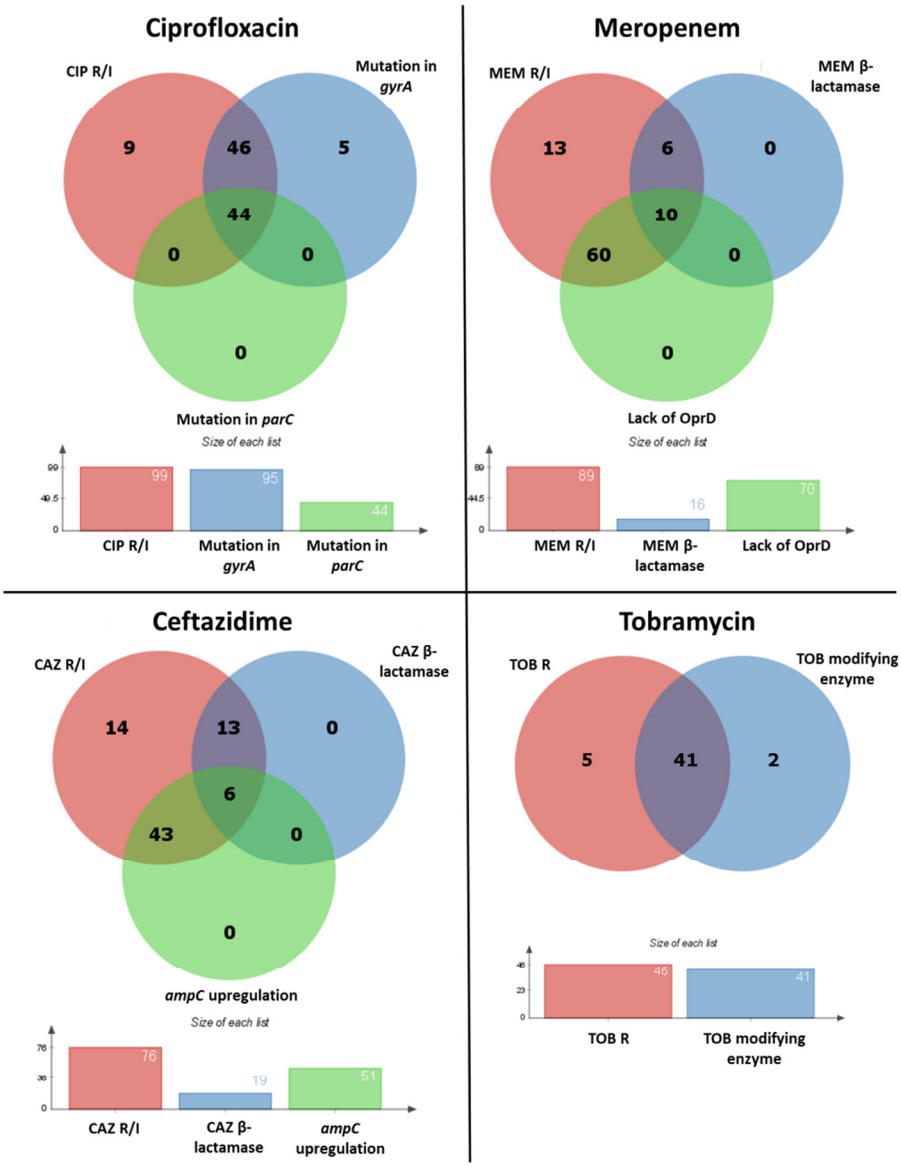
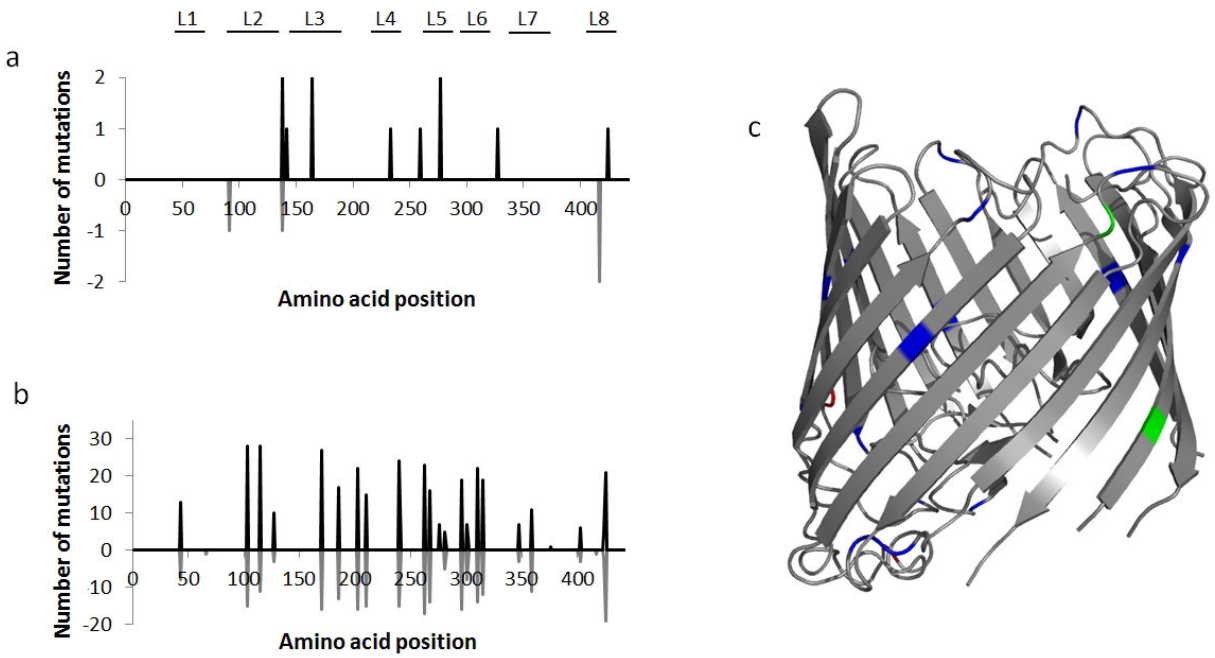


