Table S1. Concentration of tobramycin (close to MIC) that hindered -but allowed- the growth of each *P. aeruginosa* clinical isolate in glass tube (μg/ml).

ISOLATE ID	ORIGIN	ST	TOBRAMYCIN concentration (μg/ml) used in the ALE experiments
AND04-004A	Tracheal aspirate	244	0.4
ARA02-005	Blood	267	0.3
BAL02-001	Sputum	1619	0.2
BAL04-002	Blood	1816	0.3
CAN01-002	Sputum	111	0.4
CAN01-003	Sputum	111	6
CAT02-004	Blood	244	0.2
CAT06-005	Sputum	175	6
CAT09-004	Blood	244	0.3
CLE03-004	Tracheal aspirate	381	0.5
CLM01-003	Sputum	709	0.3
CVA01-006	Sputum	175	12
EXT01-004	Sputum	3353	0.8
GAL01-001	Sputum	560	0.2
GAL02-004	Sputum	217	2
ICA01-004	Sputum	698	0.2

MAD04-002	Sputum	242	0.3
MAD05-009	Sputum (CF)	27	0.2
FQSE03-1212-2	Sputum (CF)	274	0.4
FQSE10-0503	Sputum (CF)	274	0.1
FQSE110603	Sputum (CF)	701	0.3
FQSE15-0803	Sputum (CF)	274	0.4
FQSE24-0304	Sputum (CF)	1089	2

ST: sequence type, MIC: Minimal Inhibitory Concentration, CF: cystic fibrosis.

Table S2. MICs in μg/ml of tobramycin and fosfomycin in *P. aeruginosa* clinical strains after evolution in presence or absence of tobramycin.

AND04-004A	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1.5	1	1.5	1.5	1.5	8	16	8	8
FOF	256	256	256	256	256	192	256	48	64
ARA02-005	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	2	1.5	2	2	3	6	4	4
FOF	96	64	64	64	64	48	96	48	128
CAN01-002	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1.5	1.5	1.5	1.5	1.5	4	3	4	6
FOF	3	3	3	3	3	1.5	3	2	1.5
CAT02-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	0.75	0.75	0.75	1	1	6	8	4	4
FOF	256	192	256	256	256	32	≥1024	128	128
CLE03-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	2	3	0.75	2	2	2	6	8
FOF	64	96	96	64	32	96	32	32	32
CVA01-006	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	12	16	24	12	32	32	128	128	128
FOF	3	12	12	16	12	6	1.5	1.5	2
MAD05-009	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	2	2	2	1.5	6	4	4	6
FOF	96	96	96	96	128	64	48	48	48
FQSE10-0503	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1	1	2	1	1.5	4	4	4	4
FOF	256	256	256	256	256	96	96	64	96
FQSE15-0803	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	3	2	2	1	3	6	6	4	6
FOF	96	96	96	96	96	64	32	32	64
FQSE24-0304	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1.5	1	0.38	1	1	8	8	12	8
FOF	16	24	24	24	24	8	8	12	16
BAL02-001	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	0.75	0.75	0.75	0.75	0.75	4	3	4	6
FOF	64	64	48	48	48	48	64	32	48
BAL04-002	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1	1	1	0.75	1	4	4	6	4
FOF	≥1024	≥1024	≥1024	≥1024	768	≥1024	768	768	≥1024
CAN01-003	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	32	32	32	24	32	128	48	96	48
FOF	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024

CAT06-005	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	32	129	32	24	32	96	96	96	96
FOF	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024
CAT09-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	3	2	1.5	3	1	8	4	8	8
FOF	64	96	96	128	96	128	96	128	64
CLM01-003	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	2	1.5	2	3	3	4	6	24
FOF	192	192	192	256	128	128	192	256	256
EXT01-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	3	3	3	3	3	12	4	12	4
FOF	2	2	2	2	2	2	1 .5	2	1.5
GAL01-001	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1.5	1.5	2	2	0.5	8	6	6	6
FOF	32	24	48	48	256	16	48	24	48
GAL02-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	6	6	4	6	4	8	8	8	8
FOF	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024	≥1024
ICA01-004	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	1.5	2	1.5	1.5	1.5	3	4	4	3
FOF	48	48	48	32	48	128	128	128	128
MAD04-002	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	3	3	3	3	3	8	8	8	12
FOF	6	12	8	8	12	16	12	16	16
FQSE03-1212-2	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	4	1.5	1.5	2	4	4	6	4
FOF	64	96	64	96	64	96	96	96	96
FQSE110603	**parental**	**C1**	**C2**	**C3**	**C4**	**E1**	**E2**	**E3**	**E4**
TOB	2	4	2	24	8	6	4	8	6
FOF	64	128	256	48	96	96	192	96	128

