Supplemental material

Figure S1: Relative abundance of ARG genes normalized to *16S rRNA* at H1a (black), H1b (sparse), H2 (grey). Significant differences (*p* value <0.05, student t-test) were indicated by different letters (e.g., a and b, a and bc) on the top of each bar. No significant differences were indicated by the same letters (e.g., a and a, a and ab).

Figure S2. Principle component analysis of ARG, ARB and antibiotic concentrations for three groups of antibiotics in the hospital wastewater: (A) meropenem and ceftazidime, (B) sulfamethoxazole and trimethoprim, (C) amikacin and ciprofloxacin

Table S1. MDL, SPE recovery and overall recovery of the compounds

Table S2: PCR primers used in this study

Table S3. Identification of meropenem resistance isolates and PCR detection of corresponding ARG (i.e., bla_{NDM}, bla_{KPC}, bla_{CTX-M}, bla_{SHV}) and *int1* genes

Table S4. Identification of ceftazidime resistance isolates and PCR detection of corresponding ARG (i.e., bla_{NDM}, bla_{KPC}, bla_{CTX-M}, bla_{SHV}) and *int1* genes

Table S5. Identification of cotrimoxazole resistance isolates and PCR detection of corresponding ARG (i.e., *sul1*, *sul2*, and *dfrA*) and *int1* genes

Table S6. Identification of amikacin resistance isolates and PCR detection of corresponding ARG (i.e., *aac(6')-Ib*) and *int1* genes

Table S7. Identification of ciprofloxacin resistance isolates and PCR detection of corresponding ARG (i.e., *qnrA*, *qnrB*) and *int1* genes

Figure S1: Relative abundance of ARGgenes normalized to *16S rRNA* at H1a (black), H1b (sparse), H2 (grey). Significant differences (*p* value <0.05, student t-test) were indicated by different letters (e.g.,, a and b, a and bc) on the top of each bar. No significant differences were indicated by the same letters (e.g., a and a, a and ab).

