Proteus mirabilis – analysis of a concealed source of carbapenemases and development of a diagnostic algorithm for detection

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Supplementary data

Supplementary Methods

Modified zinc-supplemented CIM test (mzCIM)

The modified zinc-supplemented CIM (mzCIM) varies from the previously described zCIM [Sattler et al., 2021] by an increased inoculum and an extended incubation (4 h instead of 2 h) of the meropenem disk in tryptic soy broth (TSB). Briefly, two full 10 µl loops of bacteria are harvested from Columbia blood agar and inoculated into TSB supplemented with 1.5 mM $ZnSO_4$. A 10 µg meropenem disk (Oxoid, Wesel, Germany) is immersed in TSB and incubated for 4 h at 35±1°C, before it is placed on a Mueller Hinton plate inoculated with *E. coli* ATCC 25922 (0.5 McFarland). After 18 h of incubation at 35±1°C, the inhibition zone was measured. A cut-off of ≤18 mm was used as the threshold for carbapenemase production, 19 mm-20 mm were regarded as indeterminate and ≥21 mm as negative.

Whole-genome sequencing and bioinformatic analysis

DNA was extracted using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany). For Illumina sequencing, a v3 reagent kit was applied generating either 150 bp or 250 bp paired-end reads. Sequence data were processed and analysed as described previously. In brief, raw reads were de novo assembled using Spades (https://doi.org/10.1089/cmb.2012.0021) and analysed with the ResFinder database (https://doi.org/10.1093/jac/dkaa345). The assemblies were deposited at the NCBI database under BioProject no. PRJNA915754. Phylogenetic relatedness of the isolates was examined from raw reads with CSIPhylogeny (https://doi.org/10.1371/journal.pone.0104984), using the *P. mirabilis* strain HI4320 as a reference. The isolates were further compared to selected sequences of carbapenemase-producing *P. mirabilis* isolates obtained from the RefSeq database. Phylogenetic trees were created with iTOL (https://doi.org/10.1093/nar/gkab301).

Table S1 Susceptibility testing of isolates was performed using disk diffusion, Vitek2 (AST-N223 card) and broth microdilution. Antibiotics tested with the respective method are marked with X.

Antibiotic	Disk diffusion	Vitek2 (AST-N223)	Microdilution
Ampicillin	X	X	
Ampicillin-sulbactam	X	X	
Amoxicillin-clavulanate	X		
Ticarcillin-clavulanate	X		
Piperacillin	X	X	
Piperacillin-tazobactam	X	X	X
Temocillin	X		X
Mecillinam	X		
Cefuroxime	X	X	
Cefpodoxime		X	
Cefotaxime	X	X	
Ceftazidime	X	X	X
Ceftazidime-avibactam	X		X
Ceftolozane-tazobactam			X
Cefepime	X		X
Aztreonam	X		X
Aztreonam-avibactam			X
Ertapenem	X	X	X
Meropenem	X	X	X
Imipenem	X	X	X
Imipenem-relebactam			X
Faropenem	X		
Ciprofloxacin	X	X	X
Moxifloxacin	X	X	
Co-trimoxazole	X	X	
Tigecycline			X
Fosfomycin			
Gentamicin	X	X	
Tobramycin	X		
Amikacin	X		
Colistin			X

Supplementary Results

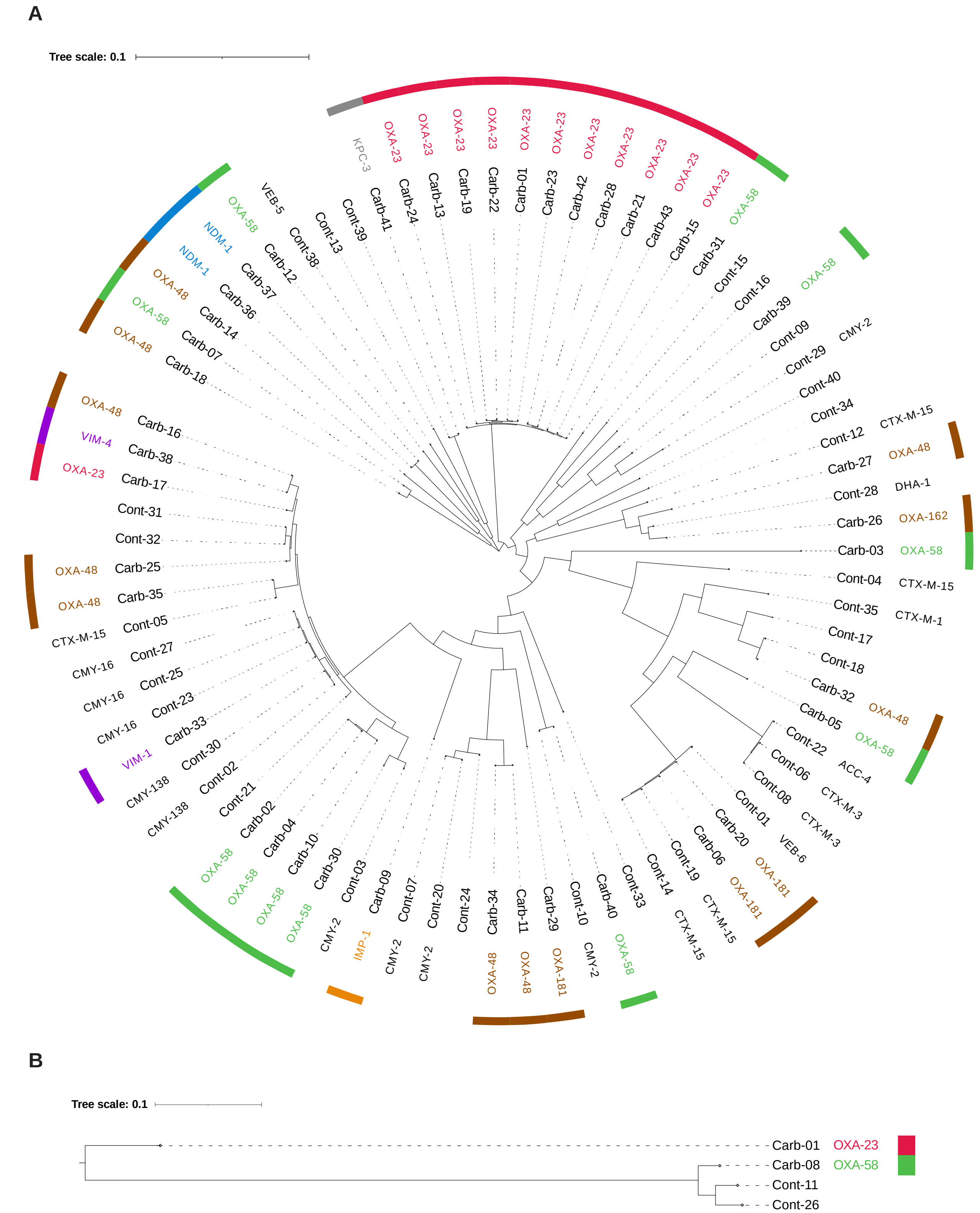


Fig. S1 Phylogeny of all 81 clinical *P. mirabilis* isolates.
The majority of the isolates are displayed in (A). Four isolates (three more distantly related isolates and Carb-01 as reference) are shown in a separate tree (B) to allow a better resolution.