C1-4: the four replicates evolved in absence of tobramycin. E1-E4: the four replicates evolved in presence of tobramycin. TOB: tobramycin, FOF: fosfomycin.

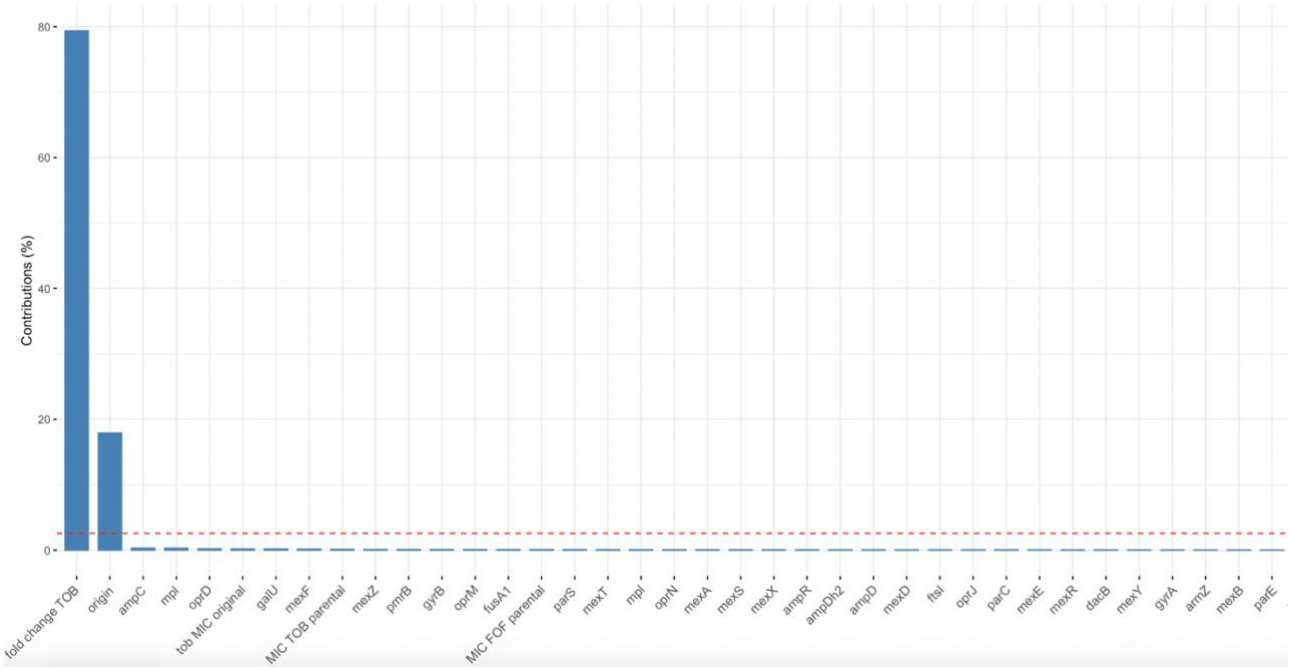

Figure S1. Contribution of different variables to the collateral sensitivity to fosfomycin in *P. aeruginosa* clinical isolates. The variables used for the analysis were: the origin of the strains, their resistome-related mutations (Table 1), their capacity of developing collateral sensitivity to fosfomycin, the MIC of the parental strain to tobramycin and fosfomycin, the fold change of these MICs after the ALE, and the tobramycin concentration used to perform said ALE. The MICs for tobramycin and fosfomycin and the tobramycin concentrations used in the ALE were classified as "high" or "low" in order to be introduced in the program as binary code. The parental MIC for fosfomycin was considered "high" if the value was ≥ 64 μg/ml and "low" if the value was ≤ 48 μg/ml. The parental MIC for tobramycin was considered high if ≥ 2 μg/ml and low if ≤ 1.5 μg/ml. The tobramycin concentrations