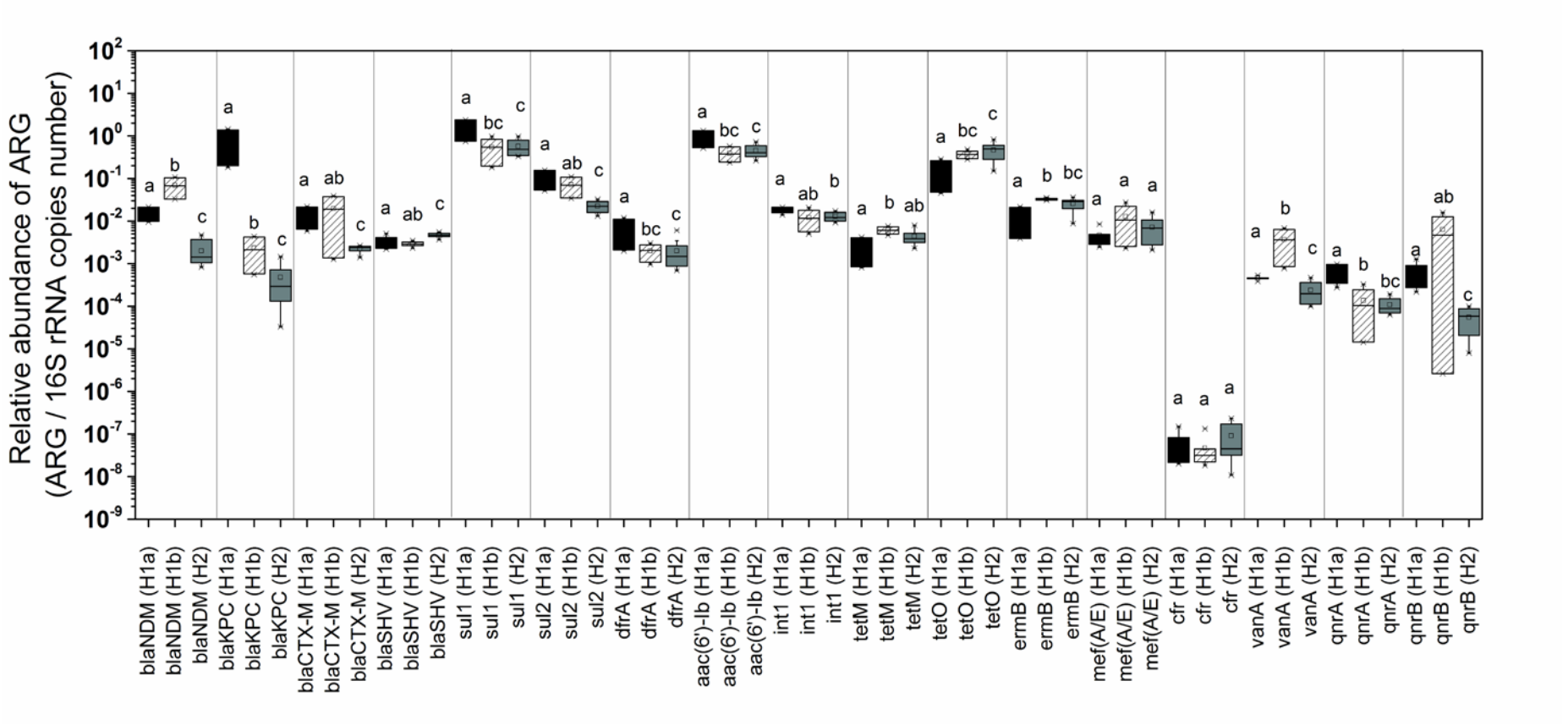
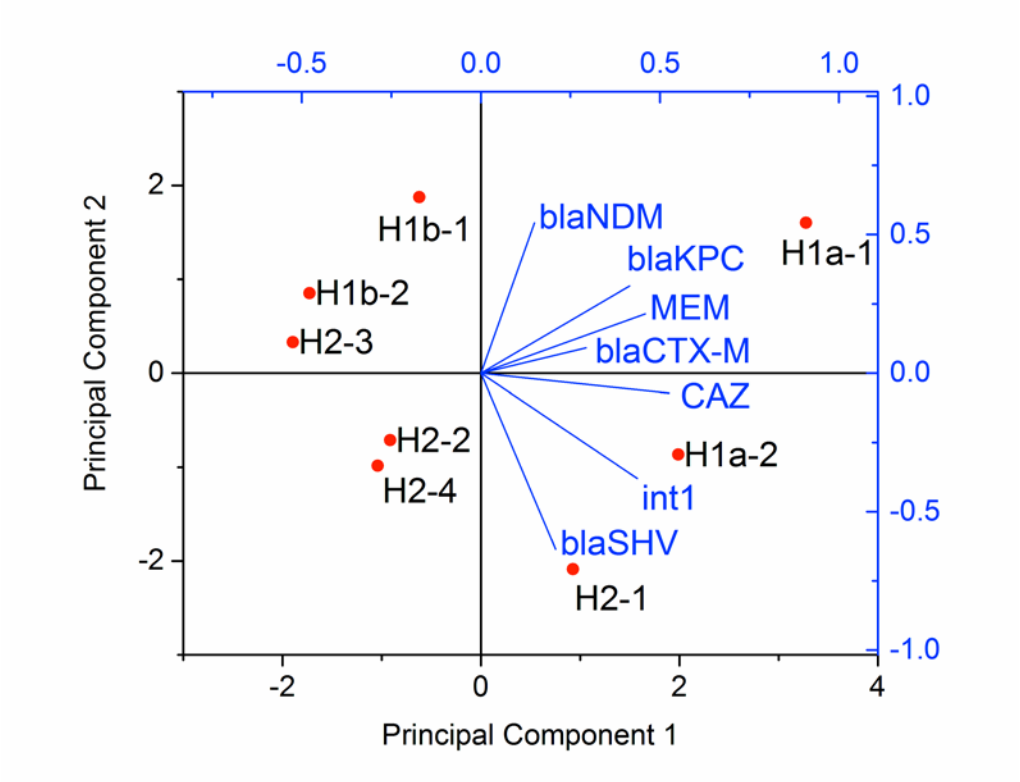