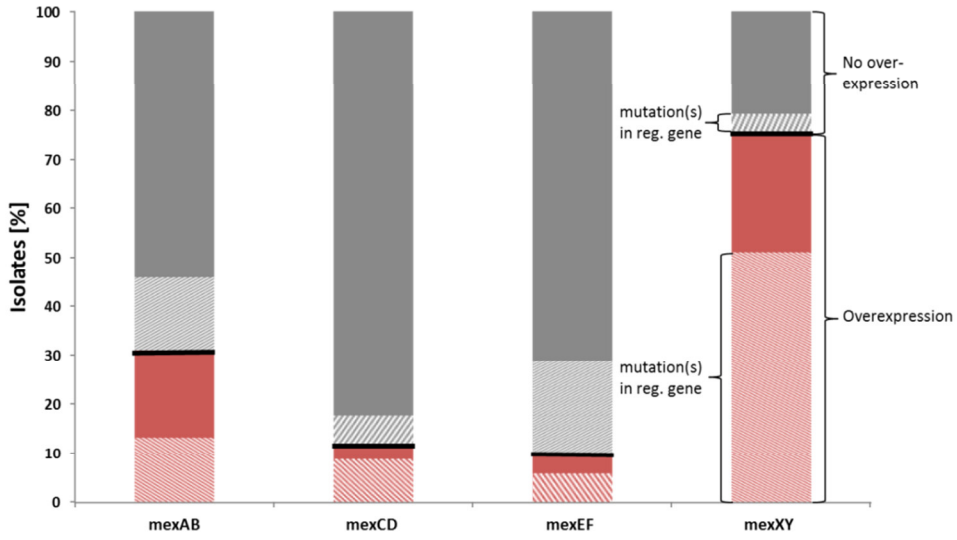
Supplementary Figure 1: Contribution of horizontally and chromosomally acquired genetic determinants to the resistance phenotype of clinical isolates

R = resistant isolates, I = intermediate isolates. Lack of OprD means loss of functional OprD protein and/or repression of the *oprD* gene. The *ampC* upregulation threshold was defined as $\log_2$FC = 3.9.