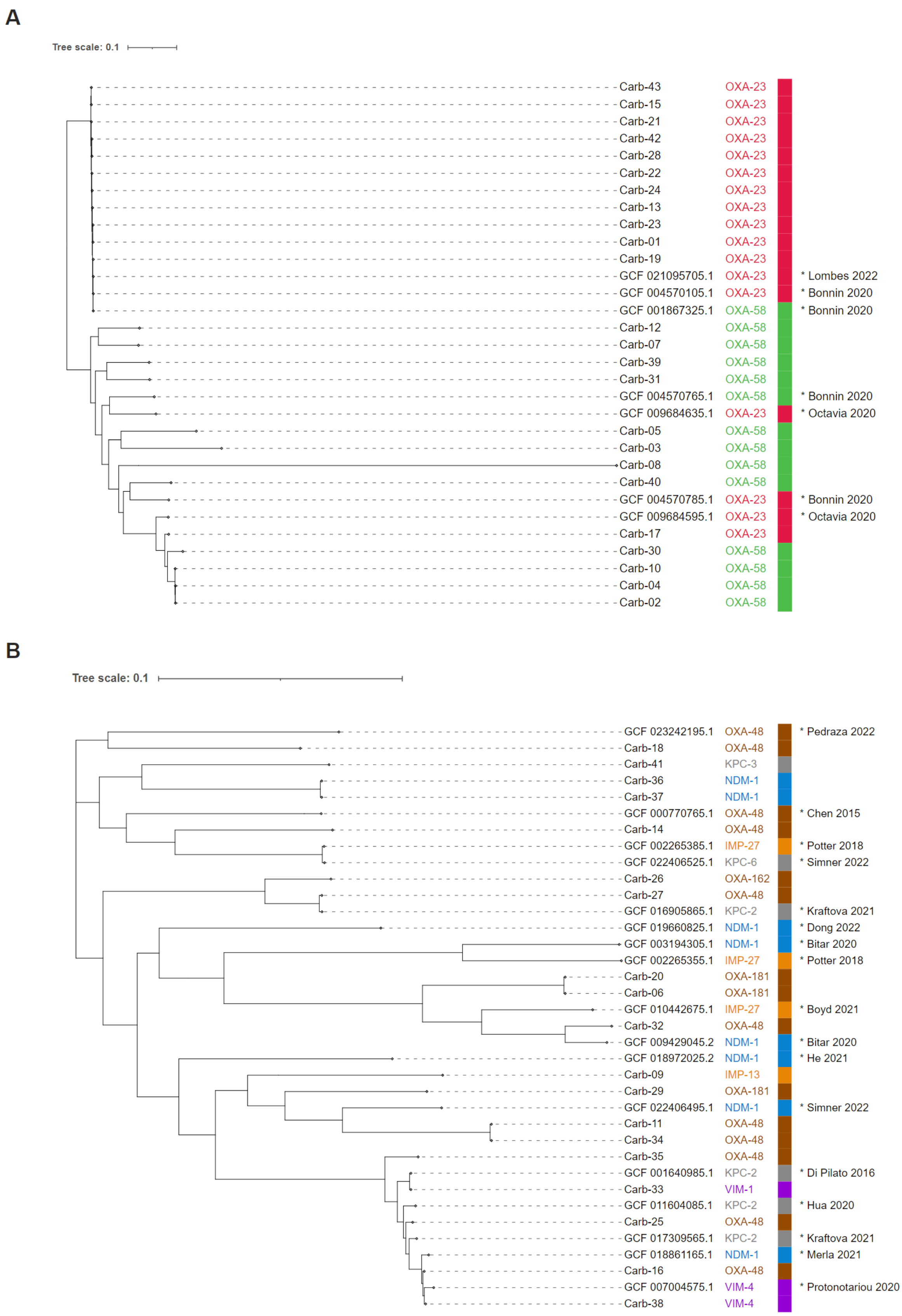


Fig. S2 Genetic relatedness of carbapenemase-harbouring *Proteus mirabilis*.

(A) Maximum likelihood tree of 31 *P. mirabilis* genomes harbouring *bla*$_{OXA-23}$ or *bla*$_{OXA-58}$, consisting of 24 isolates from this study and seven representative isolates from the literature. (B) Maximum likelihood tree of 36 *P. mirabilis* genomes harbouring genes encoding for either OXA-48-like, NDM-1, KPC, VIM or IMP, consisting of 19 isolates from this study and 17 isolates from the literature.