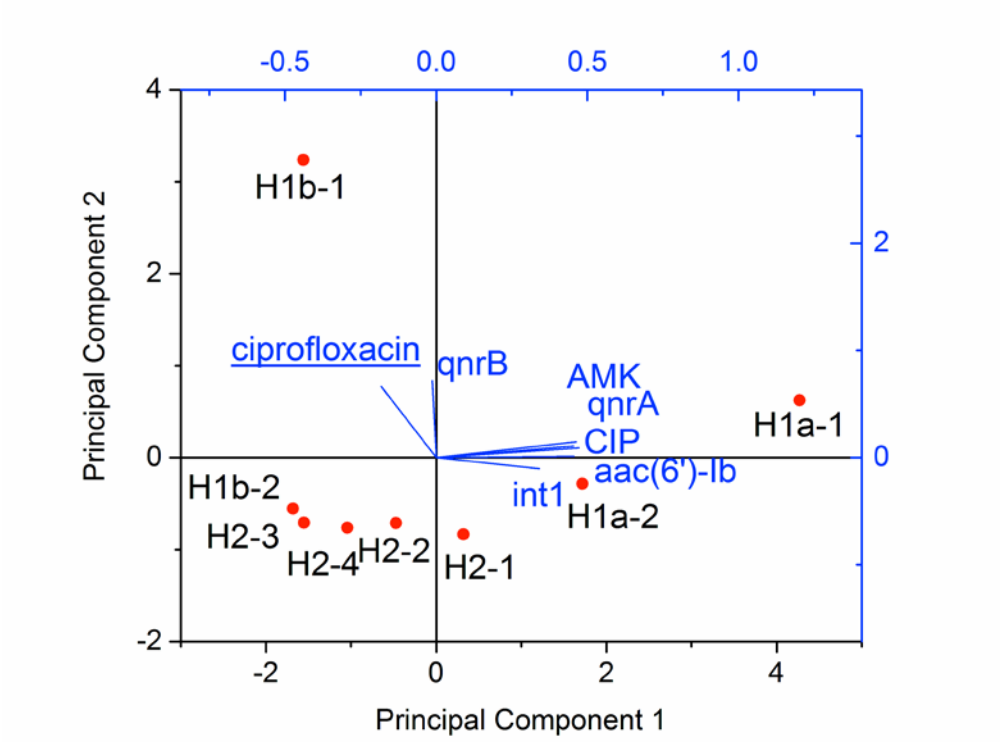