Supplementary Figure 2: OprD sequence modifications by non-synonymous SNPs

A: Nonsense mutation sites indicating premature translational stops. **B:** Total amounts of SNPs leading to single amino acid exchanges. The x-axis highlights the positions of the modified amino acids relative to protein length. Mutations in MEM resistant (n = 46) isolates are depicted above the x-axis (black), amino acid exchanges in MEM susceptible (n = 32) strains below the xaxis (grey). The positions of the eight external loops are labeled L1 to L8 (according to Huang *et al.* (1)). **C:** SNPs (excluding stop sites) mapped to the tridimensional structure of PAO1 OprD based on the model by Biswas *et al.* (2). SNPs exclusively found in the MEM susceptible isolates are labeled in green, those found in the MEM resistant in red and those found in both in blue.



Supplementary Figure 3: Distribution of efflux pump expression in our panel of clinical isolates

The relative percentage of isolates with an overexpression of one of the four efflux pumps (MexAB, MexCD, MexEF and MexXY) is depicted in red. Furthermore the relative percentage of isolates that harbor at least one mutation (non-synonymous SNP or Indel) in the corresponding negative regulatory gene (*mexR*, *nfxB*, *mexZ*, *mexS*) is indicated as shaded area. Commonly occurring SNPs between reference strains were excluded from the analysis.

Supplementary Table 1: Clinical isolates included in this study with antibiotic resistance profile and infection site

Isolate	Cystic fibrosis	Infection site	MEM MIC*		CAZ MIC*		TOB MIC*		CIP MIC*	
PSAE0613	nd	nd	S	0.25	S	8	S	0.5	R	128
PSAE1152	nd	drainage_catheter	R	64	R	128	R	32	R	32
PSAE1471	nd	repiratory tract	S	0.5	S	2	S	0.25	R	4
PSAE1640	nd	urine	S	2	S	8	S	4	R	64
PSAE1646	nd	urine	R	16	S	4	S	2	I	2
PSAE1655	nd	repiratory tract	I	8	S	4	S	1	I	2
PSAE1657	nd	repiratory tract	R	16	S	8	S	0.5	I	2
PSAE1659	nd	repiratory tract	I	8	I	16	S	4	I	2
PSAE1660	nd	repiratory tract	R	16	S	8	S	1	I	2
PSAE1661	nd	repiratory tract	R	128	R	64	R	128	R	32
PSAE1688	nd	urine	I	8	S	4	S	0.5	S	0.5
PSAE1695	nd	repiratory tract	S	1	S	4	S	4	R	64
PSAE1711	nd	nd	S	0.5	S	1	S	0.5	R	>128
PSAE1715	nd	respiratory tract	I	8	S	4	S	0.5	S	1
PSAE1716	nd	blood	I	8	R	32	R	>1024	R	32
PSAE1747	nd	respiratory tract	R	16	R	64	R	>1024	R	16
PSAE1758	nd	respiratory tract	S	0.25	S	2	S	0.25	R	4
PSAE1766	nd	respiratory tract	R	16	S	8	S	0.5	S	1
PSAE1775	nd	respiratory tract	I	8	S	4	S	1	S	0.5
PSAE1793	nd	respiratory tract	R	>32	S	8	R	8	R	8
PSAE1807	nd	respiratory tract	I	8	R	64	S	0.5	S	0.25
PSAE1829	nd	respiratory tract	I	8	S	4	S	0.5	I	2
PSAE1875	nd	respiratory tract	R	16	S	4	S	2	S	0.5
PSAE1892	nd	respiratory tract	S	2	S	4	S	0.25	R	32
PSAE1910	nd	respiratory tract	S	0.25	S	1	S	2	R	4
PSAE1928	nd	respiratory tract	S	2	R	32	S	0.125	R	32
PSAE1950	nd	respiratory tract	S	1	S	0.5	S	1	R	8
PSAE2134	nd	respiratory tract	R	32	I	16	R	256	R	16
PSAE2136	nd	respiratory tract	R	16	R	64	R	128	R	16
PSAE2162	nd	nd	R	32	R	128	R	8	R	16
PSAE2180	nd	urine	R	64	R	128	R	32	R	8
PSAE2302	nd	respiratory tract	I	8	R	64	S	2	R	4
PSAE2305	nd	respiratory tract	S	0.25	S	4	S	0.25	R	4
PSAE2307	nd	respiratory tract	R	32	R	128	S	0.5	R	64
PSAE2319	nd	nd	R	64	R	128	R	32	R	128
PSAE2324	nd	respiratory tract	S	4	S	2	S	0.5	S	0.063
PSAE2326	nd	urine	R	16	R	32	S	2	R	16
PSAE2328	nd	urine	R	8-16.	S	2	S	1	S	0.125
PSAE2335	nd	urine	R	128	R	>256	R	32	R	16
PSAE2338	nd	respiratory tract	R	64	R	128	R	> 1024	R	64
MHH6827	no	midstream urine	I	8	R	32	R	16	R	>8
MHH6829	no	midstream urine	R	16	I	16	S	1	R	>8