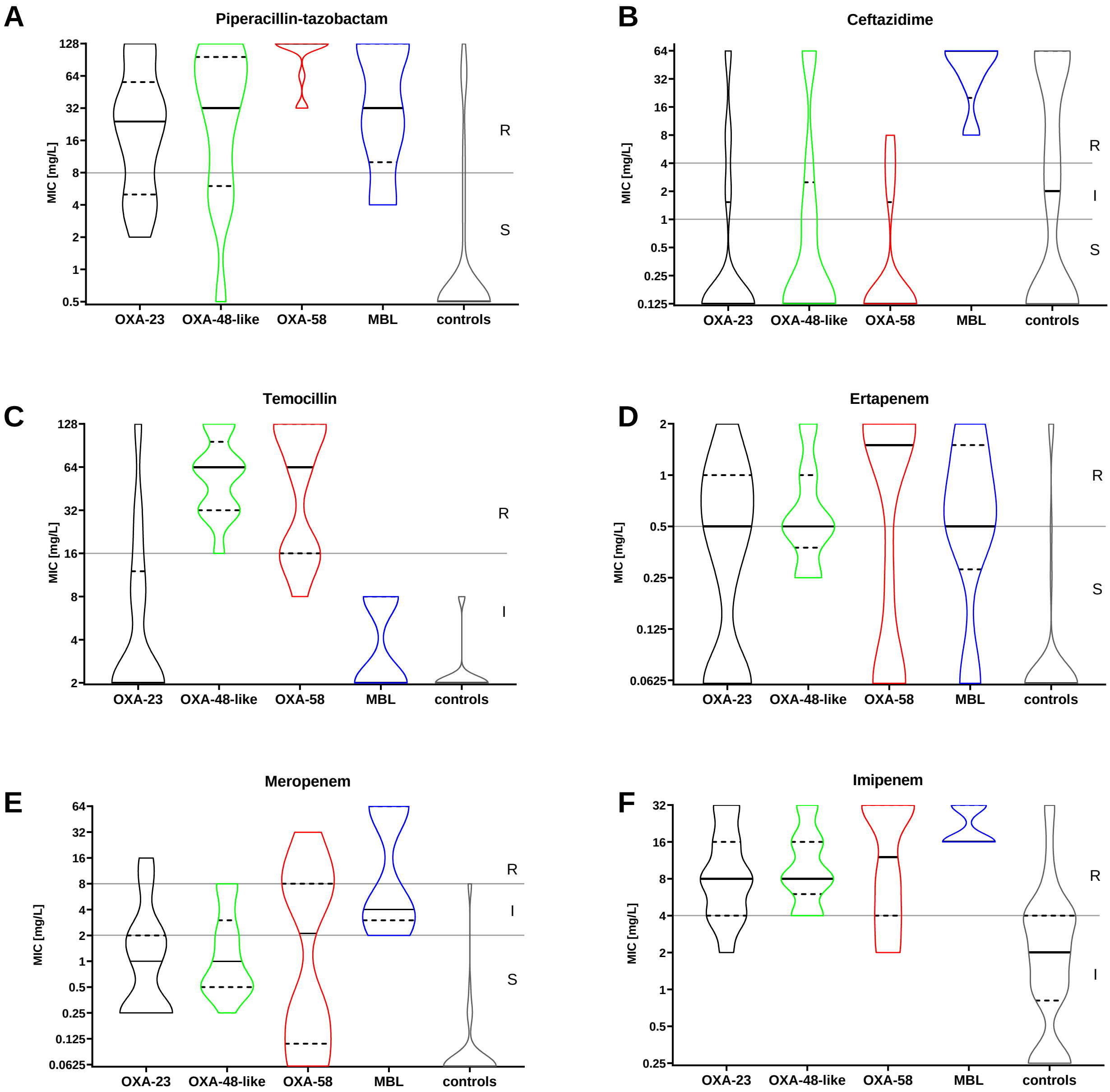
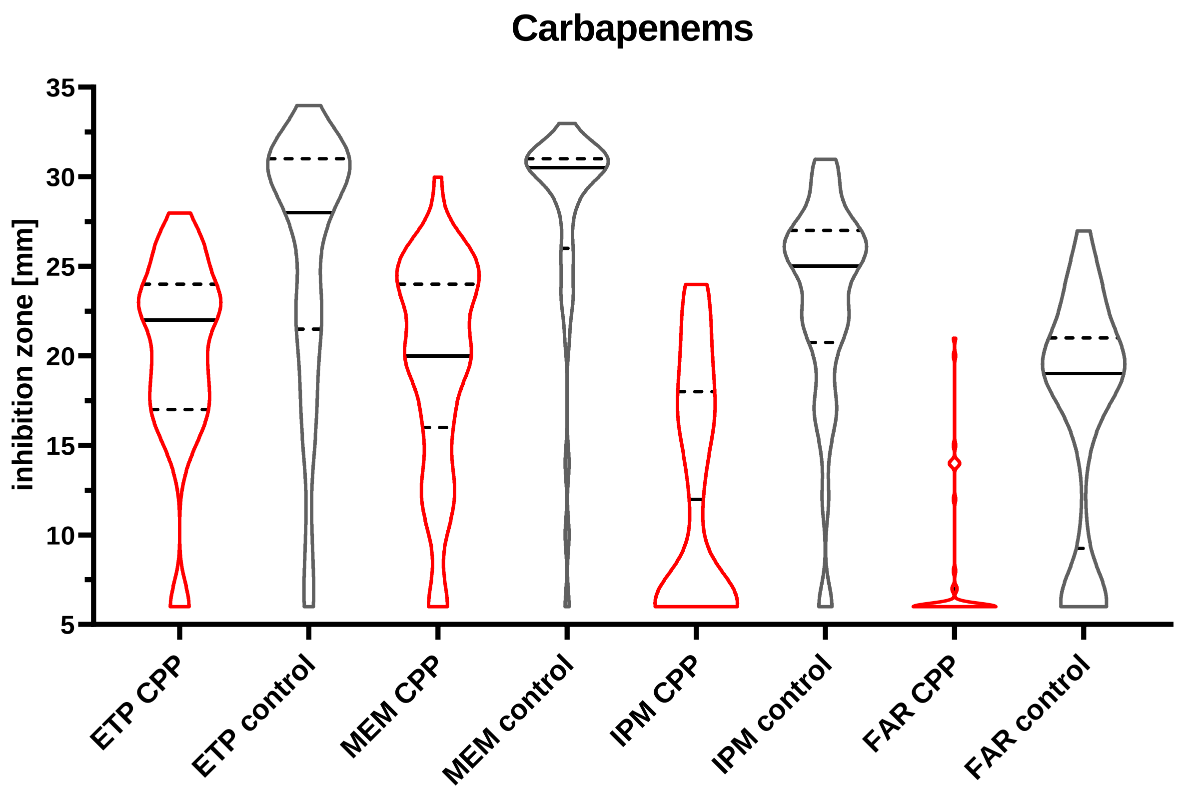


Fig. S3 MIC distributions by broth microdilution for 81 clinical *P. mirabilis* isolates
MIC distributions stratified by carbapenemase for piperacillin-tazobactam (A), ceftazidime (B), temocillin (C), ertapenem (D), meropenem (E) and imipenem (F). The solid black line denotes the median, the dashed line the quartiles; EUCAST breakpoints (S/I/R) are indicated by grey horizontal lines. For MIC values above or below the BMD assay range (e.g., >64 mg/L), the next dilution (e.g., 128 mg/L) was used for the calculation of the MIC distribution.



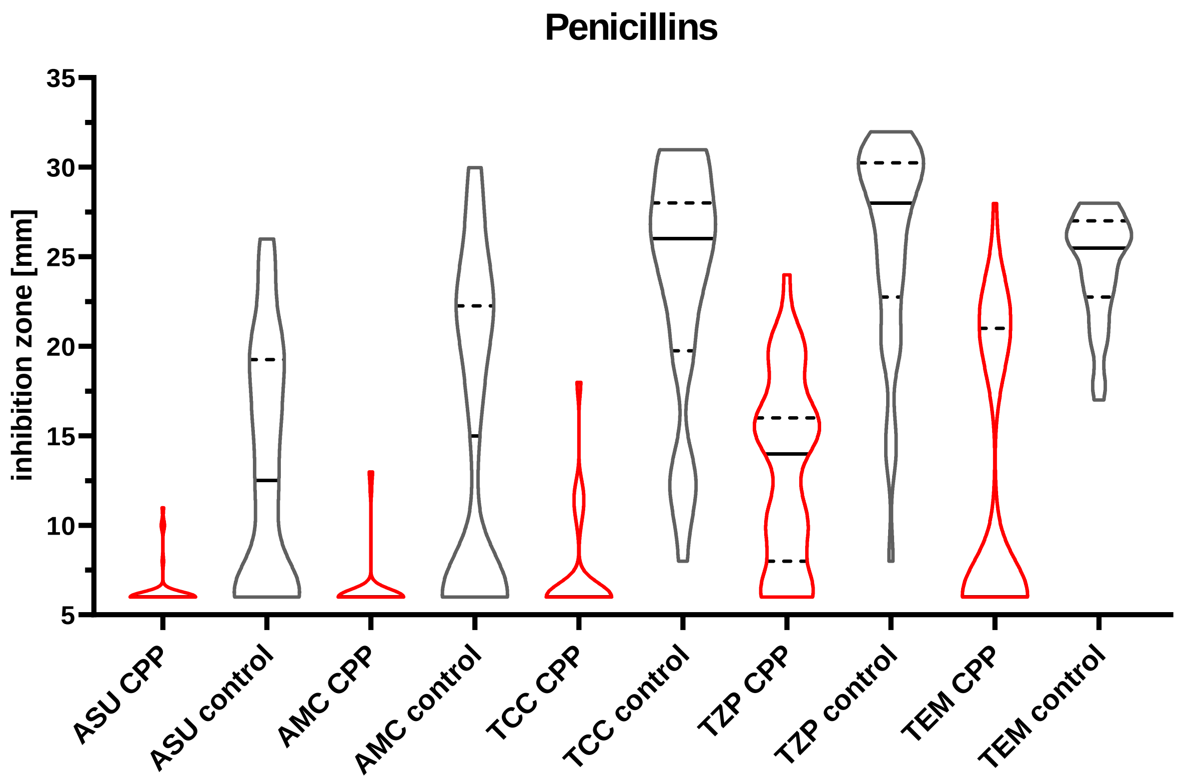


Fig. S4 Comparison of inhibition zones for A) carbapenems and B) five other β-lactam antibiotics among carbapenemase-producing *Proteus* spp. (CPP) and controls. ETP=ertapenem; MEM=meropenem; IPM=imipenem; FAR=faropenem; ASU=ampicillin-sulbactam; AMC=amoxicillin-clavulanate; TCC=ticarcillin-clavulanate; TZP=piperacillin-tazobactam; TEM=temocillin. The solid line denotes the median, the dashed line the quartiles.