Figure S2. Principle component analysis of ARG, ARB and antibiotic concentrations for three groups of antibiotics in the hospital wastewater: (A) meropenem and ceftazidime, (B) sulfamethoxazole and trimethoprim, (C) amikacin and ciprofloxacin

(A)



(B)


-0.5
0.0
0.5
1.0
4
2
0
-2
2
0
H1b-1
ciprofloxacin
qnrB
AMK
qnrA
CIP
aac(6')-Ib
int1
H1a-1
H1a-2
H1b-2
H2-3
H2-4
H2-2
H2-1
-2
0
2
4
Principal Component 2
Principal Component 1

(C)

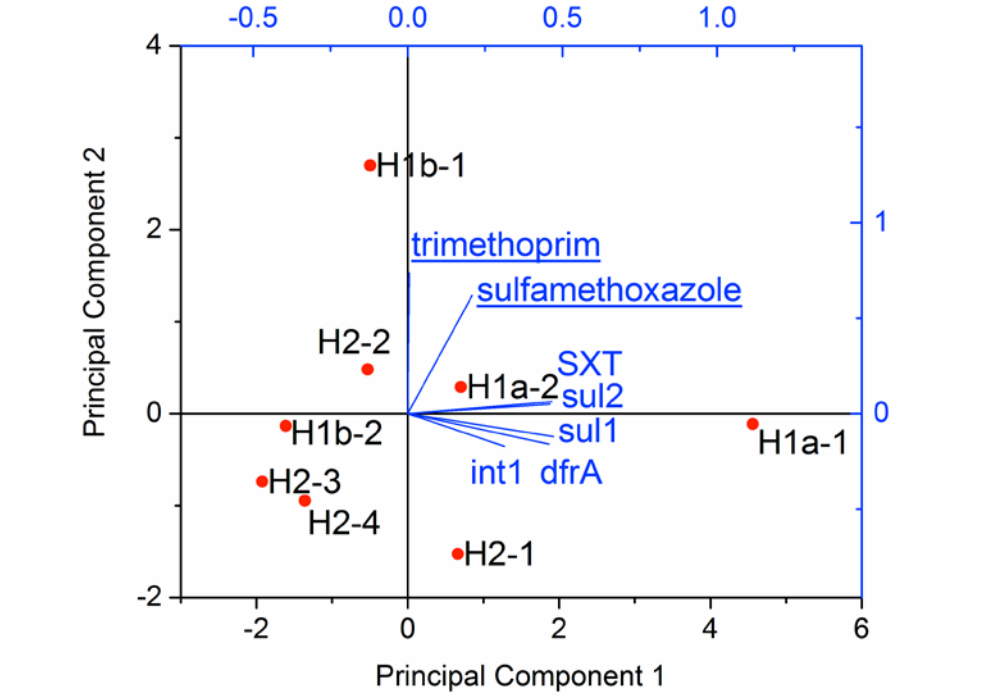

-0.5
0.0
0.5
1.0
4
2
0
-2
1
0
H1b-1
trimethoprim
sulfamethoxazole
H2-2
SXT
H1a-2
sul2
H1b-2
sul1
H1a-1
int1
dfrA
H2-3
H2-4
H2-1
-2
0
2
4
6
Principal Component 2
Principal Component 1

Table S1. MDL, SPE recovery and overall recovery of the compounds

Compound	MDL (ng/L)	SPE recovery		Overall recovery	
		Average	RSD	Average	RSD
meropenem	15.1	67%	9%	57%	10%
lincomycin	1.5	49%	7%	41%	12%
sulfamethazine	8.0	108%	2%	129%	5%
trimethoprim	4.7	80%	8%	79%	23%
tetracycline	50.8	99%	20%	88%	26%
ciprofloxacin	70.3	101%	13%	94%	24%
sulfamethoxazole	22.3	93%	12%	109%	12%
chloramphenicol	18.9	100%	11%	112%	10%
azithromycin	1.8	43%	7%	85%	24%
clindamycin	28.0	142%	13%	96%	20%
erythromycin	172.1	87%	7%	85%	17%
clarithromycin	2.4	112%	11%	102%	9%