MHH6870	no	midstream urine	I	8	R	>32	R	32	R	>8
MHH6887	no	tracheal secrete	S	0.125	R	32	S	0.5	I	2
MHH6938	no	bronchoalveolar lavage	I	4	I	16	R	32	R	>8
MHH6964	no	wound swab abdomen	I	8	S	8	R	16	R	8
MHH7032	no	venous catheter	I	8	I	16	R	32	R	>8
MHH7055	no	bronchoalveolar lavage	I	8	S	4	S	1	R	8
MHH7084	no	permanent catheter urine	I	8	R	32	R	16	R	>8
MHH7091	yes	lung transplant recipient	S	0.5	S	4	S	2	S	1
MHH7125	no	tonsil swab	I	4	S	8	R	32	R	>8
MHH7135	no	bronchial secrete	I	4	S	4	S	1	R	8
MHH7176	no	midstream urine	I	8	R	32	R	32	R	>8
MHH7200	yes	tonsil swab	I	4	R	>32	R	>32	R	>8
MHH7252	no	tracheal secrete	I	4	S	2	S	0.5	I	2
MHH7261	no	permanent catheter urine	I	8	R	32	R	16	R	>8
MHH7313	no	tracheal secrete	I	8	S	4	S	0.5	R	4
MHH7368	no	nasal swab	I	8	I	16	R	32	R	>8
MHH7444	yes	bronchoalveolar lavage	I	8	R	>32	S	2	I	2
MHH7508	yes	bronchial secrete	I	8	R	32	R	16	S	1
MHH7818	yes	tracheal secrete	I	4	S	8	R	32	S	1
MHH7823	no	tonsil swab	I	8	R	>32	R	16	R	>8
MHH7863	no	bronchial secrete	I	8	R	32	R	16	R	>8
MHH8044	yes	tracheal secrete	I	8	R	32	S	1	I	2
MHH8349	no	midstream urine	I	8	R	>32	R	>32	R	>8
MHH8478	no	midstream urine	S	1	R	32	R	32	R	>8
MHH8482	yes	bronchial secrete	I	8	R	>32	R	8	I	2
MHH8607	no	sputum	R	16	R	>32	R	16	I	2
MHH8613	no	ear swab	S	2	R	>32	R	>32	R	>8
MHH8614	no	midstream urine	S	1	R	32	R	32	R	>8
MHH8627	no	drainage bile duct	R	>32	I	16	S	0.5	S	0.25
MHH8697	no	rectal swab	R	>16	S	<1	S	2	R	>8
MHH9157	no	wound swab abdomen	S	1	R	>32	S	1	S	0.5
MHH9229	no	tonsil swab	I	4	I	16	S	1	S	1
MHH9481	no	bronchial rinsing	I	4	R	32	S	1	R	4
MHH9484	no	tonsil swab	I	8	S	8	S	2	S	1
MHH9534	no	bronchial secrete	I	8	R	>32	R	8	I	2
MHH9652	no	drainage liquid	I	8	R	32	R	16	R	>8
MHH9674	no	bronchoalveolar lavage	S	0.125	S	2	S	0.5	R	8
MHH9717	no	tracheal secrete	R	16	S	8	S	1	R	>8
MHH9748	no	tonsil swab	I	4	S	4	S	0.5	I	2
MHH9830	no	tracheal secrete	I	8	R	32	S	<=0.25	I	2
MHH9854	yes	nasal swab	I	8	R	>256	S	2	S	1