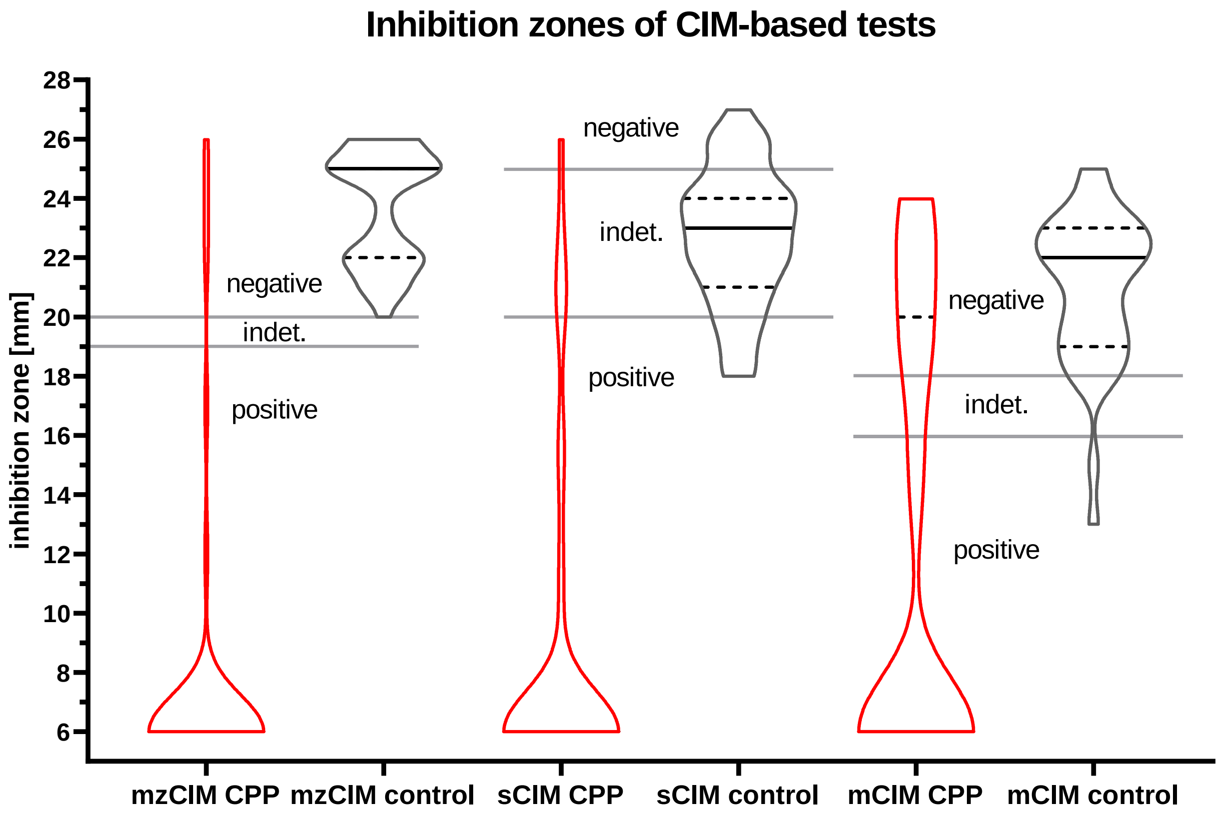


Fig. S5 Performance of mzCIM, sCIM and mCIM for detection of carbapenemase production. Inhibition zones are shown as violin plots. The horizontal grey bars indicate the cut-off of the test as described for sCIM by Jing *et al*. and for mCIM by Pierce *et al*. Indet.= indeterminate results. The solid line denotes the median, the dashed line the quartiles.

Table S2 Molecular characterization of CPP and control isolates with minimal inhibitory concentrations as determined by broth microdilution

					MIC (mg/L)									
Isolate	**Species**	**Carbapenemase**	**Other β-lactamases**	**Other resistance genes**	**TPZ**	**CAZ**	**CAZ-AVI**	**ETP**	**MEM**	**IMP**	**IMR**	**ATM-AVI**	**CIP**	**TEM**
Carb-01	*P. mirabilis*	*bla*$_{OXA-23}$		*aph(3'')-Ib, aph(6)-Id, floR, sul2, aac(3)-IIa, aph(3')-Ia, tet(J), dfrA1, ant(3'')-Ia*	16	< 0.25	< 2	> 1	16	> 16	16	< 0.5	0.125	32
Carb-02	*P. mirabilis*	*bla*$_{OXA-58}$	*bla*$_{CMY-2}$	*cat, aadA14, tet(J), sul3, ant(3'')-Ia, dfrA1*	64	8	< 2	< 0.125	0.25	4	4	< 0.5	4	64
Carb-03	*P. mirabilis*	*bla*$_{OXA-58}$		*cat, aadA14, tet(J), ant(3'')-Ia*	> 64	< 0.25	< 2	> 1	8	> 16	8	< 0.5	> 4	> 64
Carb-04	*P. mirabilis*	*bla*$_{OXA-58}$	*bla*$_{CMY-2}$	*cat, aadA14, tet(J), dfrA1, ant(3'')-Ia, sul3*	> 64	4	< 2	0.25	< 0.125	2	2	1	4	8
Carb-05	*P. mirabilis*	*bla*$_{OXA-58}$		*cat, aadA14, tet(J)*	> 64	< 0.25	< 2	> 1	32	> 16	> 16	< 0.5	0.125	> 64
Carb-06	*P. mirabilis*	*bla*$_{OXA-181}$	*bla*$_{TEM-1B}$ *bla*$_{VEB-6}$	*cat, tet(J), tet(A), aph(3')-Ia, sul2, aph(3'')-Ib, aph(6)-Id, catA1, ant(3'')-Ia, dfrA1, sul1, qacE, tet(A), sul1, qacE, dfrA1, ant(2'')-Ia, aac(6')-Ib*	> 64	> 32	< 2	> 1	4	> 16	> 16	< 0.5	> 4	> 64
Carb-07	*P. mirabilis*	*bla*$_{OXA-58}$		*qacE, sul1, mph(A), tet(A), ant(3'')-Ia, aadA14, dfrA1, tet(J), cat*	32	< 0.25	< 2	< 0.125	< 0.125	2	2	< 0.5	4	16
Carb-08	*P. mirabilis*	*bla*$_{OXA-58}$		*ant(3'')-Ia, dfrA1, qnrD1*	> 64	< 0.25	< 2	> 1	16	> 16	> 16	< 0.5	> 4	> 64
Carb-09	*P. mirabilis*	*bla*$_{IMP-1}$	*bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*cat, tet(J), sul2, aph(3'')-Ib, aph(6)-Id, aac(3)-IIa, aac(6')-Ib-cr, mph(E), msr(E), sul1, qacE, ant(3'')-Ia, sul1, qacE, aadA2, dfrA16, sul1, qnrA1*	4	8	8	1	4	16	16	< 0.5	1	< 4
Carb-10	*P. mirabilis*	*bla*$_{OXA-58}$		*cat, sul2, tet(J), sul3, ant(3'')-Ia, dfrA1, qnrD1*	> 64	2	< 2	> 1	0.25	8	8	< 0.5	> 4	16
Carb-11	*P. mirabilis*	*bla*$_{OXA-48}$	*bla*$_{CTX-M-14b}$	*cat, tet(J), aph(6)-Id, aph(3'')-Ib, ant(3'')-Ia, dfrA1, aph(3')-VIb, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, aac(3)-IIa, aph(3'')-Ib*	4	0.25	< 2	> 1	8	8	8	< 0.5	> 4	64
Carb-12	*P. mirabilis*	*bla*$_{OXA-58}$		*cat, aadA14, tet(J), qnrD1*	32	< 0.25	< 2	< 0.125	< 0.125	4	4	< 0.5	0.125	16
Carb-13	*P. mirabilis*	*bla*$_{OXA-23}$		*sul2, floR, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	8	< 0.25	< 2	< 0.125	0.25	4	4	< 0.5	< 0.06	< 4
Carb-14	*P. mirabilis*	*bla*$_{OXA-48}$	*bla*$_{TEM-1B}$	*cat, tet(J), sul1, qacE, ant(3'')-Ia, dfrA1, aph(6)-Id, aph(3'')-Ib, sul2, catA1*	64	< 0.25	< 2	0.5	0.5	8	4	< 0.5	< 0.06	64
Carb-15	*P. mirabilis*	*bla*$_{OXA-23}$		*sul2, aph(6)-Id, aph(3'')-Ib, aph(3')-VIa, aph(3')-Ia, aac(3)-IIa, tet(J), ant(3'')-Ia, dfrA1*	64	8	8	1	8	8	8	< 0.5	0.125	8