Table S2: PCR primers used in this study

Target genes	Primer	Sequences (5'-3')	Amplicon length (bp)	Ta (^{o}C)	References
16S rRNA	16S EUB-f	ACT CCT ACG GGA GGC AGC AG	193	64	(1)
	16S EUB-r	ATT ACC GCG GCT GCT GG			(2)
aac(6')-Ib	aac6-f	CAT ATC GTC GAG TGG TGG GG	264	62	This study
	aac6-r	CTT GGT TCC CAA GCC TTT GC			This study
bla$_{SHV}$	bla SHVf	CGC TTT CCC ATG ATG AGC ACC TTT	109	60	(3)
	bla SHVr	TCC TGC TGG CGA TAG TGG ATC TTT			(3)
bla$_{CTX-M}$	blaCTX-Mf	ATG TGC AGY ACC AGT AAR GTK ATG GC	300	60	(4)
	blaCTX-Mr	ATC ACK CGG RTC GCC XGG RAT			(4)
bla$_{KPC}$	blaKPC-f	GAT ACC ACG TTC CGT CTG G	246	60	(5)
	blaKPC-r	GCA GGT TCC GGT TTT GTC TC			(5)
bla$_{NDM}$	blaNDM-f	AAT GGC TCA TCA CGA TCA TGC	220	60	This study
	blaNDM-r	GGC CCG CTC AAG GTA TTT TAC			This study
cfr	cfr-h2f	TGA CCA CAA GCA GCG TCA AT	104	60	(6)
	cfr-h2r	AAC GAA GGG CAG GTA GAA GC			(6)
dfrA	dfrA-f	ACG GAT CCT GGC TGT TGG TTG GAC GC	237	60	(7)
	dfrA-r	CGG AAT TCA CCT TCC GGC TCG ATG TC			(7)
erm(B)	ermB-f	GGT TGC TCT TGC ACA CTC AAG	191	62	(8)
	ermB-r	CAG TTG ACG ATA TTC TCG ATT G			(8)
int1	int1-LC1	GCC TTG ATG TTA CCC GAG AG	196	60	(9)
	int1-LC5	GAT CGG TCG AAT GCG TGT			(9)
mef(A/E)	mefAE-f	AGT ATC ATT AAT CAC TAG TGC	346	60.5	(10)
	mefAE-r	TTC TTC TGG TAC TAA AAG TGG			(10)
qnrA	qnrAf	ATT TCT CAC GCC AGG ATT TG	158	60	(11)
	qnrAr	GCA GAT CGG CAT AGC TGA AG			(11)

Target genes	Primer	Sequences (5'-3')	Amplicon length (bp)	Ta (^{o}C)	References
qnrB	qnrBf	GGM ATH GAA ATT CGC CAC TG	263	60	(11)
	qnrBr	TTY GCB GYY CGC CAG TCG AA			(11)
sul1	sul1-f	CAC CGT TGG CCT TCC TGT AA	180	61	This study
	sul1-r	TCT AAC CCT CGG TCT CTG GC			This study
sul2	sul2-f	GCG CTC AAG GCA GAT GGC ATT	293	60	(12)
	sul2-r	GCG TTT GAT ACC GGC ACC CGT			(12)
tet(M)	tetM-f	CC[TA] AC[AT] GTC ATT TAT ATG GA[GA] AGA CC	304	62.5	(13)
	tetM-r	CGA AAA TCT GCT GG[CGA] GTA CT[GA] ACA GGG C			(13)
tet(O)	tetO-f	AAG AAA ACA GGA GAT TCC AAA ACG	75	60	(14)
	tetO-r	CGA GTC CCC AGA TTG TTT TTA GC			(14)
vanA	vanA-h2f	CAG CCT GAT TTG GTC CAC CT	183	60	This study
	vanA-h2r	GGC TCA TCC TTC GGT GTG AA			This study

Table S3. Identification of meropenem resistance isolates and PCR detection of corresponding ARGs (i.e., bla_{NDM}, bla_{KPC}, bla_{CTX-M}, bla_{SHV}) and *int1* genes

Location	**Identification of bacterial isolates (n=24)**	**Meropenem resistance**	***bla*$_{NDM}$**	***bla*$_{KPC}$**	***bla*$_{CTX-M}$**	***bla*$_{SHV}$**	***int1***
H1a	*Enterobacter / Pantoea*	*5*	1	5	2	1	5
H1a	*Kluyvera georgiana*	*1*	0	1	1	0	1
H1b	*Acinetobacter junii*	*1*	1	0	0	0	0
H1b	*Comamonas testosteroni*	*1*	1	0	1	1	1
H1b	*Enterobacter / Pantoea*	*1*	1	1	1	0	0
H1b	*Pseudomonas spp.*	*3*	3	0	0	0	3
H2	*Acinetobacter spp.*	*3*	1	0	0	0	2
H2	*Aeromonas hydrophila*	*1*	0	0	0	1	0
H2	*Elizabethkingia anophelis*	*2*	0	0	0	0	2
H2	*Klebsiella pneumoniae*	*3*	1	0	0	3	2
H2	*Pseudomonas spp.*	*2*	1	0	0	0	2
H2	*Stenotrophomonas pavanii*	*1*	0	0	0	0	0
	Percentage positive (%)	100.0%	41.7%	31.8%	20.8%	25.0%	75%