MHH10047	no	tonsil swab	S	1	S	8	S	0.5	S	1
MHH10049	yes	nasal swab	S	2	S	8	S	2	S	0.5
MHH10660	yes	lung transplant donor	R	>16	R	>256	S	4	I	2
MHH10978	yes	tonsil swab	I	4	R	>32	S	4	R	8
MHH11148	no	tonsil swab	S	1	I	16	S	1	R	4
MHH11444	yes	tonsil swab	R	16	S	8	S	2	S	1
MHH11445	yes	tonsil swab	I	4	S	8	S	4	S	1
MHH11540	no	midstream urine	I	8	R	32	R	16	R	>8
MHH11572	no	midstream urine	R	16	R	>256	R	16	R	>8
MHH11785	yes	lung transplant donor	I	8	R	>256	S	2	R	>8
MHH11935	no	bronchoalveolar lavage	I	8	S	8	S	1	S	1
MHH11989	yes	tonsil swab	I	8	S	8	S	4	R	4
MHH12178	no	catheter swab abdomen	R	16	I	16	S	0.5	S	0.5
MHH12207	yes	bronchoalveolar lavage	I	8	R	>32	S	1	I	2
MHH12269	yes	sputum	R	16	R	>32	S	0.5	S	1
MHH12274	no	bronchoalveolar lavage	S	0.5	I	16	S	0.5	R	4
MHH13062	no	permanent catheter urine	I	4	S	8	R	16	R	8
MHH13224	no	bronchial rinsing	I	8	S	8	S	2	I	2
MHH13305	yes	bronchial secrete	R	>16	R	>32	R	16	R	4
MHH13395	no	bronchoalveolar lavage	I	8	S	8	S	1	S	0.5
MHH13428	no	swab intraop abdomen	S	2	R	32	S	4	I	2
MHH13633	no	tracheal secrete	S	1	S	2	S	2	R	>8
MHH13682	yes	lung transplant donor	I	8	R	>256	S	2	R	>8
MHH13684	yes	lung transplant recipient	I	4	R	>256	S	2	R	8
MHH13714	no	permanent catheter urine	S	0.25	S	8	S	1	R	4
MHH14088	no	perfusate	S	2	S	8	S	4	I	2
MHH14103	no	swab heel	I	8	R	>32	R	32	R	>8
MHH14322	no	bronchial rinsing	S	0.5	S	8	S	1	R	8
MHH14387	yes	lung transplant recipient	I	4	S	4	S	4	I	2
MHH14449	no	bronchial secrete	R	>16	I	16	S	<=1	S	0.5
RKI_82_10	nd	nd	R	>32	R	>32	R	64	R	32
RKI_12_11	nd	nd	R	>32	S	8	S	2	R	16
RKI_24_11	nd	nd	R	>32	R	>32	R	8	R	16
RKI_37_11	nd	nd	R	>32	I	16	S	2	S	2
RKI_53_11	nd	nd	S	4	R	>32	R	8	R	>64
RKI_359_11	nd	nd	R	>32	R	32	R	96	R	64
RKI_360_11	nd	nd	R	>32	I	16	R	64	R	64
RKI_392_11	nd	nd	S	0.25	S	8	S	0.125	S	0.063
RKI_395_11	nd	nd	I	8	R	32	S	0.25	S	1

