	4 mg/L DOX	9,75E+06	3,10E+07	2,52E+07	1,33E+08	4,96E+07	4,85E+07	7,60E+06	5,24E+07	2,68E+08	1,99E+07	8,70E+07	1,06E+08
	Blank	1,45E+05	4,34E+05	8,66E+04	4,96E+05	2,90E+05	1,77E+05	0,00E+00	1,24E+06	1,68E+07	1,04E+07	7,10E+06	6,88E+06
	1 mg/L DOX	1,61E+07	2,58E+07	2,00E+07	1,11E+08	4,31E+07	3,91E+07	5,20E+07	3,91E+07	2,10E+08	1,51E+08	1,13E+08	7,06E+07
	4 mg/L DOX	1,25E+07	3,24E+07	3,51E+07	1,74E+08	6,33E+07	6,42E+07	2,36E+07	4,00E+07	1,53E+08	1,29E+08	8,63E+07	5,55E+07
Total number of Recipient (EC 588RIF)		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	1,95E+08	1,32E+08	3,35E+08	1,11E+09	4,44E+08	3,94E+08	2,65E+08	6,93E+07	1,99E+09	9,60E+09	2,98E+09	3,89E+09
	1 mg/L DOX	1,68E+08	3,40E+07	1,63E+08	3,61E+08	1,81E+08	1,17E+08	1,50E+07	2,55E+07	4,60E+07	3,45E+08	1,08E+08	1,37E+08
	4 mg/L DOX	4,95E+07	6,20E+07	1,23E+08	2,85E+08	1,30E+08	9,36E+07	1,00E+06	2,32E+06	4,85E+06	6,65E+06	3,71E+06	2,19E+06
	Blank	7,50E+07	2,88E+08	7,25E+07	3,20E+09	9,08E+08	1,32E+09	1,70E+08	9,00E+07	1,54E+09	1,55E+09	8,36E+08	7,07E+08
	1 mg/L DOX	1,70E+08	2,54E+08	1,22E+08	1,25E+09	4,49E+08	4,65E+08	6,15E+07	1,51E+07	1,55E+07	4,30E+06	2,41E+07	2,21E+07
	4 mg/mL DOX	3,50E+06	2,47E+08	2,82E+08	1,44E+08	1,69E+08	1,08E+08	1,00E+06	5,20E+05	8,70E+05	7,30E+05	7,80E+05	1,78E+05
	Blank	7,50E+07	4,30E+07	1,07E+08	1,22E+09	3,61E+08	4,96E+08	1,60E+08	8,80E+07	1,47E+09	1,58E+10	4,37E+09	6,60E+09
	1 mg/L DOX	3,30E+08	2,10E+08	1,85E+08	5,18E+08	3,11E+08	1,32E+08	5,20E+07	2,44E+08	3,45E+08	1,12E+08	1,88E+08	1,14E+08
	4 mg/L DOX	5,45E+07	3,07E+08	6,95E+07	2,11E+08	1,60E+08	1,04E+08	2,36E+07	6,33E+06	8,35E+07	7,75E+08	2,22E+08	3,21E+08
Number of Transconjugants		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	0,00E+00	2,55E+02	1,95E+02	4,21E+03	1,17E+03	1,76E+03	8,10E+03	1,63E+04	9,15E+03	4,52E+04	1,97E+04	1,50E+04
	1 mg/L DOX	5,00E+02	3,30E+02	2,00E+01	2,25E+02	2,69E+02	1,74E+02	4,70E+03	3,69E+03	3,55E+02	3,00E+03	2,94E+03	1,61E+03
	4 mg/L DOX	0,00E+00	1,80E+02	0,00E+00	1,00E+01	4,75E+01	7,66E+01	1,60E+03	3,04E+03	2,05E+02	2,35E+02	1,27E+03	1,17E+03
	Blank	3,00E+03	5,55E+03	2,70E+02	2,29E+03	2,78E+03	1,89E+03	0,00E+00	9,70E+04	4,54E+05	1,13E+03	1,38E+05	1,86E+05
	1 mg/L DOX	2,00E+03	2,76E+03	1,85E+02	1,40E+02	1,27E+03	1,14E+03	1,30E+03	5,75E+04	1,95E+02	7,00E+01	1,48E+04	2,47E+04
	4 mg/L DOX	0,00E+00	1,50E+01	5,50E+01	7,00E+01	3,50E+01	2,85E+01	0,00E+00	0,00E+00	5,00E+00	4,00E+01	1,13E+01	1,67E+01
	Blank	3,00E+01	6,50E+01	1,00E+01	1,50E+01	3,00E+01	2,15E+01	2,55E+03	8,10E+02	3,35E+02	1,77E+03	1,37E+03	8,55E+02
	1 mg/L DOX	2,08E+03	2,22E+04	1,00E+01	6,65E+02	6,24E+03	9,25E+03	2,75E+04	7,53E+05	1,96E+04	1,76E+03	2,00E+05	3,19E+05
	4 mg/L DOX	2,45E+02	2,19E+04	5,00E+00	6,30E+02	5,70E+03	9,36E+03	1,09E+04	3,83E+05	3,63E+03	6,00E+02	9,95E+04	1,64E+05