Table S4: Identification of ceftazidime resistance isolates and PCR detection of corresponding ARGs (i.e., *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$) and *int1* genes

Location	**Identification of bacterial isolates (n=24)**	**Ceftazidime resistance**	***bla*$_{NDM}$**	***bla*$_{KPC}$**	***bla*$_{CTX-M}$**	***bla*$_{SHV}$**	***int1***
H1a	*Aeromonas spp.*	*2*	0	0	0	0	2
H1a	*Enterobacter / Pantoea*	*2*	0	1	1	0	2
H1a	*Klebsiella pneumoniae*	*2*	0	2	1	2	2
H1b	*Acinetobacter junii*	*3*	3	0	0	2	0
H1b	*Shigella/E.coli*	*3*	0	0	3	0	3
H2	*Aeromonas spp.*	*5*	0	0	0	0	4
H2	*Elizabethkingia anophelis*	*1*	0	0	0	0	0
H2	*Klebsiella pneumoniae*	*4*	0	1	2	3	3
H2	*Pseudomonas spp.*	*2*	0	0	0	0	2
	Percentage positive (%)	100.0%	12.5%	17.4%	29.2%	29.2%	75%

Table S5: Identification of cotrimoxazole resistance isolates and PCR detection of corresponding ARGs (i.e., *sul1*, *sul2*, and *dfrA*) and *int1* genes

Location	**Identification of bacterial isolates (n=24)**	**Cotrimoxazole resistance**	***sul1***	***sul2***	***dfrA***	***int1***
H1a	*Enterobacter spp.*	*3*	1	0	0	2
H1a	*Klebsiella pneumoniae*	*3*	2	0	0	3
H1b	*Acinetobacter spp.*	*3*	3	0	0	1
H1b	*Shigella / E.coli*	*3*	3	0	0	3
H2	*Aeromonas spp.*	*2*	2	0	0	2
H2	*Citrobacter amalonaticus*	*1*	1	0	0	1
H2	*Enterobacter spp.*	*1*	1	0	0	1
H2	*Klebsiella pneumoniae*	*3*	2	0	0	3
H2	*Pseudomonas spp.*	*2*	2	0	0	2
H2	*Shigella/E.coli*	*3*	3	0	0	3
	Percentage positive (%)	100.0%	83.3%	0.0%	0.0%	95.5%

Table S6: Identification of amikacin resistance isolates and PCR detection of corresponding ARGs (i.e., *aac(6')-Ib*) and *int1* genes

Location	**Identification of bacterial isolates (n=23)**	**Amikacin resistance**	***aac(6')-Ib***	***int1***
H1a	*Aeromonas spp.*	*1*	1	1
H1a	*Enterobacter / Pantoea*	*3*	3	3
H1a	*Klebsiella spp.*	*2*	2	2
H1b	*Acinetobacter spp.*	*2*	2	1
H1b	*Shigella / E.coli*	*3*	3	2
H2	*Aeromonas spp.*	*3*	3	3
H2	*Elizabethkingia anophelis*	*1*	1	0
H2	*Enterobacter hormaechei*	*1*	0	1
H2	*Klebsiella pneumoniae*	*5*	5	5
H2	*Proteus mirabilis*	*1*	1	1
	Percentage positive (%)	100.0%	95.5%	86.4%

Table S7: Identification of ciprofloxacin resistance isolates and PCR detection of corresponding ARGs (i.e., *qnrA*, *qnrB*) and *int1* genes