RKI_100_12	nd	nd	R	>32	R	32	S	0.5	S	0.25
RKI_96_12	nd	nd	I	8	R	>32	R	512	S	0.5
RKI_98_12	nd	nd	R	16	S	4	S	0.5	R	8
RKI_99_12	nd	nd	R	16	R	32	S	1	R	8
RKI_339_12	nd	nd	R	>32	R	>256	S	1.5	S	0.25
B34	nd	nd	S	<=0.25	R	>265	R	64	I	0.5
B197	nd	nd	R	>=16	S	8	S	1	S	<=0.125
B214	nd	nd	I	8	S	8	S	2	S	0.5
B271	nd	nd	S	0.5	S	<=1	S	0.5	S	<=0.125
B266	nd	nd	R	>=16	I	16	S	1	I	1
B337	nd	nd	I	8	S	<=1	S	0.5	S	<=0.125
B428	nd	nd	R	>32	S	8	S	1	S	0.5
B445	nd	nd	R	32	S	<=1	S	0.5	S	<=0.25

R= resistant, I= intermediate resistant, S= susceptible; nd= not determined

*The MIC values are given in µg/ml, and the classification of resistance and susceptibility is according to CLSI guidelines.

Supplementary Table 2: Resistance-associated markers for gene expression and sequence variations

All genetic markers found to be directly associated with resistance in either the receiver operating characteristic analyses (expression association) or the linear mixed model analyses (SNP association) are listed with their respective AUC* or p-value.

	Isolates [n]		Expression association		SNP association	
Antibiotic	**Susceptible**	**Resistant**	**Gene**	**AUC[#]**	**Gene/SNP**	**p-value**
CIP	38	77 (0)*	PA14_46470, *pdxB*	0.760	*gyrA* T83I	5.88E-18
			PA14_31480	0.759	*parC* S87L/W	4.07E-07
			PA14_53010, *phhC*	0.759		
			PA14_62530, *cbrA*	0.742		
			PA14_63890	0.741		
			PA14_33130	0.739		
CAZ	59	44 (17)*	PA14_10790, *ampC*	0.837	*ampC* T55A	2.92E-07
			PA14_10780	0.765		
			PA14_21130	0.753		
			PA14_62920	0.742		
MEM	46	31 (14)*	PA14_46110	0.736	0	
			PA14_72460, *cc4*	0.731		
			PA14_46070, *gbuA*	0.731		

*Number of isolates with acquired resistance enzymes, which were excluded for the association study of CAZ and MEM

[#]Area under the curve: probability that the classifier will rank a randomly chosen positive example higher than a randomly chosen negative one (maximum achievable value = 1). The significance threshold was calculated based on a corrected maximal p-value of 0.05 for each dataset.

1. **Huang H, Jeanteur D, Pattus F, Hancock RE.** 1995. Membrane topology and site-specific mutagenesis of Pseudomonas aeruginosa porin OprD. Molecular microbiology **16:**931-941.
2. **Biswas S, Mohammad MM, Patel DR, Movileanu L, van den Berg B.** 2007. Structural insight into OprD substrate specificity. Nature structural & molecular biology **14:**1108-1109.