					MIC (mg/L)									
Isolate	**Species**	**Carbapenemase**	**Other β-lactamases**	**Other resistance genes**	**TPZ**	**CAZ**	**CAZ-AVI**	**ETP**	**MEM**	**IMP**	**IMR**	**ATM-AVI**	**CIP**	**TEM**
Carb-16	*P. mirabilis*	bla_{OXA-48}	bla_{TEM-1B}, bla_{TEM-2}, $bla_{CTX-M-14b}$	*cat, tet(J), catA1, dfrA12, aadA2, qacE, sul1, mph(A), sul2, aph(3'')-Ib, aph(6)-Id, aac(3)-IId, sul1, qacE, aadA5, dfrA17, ant(3'')-Ia, dfrA1*	32	1	< 2	1	2	8	4	< 0.5	> 4	32
Carb-17	*P. mirabilis*	bla_{OXA-23}	$bla_{CMY-138}$, bla_{VEB-6}, bla_{TEM-1B}, bla_{TEM-2}	*cat, tet(J), ant(3'')-Ia, dfrA1, aph(6)-Id, ant(2'')-Ia, dfrA1, qacE, sul1, tet(A), qacE, sul1, aph(3')-Ia, qacE, cmlA1, ant(2'')-Ia, aac(6')-Ib*	> 64	> 32	< 2	0.5	2	16	> 16	< 0.5	> 4	16
Carb-18	*P. mirabilis*	bla_{OXA-48}	bla_{CARB-2}, bla_{OXA-10}, $bla_{CTX-M-14b}$	*ant(3'')-Ia, dfrA1, cat, tet(J), ant(3'')-Ia, dfrA1, sul1, qacE, tet(B), sul1, qacE, ant(3'')-Ia, ant(2'')-Ia, aph(3')-Ia, aph(3'')-Ib, aph(3')-VIb, aph(3'')-Ib*	> 64	1	< 2	0.5	8	> 16	8	< 0.5	> 4	64
Carb-19	*P. mirabilis*	bla_{OXA-23}		*sul2, floR, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	4	< 0.25	< 2	< 0.125	0.25	4	4	< 0.5	< 0.06	< 4
Carb-20	*P. mirabilis*	$bla_{OXA-181}$	bla_{TEM-1B}, bla_{VEB-6}	*ant(3'')-Ia, dfrA1, cat, tet(J), tet(A), aph(6)-Id, aph(3'')-Ib, sul2, catA1, ant(3'')-Ia, dfrA1, sul1, qacE, tet(A), sul1, qacE, qnrA1, sul1, qacE, dfrA1, ant(2'')-Ia, aac(6')-Ib*	> 64	> 32	< 2	1	2	16	16	< 0.5	> 4	> 64
Carb-21	*P. mirabilis*	bla_{OXA-23}		*sul2, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	32	2	< 2	0.5	1	8	8	< 0.5	< 0.06	< 4
Carb-22	*P. mirabilis*	bla_{OXA-23}		*sul2, floR, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	4	< 0.25	< 2	< 0.125	0.25	2	2	< 0.5	< 0.06	< 4
Carb-23	*P. mirabilis*	bla_{OXA-23}		*tet(J), aph(3')-Ia, aac(3)-IIa, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, floR, sul2, ant(3'')-Ia, dfrA1*	16	< 0.25	< 2	0.25	0.25	8	4	< 0.5	0.125	< 4
Carb-24	*P. mirabilis*	bla_{OXA-23}		*aph(3'')-Ib, aph(6)-Id, floR, sul2, aac(3)-IIa, aph(3')-Ia, tet(J), dfrA1, ant(3'')-Ia*	32	< 0.25	< 2	0.5	1	8	8	< 0.5	< 0.06	< 4
Carb-25	*P. mirabilis*	bla_{OXA-48}	$bla_{CTX-M-65}$, bla_{TEM-1B}, bla_{OXA-1}	*cat, tet(J), catA1, dfrA17, aadA5, qacE, sul1, fosA3, aph(3')-Ia, sul2, aph(3'')-Ib, aph(6)-Id, aac(3)-IId, sul1, qacE, ARR-3, catB3, aac(6')-Ib-cr, aac(3)-IVa, aph(4)-Ia, sul2, floR, ant(3'')-Ia, dfrA1*	32	< 0.25	< 2	0.25	0.5	4	4	< 0.5	> 4	64
Carb-26	*P. mirabilis*	$bla_{OXA-162}$	$bla_{CTX-M-15}$	*tet(J)*	64	4	< 2	0.5	0.5	8	4	< 0.5	0.25	> 64