used in ALE assays were considered high if ≥ 2 μg/ml and low if ≤ 1 μg/ml. The MICs' fold change was calculated as the average of MICs of the strain evolved in presence of tobramycin, divided by the average of MICs of the same strain evolved without antibiotic. Resistome-related mutations in genes that were included: *oprD*, *pmrB*, *oprM*, *fusA*, *gyrB*, *mexA*, *mexX*, *mpl*, *gyrA*, *oprN*, *mexZ*, *ampC*, *mexS*, *mexY*, *armZ*, *mexB*, *ampR*, *parC* and *mexT*. TOB: tobramycin; FOF: fosfomycin.

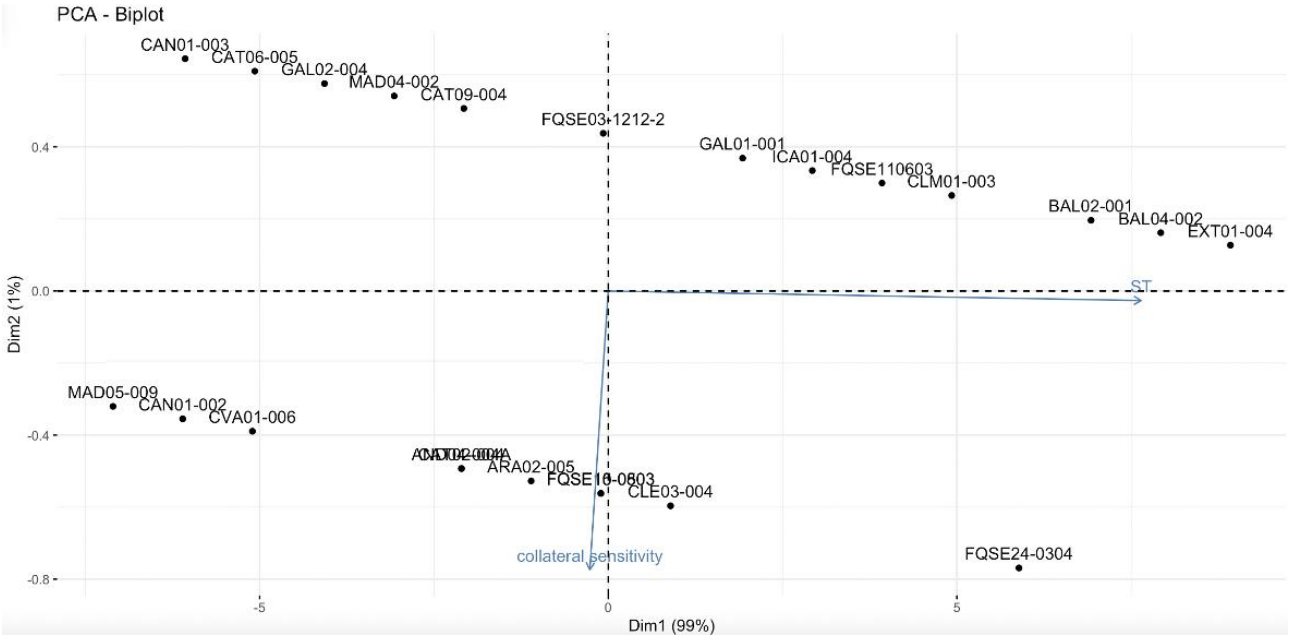

Figure S2. Principal component analysis of fosfomycin collateral sensitivity and sequence type (ST) as unique variables. The principal component analysis was done using as Dim1 the fosfomycin collateral sensitivity and the ST as Dim2. In the upper part of the biplot the strains that did not develop collateral sensitivity were plotted, whereas in the bottom there were the ones that developed said phenotype. The contribution to this division in two groups of the variable collateral sensitivity was 99%, while the one of the ST was 1%.