LOG10 (Resistant/Susceptible)*		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	-3,29	-4,23	-3,34	-3,60	-3,62	0,37	-3,33	-3,06	nd	-3,23	-3,20	0,11
	1 mg/L DOX°	-0,65	0,08	-0,61	-0,92	-0,53	0,37	0,88	0,51	1,04	-0,40	0,51	0,56
	4 mg/L DOX°	-0,54	-0,10	-0,51	-0,83	-0,49	0,26	2,02	1,30	1,62	1,36	1,58	0,28
	Blank	-3,97	-3,84	-4,12	-4,37	-4,08	0,19	-4,12	-2,90	-3,13	-4,50	-3,66	0,67
	1 mg/L DOX°	-1,06	-0,95	-1,10	-1,36	-1,12	0,15	-0,31	0,17	0,89	1,13	0,47	0,57
	4 mg/L DOX°	0,44	-0,90	-1,05	-0,04	-0,39	0,62	0,88	2,00	2,49	1,43	1,70	0,60
	Blank	-2,71	-2,00	-3,09	-3,39	-2,80	0,52	-4,80	-1,85	-1,94	-3,18	-2,94	1,19
	1 mg/L DOX°	-1,31	-0,91	-0,97	-0,67	-0,96	0,23	0,00	-0,78	-0,22	0,13	-0,22	0,35
	4 mg/L DOX°	-0,64	-0,98	-0,30	-0,08	-0,50	0,34	0,00	0,83	0,26	-0,78	0,08	0,58
Plasmid transfer frequency		1-24h	2-24h	3-24h	4-24h	Mean	SD	1-48h	2-48h	3-48h	4-48h	Mean	SD
	Blank	0,00E+00	1,93E-06	5,83E-07	3,78E-06	1,57E-06	1,45E-06	3,06E-05	2,35E-04	4,61E-06	4,70E-06	6,88E-05	9,67E-05
	1 mg/L DOX	2,98E-06	9,71E-06	1,23E-07	6,24E-07	3,36E-06	3,82E-06	3,13E-04	1,45E-04	7,72E-06	8,71E-06	1,19E-04	1,25E-04
	4 mg/L DOX	0,00E+00	2,90E-06	0,00E+00	3,51E-08	7,35E-07	1,25E-06	1,60E-03	1,31E-03	4,23E-05	3,53E-05	7,46E-04	7,15E-04
	Blank	4,00E-05	1,93E-05	3,72E-06	7,17E-07	1,59E-05	1,56E-05	0,00E+00	1,08E-03	2,95E-04	7,26E-07	3,43E-04	4,41E-04
	1 mg/L DOX	1,18E-05	1,09E-05	1,52E-06	1,12E-07	6,08E-06	5,29E-06	2,11E-05	3,82E-03	1,26E-05	1,63E-05	9,68E-04	1,65E-03
	4 mg/L DOX	0,00E+00	6,09E-08	1,95E-07	4,86E-07	1,85E-07	1,87E-07	0,00E+00	0,00E+00	5,75E-06	5,48E-05	1,51E-05	2,30E-05
	Blank	4,00E-07	1,51E-06	9,35E-08	1,23E-08	5,04E-07	5,99E-07	1,59E-05	9,20E-06	2,29E-07	1,12E-07	6,36E-06	6,63E-06
	1 mg/L DOX°	6,30E-06	1,06E-04	5,42E-08	1,28E-06	2,83E-05	4,47E-05	5,29E-04	3,09E-03	5,69E-05	1,57E-05	9,24E-04	1,27E-03
	4 mg/L DOX°	4,50E-06	7,13E-05	7,19E-08	2,99E-06	1,97E-05	2,98E-05	4,63E-04	6,06E-02	4,35E-05	7,74E-07	1,53E-02	2,61E-02

The table shows the results (+ mean values and SD of quadruplicates 1- to 4-) of enumerations (after 24h and 48h of incubation) of resistant bacteria, susceptible bacteria and transconjugants and the results that were used for statistical analysis, namely LOG10 (Resistant/Susceptible). DOX concentrations that resulted in significant higher LOG10 (Resistant/Susceptible) values and thus have a significant higher selective effect than blank medium are denoted by an °. DOX concentrations that resulted in significantly higher transfer frequencies are also indicated with an °

*Resistant = donor+ transconjugants ; Susceptible = recipient-transconjugants