Isolate	Species	Carbapenemase	Other β-lactamases	Other resistance genes	MIC (mg/L)									
					TPZ	CAZ	CAZ-AVI	ETP	MEM	IMP	IMR	ATM-AVI	CIP	TEM
Carb-27	*P. mirabilis*	*bla*$_{\text{OXA-48}}$	*bla*$_{\text{CTX-M-14b}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CTX-M-14b}}$	*tet(J), rmtB, dfrA17, aadA5, qacE, sul1, mph(A), aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, qnrD1*	< 1	< 0.25	< 2	0.25	0.25	4	4	< 0.5	> 4	64
Carb-28	*P. mirabilis*	*bla*$_{\text{OXA-23}}$		*sul2, floR, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	2	< 0.25	< 2	< 0.125	0.25	4	2	< 0.5	< 0.06	< 4
Carb-29	*P. mirabilis*	*bla*$_{\text{OXA-181}}$		*tet(J), qnrS1*	16	< 0.25	< 2	0.5	1	16	8	< 0.5	< 0.06	32
Carb-30	*P. mirabilis*	*bla*$_{\text{OXA-58}}$		*tet(J), sul3, ant(3'')-Ia, cmlA1, aadA2, ant(3'')-Ia, dfrA1, aadA14, qnrD1*	> 64	< 0.25	< 2	< 0.125	0.25	8	8	< 0.5	> 4	16
Carb-31	*P. mirabilis*	*bla*$_{\text{OXA-58}}$		*tet(J), aadA14*	> 64	< 0.25	< 2	> 1	8	> 16	> 16	< 0.5	0.25	> 64
Carb-32	*P. mirabilis*	*bla*$_{\text{OXA-48}}$		*cat, tet(J), catA3, dfrA1*	4	< 0.25	< 2	0.5	0.5	8	2	< 0.5	< 0.06	32
Carb-33	*P. mirabilis*	*bla*$_{\text{VIM-1}}$	*bla*$_{\text{CMY-99}}$, *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{OXA-9}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1B}}$	*cat, tet(J), ant(3'')-Ia, aac(6')-Ib, aph(6)-Id, aph(3'')-Ib, sul2, ant(3'')-Ia, dfrA1, aac(6')-Il, mph(E), msr(E), armA, sul1, qacE, aadA2, dfrA12, aac(6')-Il, dfrA1, ant(3'')-Ia, sul2, aph(3'')-Ib, aph(6)-Id, catA1, ant(3'')-Ia, dfrA1*	32	> 32	4	< 0.125	2	16	16	< 0.5	> 4	8
Carb-34	*P. mirabilis*	*bla*$_{\text{OXA-48}}$	*bla*$_{\text{CTX-M-14b}}$	*tet(J), cat, dfrA1, ant(3'')-Ia, aac(3)-IIa, aph(3')-Ia, aph(6)-Id, aph(3'')-Ib, aph(3')-VIb, aph(3'')-Ib, aph(3'')-Ib, aph(6)-Id*	8	< 0.25	< 2	0.5	1	16	8	< 0.5	4	16
Carb-35	*P. mirabilis*	*bla*$_{\text{OXA-48}}$		*tet(J), dfrA1, cat*	64	< 0.25	< 2	0.25	0.5	4	4	< 0.5	< 0.06	32
Carb-36	*P. mirabilis*	*bla*$_{\text{NDM-1}}$	*bla*$_{\text{TEM-1B}}$	*cat, tet(J), aph(6)-Id, aph(3'')-Ib, sul2, sul1, qacE, dfrA7, catA1*	16	32	> 32	0.5	> 32	> 16	> 16	< 0.5	< 0.06	< 4
Carb-37	*P. mirabilis*	*bla*$_{\text{NDM-1}}$	*bla*$_{\text{TEM-1B}}$	*aph(6)-Id, aph(3'')-Ib, sul2, sul1, qacE, dfrA7, catA1, cat, tet(J)*	> 64	> 32	> 32	> 1	> 32	> 16	> 16	< 0.5	< 0.06	< 4
Carb-38	*P. mirabilis*	*bla*$_{\text{VIM-4}}$	*bla*$_{\text{TEM-2}}$	*cat, tet(J), sul2, qacE, sul1, qnrA1, ant(3'')-Ia, aph(3')-VI, catA1, aph(3')-Ia, aac(3)-IVa, lnu(F), aac(6')-IIc, dfrA1*	> 64	> 32	32	0.5	4	16	16	< 0.5	> 4	8
Carb-39	*P. mirabilis*	*bla*$_{\text{OXA-58}}$		*sul2, aadA14, cat, tet(J)*	> 64	< 0.25	< 2	1	4	16	> 16	< 0.5	1	64
Carb-40	*P. mirabilis*	*bla*$_{\text{OXA-58}}$		*aadA14, ant(3'')-Ia, tet(J), cat*	> 64	< 0.25	< 2	> 1	8	> 16	> 16	< 0.5	> 4	> 64
Carb-41	*P. mirabilis*	*bla*$_{\text{KPC-3}}$		*dfrA1, catA1, sul1, qacE, cat, tet(J)*	16	4	< 2	< 0.125	0.25	4	2	< 0.5	< 0.06	< 4

					MIC (mg/L)									
Isolate	**Species**	**Carbapenemase**	**Other β-lactamases**	**Other resistance genes**	**TPZ**	**CAZ**	**CAZ-AVI**	**ETP**	**MEM**	**IMP**	**IMR**	**ATM-AVI**	**CIP**	**TEM**
Carb-42	*P. mirabilis*	bla_{OXA-23}		*aph(6)-Id, aph(3'')-Ib, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	> 64	< 0.25	< 2	1	2	> 16	> 16	< 0.5	< 0.06	< 4
Carb-43	*P. mirabilis*	bla_{OXA-23}		*aph(3')-VIa, aph(3'')-Ib, aph(6)-Id, sul2, aac(3)-IIa, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	32	< 0.25	< 2	1	2	16	16	< 0.5	< 0.06	8
Cont-01	*P. mirabilis*		bla_{VEB-6}, bla_{TEM-1B}	*ant(2'')-Ia, aac(6')-Ib, cat, ant(3'')-Ia, tet(J), catA1, aph(6)-Id, aph(3'')-Ib, sul2, qnrA1, qacE, sul1, aph(3')-Ia, tet(A), dfrA1*	< 1	32	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	> 4	< 4
Cont-02	*P. mirabilis*		bla_{TEM-1A}, $bla_{CMY-138}$	*cat, sul2, aph(3'')-Ib, aph(6)-Id, catA1, aac(3)-IIa, aph(3')-Ia, ant(3'')-Ia, dfrA1, tet(J)*	> 64	> 32	< 2	< 0.125	< 0.125	2	< 0.5	< 0.5	< 0.06	< 4
Cont-03	*P. mirabilis*		bla_{CMY-2}	*tet(J), dfrA1, ant(3'')-Ia, sul3*	< 1	4	< 2	< 0.125	< 0.125	4	2	< 0.5	4	< 4
Cont-04	*P. mirabilis*		$bla_{CTX-M-15}$	*tet(J), ant(2'')-Ia, aadA3, qacE, sul1, dfrA1, ant(3'')-Ia*	< 1	16	< 2	< 0.125	< 0.125	1	2	< 0.5	2	< 4
Cont-05	*P. mirabilis*		$bla_{CTX-M-15}$	*cat, dfrA1, tet(J)*	< 1	2	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-06	*P. mirabilis*		$bla_{CTX-M-3}$	*tet(J), cat, qnrD1, ant(3'')-Ia, dfrA1*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-07	*P. mirabilis*		bla_{CMY-2}	*cat, tet(J), ant(3'')-Ia, dfrA1*	64	> 32	< 2	> 1	8	> 16	< 0.5	< 0.5	> 4	8
Cont-08	*P. mirabilis*		$bla_{CTX-M-3}$	*cat, tet(J), catA1, qnrD1, dfrA1, ant(3'')-Ia, aph(3')-Ia*	< 1	< 0.25	< 2	< 0.125	< 0.125	1	1	< 0.5	> 4	< 4
Cont-09	*P. mirabilis*		bla_{TEM-1B}	*catA1, aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, cat, ant(3'')-Ia, dfrA1, lnu(F), sul1, qacE, aadA2, ant(2'')-Ia, tet(J)*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-10	*P. mirabilis*		bla_{CMY-2}	*tet(J), ant(3'')-Ia*	< 1	2	< 2	< 0.125	< 0.125	1	2	< 0.5	> 4	< 4
Cont-11	*P. mirabilis*		$bla_{CTX-M-15}$, bla_{TEM-1B}	*tet(A)*	< 1	2	< 2	< 0.125	< 0.125	2	2	< 0.5	2	< 4
Cont-12	*P. mirabilis*		$bla_{CTX-M-15}$, bla_{TEM-2}	*tet(J)*	2	8	< 2	> 1	< 0.125	16	1	< 0.5	> 4	< 4
Cont-13	*P. mirabilis*		bla_{TEM-1B}	*tet(J), catA1, aph(6)-Id, aph(3'')-Ib, sul2, dfrA7, qacE, sul1, cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	2	2	< 0.5	< 0.06	8
Cont-14	*P. mirabilis*		$bla_{CTX-M-15}$, bla_{TEM-1B}	*qacE, sul1, tet(J), ant(3'')-Ia, catA1, aph(6)-Id, aph(3'')-Ib, sul2, rmtB, aac(6')-Ib, ant(2'')-Ia, aph(3')-Ia, dfrA1, cat*	< 1	8	< 2	< 0.125	< 0.125	1	1	< 0.5	4	8