Location	**Identification of bacterial isolates (n=24)**	**Ciprofloxacin resistance**	***qnrA***	***qnrB***	***int1***
H1a	*Aeromonas hydrophila*	*3*	0	0	3
H1a	*Klebsiella pneumoniae*	*3*	0	0	3
H1b	*Citrobacter freundii*	*1*	1	1	1
H1b	*Shigella / E.coli*	*5*	0	1	3
H2	*Aeromonas hydrophila*	*2*	0	1	2
H2	*Citrobacter freundii*	*1*	0	1	1
H2	*Comamonas testosteroni*	*1*	0	0	1
H2	*Enterobacter spp.*	*3*	1	0	2
H2	*Klebsiella pneumoniae*	*3*	0	0	3
H2	*Pseudomonas spp.*	*1*	0	0	1
	Percentage positive (%)	100.0%	8.7%	17.4%	87.0%

References

1. **Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA.** 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology **56:**7.
2. **Muyzer G, de Waal EC, Uitterlinden AG.** 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology **59:**6.
3. **Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, Nriagu J.** 2009. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl Environ Microbiol **75:**5714-5718.
4. **Birkett CI, Ludlam HA, Woodford N, Brown DFJ, Brown NM, Roberts MTM, Milner N, Curran MD.** 2007. Real-time TaqMan PCR for rapid detection and typing of genes encoding CTX-M extended-spectrum -lactamases. Journal of Medical Microbiology **56:**52-55.
5. **Hindiyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, Vax M, Ben David D, Tal I, Rahav G, Shamiss A, Mendelson E, Keller N.** 2008. Rapid Detection of blaKPC Carbapenemase Genes by Real-Time PCR. Journal of Clinical Microbiology **46:**2879-2883.
6. **Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B.** 2006. The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. Antimicrob Agents Chemother **50:**2500-2505.
7. **Lee JC, Oh JY, Cho JW, Park JC, Kim JM, Seo SY, Cho DT.** 2001. The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of Escherichi coli in Korea. Journal of Antimicrobial Chemotherapy **47:**6.
8. **Koike S, Aminov RI, Yannarell AC, Gans HD, Krapac IG, Chee-Sanford JC, Mackie RI.** 2010. Molecular ecology of macrolide-lincosamide-streptogramin B methylases in waste lagoons and subsurface waters associated with swine production. Microbial Ecology **59:**12.
9. **Barraud O, Baclet MC, Denis F, Ploy MC.** 2010. Quantitative multiplex real-time PCR for detecting class 1, 2 and 3 integrons. J Antimicrob Chemother **65:**1642-1645.
10. **Gygax SE, Schuyler JA, Kimmel LE, Trama JP, Mordechai E, Adelson ME.** 2006. Erythromycin and Clindamycin Resistance in Group B Streptococcal Clinical Isolates. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY **50:**1875-1877.
11. **Marti E, Balcazar JL.** 2013. Real-Time PCR assays for quantification of qnr genes in environmental water samples and chicken feces. Appl Environ Microbiol **79:**1743-1745.

12. **Dahmen S, Mansour W, Boujaafar N, Arlet G, Boualle`gue O.** 2010. Distribution of Cotrimoxazole Resistance Genes Associated with Class 1 Integrons in Clinical Isolates of Enterobacteriaceae in a University Hospital in Tunisia. MICROBIAL DRUG RESISTANCE **16:**5.
13. **Dorsch MR.** 2007. Rapid Detection of Bacterial Antibiotic Resistance: Preliminary Evaluation of PCR Assays Targeting Tetracycline Resistance Genes. Human Protection and Performance Division. DSTO Defence Science and Technology Organisation, Australia.
14. **Smith MS, Yang RK, Knapp CW, Niu Y, Peak N, Hanfelt MM, Galland JC, Graham DW.** 2004. Quantification of Tetracycline Resistance Genes in Feedlot Lagoons by Real-Time PCR. Applied and Environmental Microbiology **70:**7372-7377.