					MIC (mg/L)									
Isolate	**Species**	**Carbapenemase**	**Other β-lactamases**	**Other resistance genes**	**TPZ**	**CAZ**	**CAZ-AVI**	**ETP**	**MEM**	**IMP**	**IMR**	**ATM-AVI**	**CIP**	**TEM**
Cont-15	*P. mirabilis*		bla_{TEM-1B}	*catA1, aph(6)-Id, aph(3'')-Ib, sul2, tet(J), dfrA1, ant(3'')-Ia*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-16	*P. mirabilis*		bla_{TEM-1B}	*catA1, sul2, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	< 1	< 0.25	< 2	< 0.125	< 0.125	4	2	< 0.5	< 0.06	< 4
Cont-17	*P. mirabilis*		.	*tet(J), dfrA1, floR, aph(6)-Id, aph(3'')-Ib, sul2, cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-18	*P. mirabilis*		bla_{TEM-1B}	*cat, ant(3'')-Ia, dfrA1, aph(6)-Id, aph(3'')-Ib, sul2, catA1, aph(3')-Ia, tet(J)*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-19	*P. mirabilis*		$bla_{CTX-M-15}$, bla_{TEM-1B}	*cat, sul1, qacE, tet(J), ant(3'')-Ia, catA1, aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, dfrA1, ant(2'')-Ia, aac(6')-Ib*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-20	*P. mirabilis*		bla_{CMY-2}	*tet(J), ant(3'')-Ia, dfrA1, cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	< 0.5	< 0.5	< 0.5	< 0.06	< 4
Cont-21	*P. mirabilis*		bla_{TEM-1B}	*cat, catA1, sul2, aph(3'')-Ib, aph(6)-Id, dfrA1, ant(3'')-Ia, tet(J)*	< 1	< 0.25	< 2	< 0.125	< 0.125	1	2	< 0.5	< 0.06	< 4
Cont-22	*P. mirabilis*		bla_{ACC-4}, bla_{TEM-1B}	*cat, sul2, aph(3'')-Ib, aph(6)-Id, catA1, aac(3)-IIa, dfrA1, ant(3'')-Ia, aph(3')-Ia, tet(J)*	64	> 32	< 2	< 0.125	0.25	1	1	< 0.5	1	< 4
Cont-23	*P. mirabilis*		bla_{CMY-16}, bla_{TEM-1B}	*cat, qacE, sul1, tet(A), catA1, sul2, aph(3'')-Ib, aph(6)-Id, aac(3)-Ia, aph(3')-Ia, tet(J), dfrA1*	16	> 32	< 2	< 0.125	< 0.125	4	1	< 0.5	> 4	< 4
Cont-24	*P. mirabilis*		bla_{TEM-1B}	*aac(3)-IIa, catA1, aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, tet(J), dfrA1, ant(3'')-Ia, cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	4	4	< 0.5	< 0.06	< 4
Cont-25	*P. mirabilis*		bla_{CMY-16}, bla_{TEM-1B}	*cat, qacE, sul1, tet(A), catA1, aph(6)-Id, aph(3'')-Ib, sul2, aac(3)-Ia, aph(3')-Ia, tet(J), dfrA1*	4	> 32	< 2	0.5	0.5	4	2	< 0.5	> 4	< 4
Cont-26	*P. mirabilis*		bla_{CMY-2}	.	64	> 32	< 2	> 1	8	> 16	1	< 0.5	> 4	< 4
Cont-27	*P. mirabilis*		bla_{CMY-16}, bla_{TEM-1B}	*tet(J), cat, qacE, sul1, tet(A), catA1, sul2, aph(3'')-Ib, aph(6)-Id, aac(3)-Ia, aph(3')-Ia, dfrA1*	8	> 32	< 2	0.25	0.25	4	2	< 0.5	4	< 4
Cont-28	*P. mirabilis*		bla_{OXA-10}, bla_{DHA-1}	*tet(B), lnu(G), dfrA14, ARR-3, ant(3'')-Ia, qacE, sul1, sul2, aph(3'')-Ib, aph(6)-Id, floR, aac(3)-IId, qnrD1, aph(3')-Ia, tet(J)*	< 1	32	< 2	< 0.125	< 0.125	8	2	< 0.5	> 4	< 4
Cont-29	*P. mirabilis*		bla_{CMY-2}	*cat, sul1, qacE, aadA2, ant(2'')-Ia, tet(J)*	< 1	4	< 2	< 0.125	< 0.125	4	2	< 0.5	2	< 4

Isolate	Species	Carbapenemase	Other β-lactamases	Other resistance genes	MIC (mg/L)									
					TPZ	CAZ	CAZ-AVI	ETP	MEM	IMP	IMR	ATM-AVI	CIP	TEM
Cont-30	*P. mirabilis*		$\mathit{bla}_{\text{CMY-138}}$, $\mathit{bla}_{\text{TEM-1B}}$	*cat, tet(A), aac(3)-IIa, sul2, aph(3'')-Ib, aph(6)-Id, catA1, aph(3')-Ia, dfrA1, ant(3'')-Ia, tet(J)*	< 1	> 32	< 2	< 0.125	0.25	4	2	< 0.5	< 0.06	< 4
Cont-31	*P. mirabilis*		$\mathit{bla}_{\text{TEM-122}}$	*cat, catA1, dfrA17, aadA5, qacE, sul1, aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, aac(3)-IIa, tet(J), ant(3'')-Ia, dfrA1*	< 1	< 0.25	< 2	< 0.125	< 0.125	4	2	< 0.5	> 4	< 4
Cont-32	*P. mirabilis*		$\mathit{bla}_{\text{TEM-1B}}$	*cat, catA1, dfrA17, aadA5, qacE, sul1, aac(3)-IId, aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, tet(J), ant(3'')-Ia, dfrA1*	< 1	< 0.25	< 2	< 0.125	< 0.125	2	2	< 0.5	> 4	< 4
Cont-33	*P. mirabilis*		$\mathit{bla}_{\text{TEM-1D}}$	*tet(J), aac(3)-IIa, cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	2	2	< 0.5	< 0.06	< 4
Cont-34	*P. mirabilis*		$\mathit{bla}_{\text{TEM-92}}$	*aac(3)-IIa, qacE, sul1, tet(J), dfrA1, catA1, aph(3')-VIa*	< 1	> 32	< 2	< 0.125	< 0.125	2	2	< 0.5	> 4	< 4
Cont-35	*P. mirabilis*		$\mathit{bla}_{\text{CTX-M-1}}$	*cat, tet(J), aph(6)-Id, aph(3'')-Ib, mph(A)*	< 1	1	< 2	< 0.125	< 0.125	4	2	< 0.5	< 0.06	< 4
Cont-38	*P. mirabilis*		$\mathit{bla}_{\text{VEB-5}}$	*tet(J), aac(3)-IId, catA1, cat, tet(A), dfrA1, aac(6')-Ib, sul1, qacE*	< 1	> 32	< 2	< 0.125	< 0.125	1	1	< 0.5	> 4	< 4
Cont-39	*P. mirabilis*		$\mathit{bla}_{\text{TEM-1B}}$	*dfrA1, aac(3)-IIa, aph(6)-Id, aph(3'')-Ib, sul2, catA1, qacE, sul1, aph(3')-Ia, tet(J), cat*	< 1	< 0.25	< 2	< 0.125	< 0.125	8	8	< 0.5	< 0.06	8
Cont-40	*P. mirabilis*		$\mathit{bla}_{\text{TEM-1B}}$	*tet(J), catA1, aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia*	< 1	< 0.25	< 2	< 0.125	< 0.125	2	2	< 0.5	< 0.06	< 4

TPZ=piperacillin-tazobactam; CAZ=ceftazidime; CAZ-AVI=ceftazidime-avibactam; ETP=ertapenem; MEM=meropenem; IMP=imipenem; IMR=imipenem-relebactam; ATM-AVI=aztreonam-avibactam; CIP=ciprofloxacin; TEM=temocillin

Table S3 Number of correctly detected carbapenemases by different assays, stratified by carbapenemase family

	OXA-23 (n=12)	OXA-48-like (n=13)	OXA-58 (n=12)	NDM (n=2)	VIM (n=2)	IMP (n=1)	KPC (n=1)	Total
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
CARBA NP	2 (17)	5 (38)	2 (17)	1 (50)	1 (50)	1 (100)	1 (100)	13 (30)
Faropenem	9 (75)	10 (77)	10 (83)	2 (100)	2 (100)	1 (100)	1 (100)	35 (81)
Temocillin*	1 (8)	13 (100)	12 (100)	0 (0)	0 (0)	0 (0)	0 (0)	26 (60)
mCIM	10 (83)	11 (85)	1 (8)	2 (100)	2 (100)	1 (100)	0 (0)	27 (63)
sCIM	12 (100)	10 (77)	9 (75)	2 (100)	2 (100)	1 (100)	1 (100)	39 (91)
mzCIM	12 (100)	12 (92)	10 (83)	2 (100)	2 (100)	1 (100)	1 (100)	40 (93)
RESIST-5	0 (0)	12 (92)	0 (0)	2 (100)	1 (50)	1 (100)	1 (100)	17 (40)
CARBA-5	0 (0)	12 (92)	0 (0)	2 (100)	2 (100)	1 (100)	1 (100)	18 (42)
mSuperCarba	7 (58)	11 (85)	8 (67)	2 (100)	1 (50)	1 (100)	0 (0)	30 (70)

*Cut-off for temocillin <14 mm

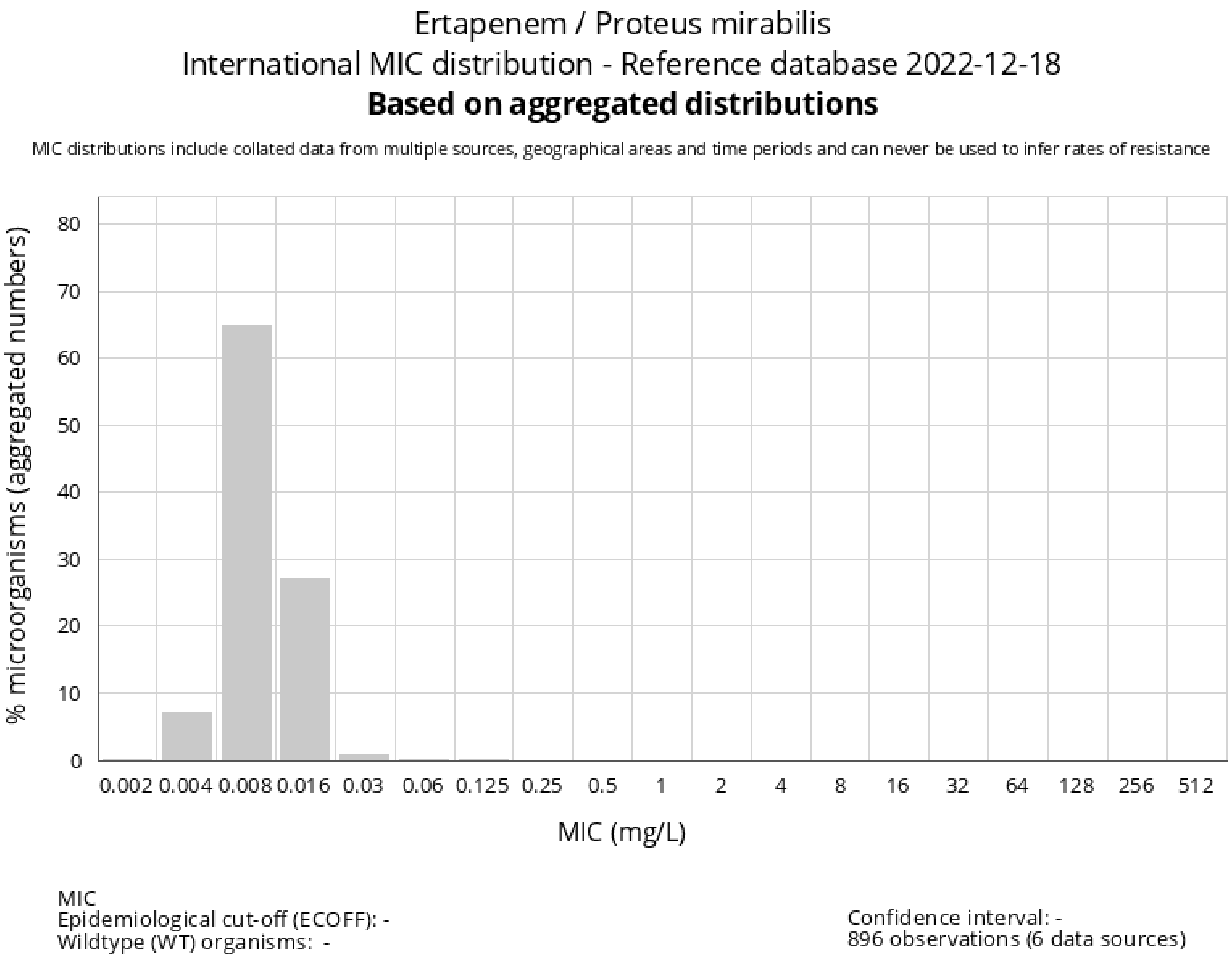


Fig. S5 MIC distribution of ertapenem in *P. mirabilis* (https://mic.eucast.org/), last accessed 18.12.2022

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