

Supplementary Material

Antibiotic-resistant bacteria, antibiotic resistance genes, and antibiotic residues in wastewater from a poultry slaughterhouse after conventional and advanced treatments

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Table S1 Temperature profile for every qPCR run, annealing temperature was the same for every primer used.

Temperature	Time	Reaction step	Cycle
90 °C	10 min	Initial denaturation and enzym-activation	x 1
90 °C	15 sec	Denaturation	x 40
60 °C	1 min	Annealing/Elongation	
60 – 90 °C	5 sec / 0,5 °C	Melt curve	x 1

PCR reagent ratio for each qPCR reaction volume was: 10 μ L 2x Maxima Sybr Green Master Mix, 0.5 μ L forward and 0.5 μ L reverse primer (final concentration was 250 nM each), 8 μ L nuclease free PCR water and 1 μ L template DNA.

Table S2 Full primer sequences of investigated FPB and ARGs with corresponding calibration curve, efficiency and LOD.

target	primer sequence	calibration curve	amplicon	efficiency	R ²	LOD	reference strain	literature
facultative pathogenic bacteria								
enterococci	Fwd: AGAAATCCAAACGAACTT G Rev: CAGTGCTCTACCTCCATCAT T	$F(x) = -3.585x + 35.283$	93 bp	90.1 %	1.000	65	<i>E. faecium</i> DSM20477	(Frahm et al. 2003)
<i>P. aeruginosa</i>	Fwd: AGCGTTCGTCCTGCACAAGT Rev: TCCACCATGCTCAGGGAGAT	$F(x) = -3.282x + 35.276$	81 bp	101.7 %	0.999	3	<i>P. aeruginosa</i> DSM1117	(Clifford et al. 2012)
<i>K. pneumoniae</i>	Fwd: ACGGCCGAATATGACGAATT C Rev: AGAGTGATCTGCTCATGAA	$F(x) = -3.387x + 38.844$	68 bp	97.4 %	0.998	18	<i>K. pneumoniae</i> DSM30104	(Clifford et al. 2012)
<i>A. baumannii</i>	Fwd: GTTGTGGCTTTAGGTTTATTATACG Rev: AAGTTACTCGACGCAATTCG	$F(x) = -3.380x + 35.679$	94 bp	97.6 %	1.000	31	<i>A. baumannii</i> DSM30007	(Clifford et al. 2012)
<i>E. coli</i>	Fwd: GCATCGTGACCACCTTGA Rev: CAGCGTGGTGGCAAAA	$F(x) = -3.361x + 35.797$	59 bp	98.4 %	0.994	4	<i>E. coli</i> DSM1103	(Clifford et al. 2012)
antibiotic resistance genes								
<i>ermB</i>	Fwd: TGAATCGAGACTTGAGTGTGCAA Rev: GGATTCTACAAGCGTACCTT	$F(x) = -3.328x + 35.901$	71 bp	100 %	1.000	16	<i>S. hyointestinalis</i> DSM20770	(Alexander et al. 2015)
<i>tetM</i>	Fwd:GGTTTCTCTTGGATACTTAAATC AATC Rev: CCAACCATAAATCCTTGTTTCRC	$F(x) = -3.424x + 38.747$	88 bp	95.9 %	0.998	4	<i>E. coli</i> DH5 α	(Peak et al. 2007)
<i>bla</i> _{TEM}	Fwd: TTCCTGTTTTTGCTCACCCAG Rev: CTCAAGGATCTTACCGCTGTTG	$F(x) = -3.303x + 38.559$	112 bp	100.8 %	0.999	80	<i>E. coli</i> pNORM	(Rocha et al. 2018)

<i>sul1</i>	Fwd: CGCACCGGAAACATCGCTGCAC Rev: TGAAGTCCGCCGCAAGGCTCG	$F(x) = -3.387x + 39.802$	161 bp	97.6 %	0.999	80	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>bla_{CMY-2}</i>	Fwd: CGTTAATCGCACCATCACC Rev: CGTCTTACTAACCGATCCTA GC	$F(x) = -3.591x + 34.026$	172 bp	89.9 %	0.998	71	<i>K. pneumoniae</i> NRZ-01013	(Kurpiel and Hanson 2011)
<i>vanA</i>	Fwd: TCTGCAATAGAGATAGCCG C Rev: GGAGTAGCTATCCCAGCAT T	$F(x) = -3.541x + 33.078$	376 bp	91.6 %	1.000	43	<i>E. faecium</i> B7641	(Klein et al. 1998)
<i>mcr-1</i>	Fwd: GGGCCTGCGTATTTTAAGC G Rev: CATAGGCATTGCTGTGCGT C	$F(x) = -3.386x + 35.349$	183 bp	97.4 %	0.999	8	<i>E. coli</i> NRZ-14408	(Hembach et al. 2017)
<i>bla_{NDM-1}</i>	Fwd: TTGGCCTTGCTGTCCTTG Rev: ACACCAGTGACAATATCACCG	$F(x) = -3.293x + 35.877$	82 bp	101.2 %	0.999	66	<i>K. pneumoniae</i> ATCC BAA-2146	(Monteiro et al. 2012)
<i>bla_{CTX-M15}</i>	Fwd: CGCTTTGCGATGTGCAG Rev: ACCGGATATCGTTGGT	$F(x) = -3.504x + 34.255$	551 bp	92.9 %	1.000	93	<i>E. coli</i> pNORM	(Paterson et al. 2003, Rocha et al. 2018)
<i>bla_{CTX-M-32}</i>	Fwd: CGTCACGCTGTTGTTAGGA A Rev: CGCTCATCAGCACGATAAA G	$F(x) = -3.517x + 37.800$	155 bp	92.5 %	1.000	235	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>bla_{OXA-48}</i>	Fwd: TGTTTTTGGTGGCATCGAT Rev: GTAAMRATGCTTGGTTCCG	$F(x) = -3.540x + 36.913$	177 bp	91.6 %	0.998	92	<i>K. pneumoniae</i> TGH Isolate 2	(Monteiro et al. 2012)

Table S3 List of all investigated antibiotics and their metabolites (limit of quantification, ng/L)

β-Lactams				
Penicillins				
Amoxicillin (50)	Ampicillin (200)	Penicillin G (50)	Cloxacillin (20)	Dicloxacillin (20)
Flucloxacillin (20)	Methicillin (10)	Mezlocillin (20)	Nafcillin (20)	Oxacillin (10)
Penicilin V (20)	Piperacillin (100)			
Carbapenems				
Meropenem (200)				
Cephalosporines				
Cefaclor (50)	Cefotaxime (50)	Ceftazidime (100)		
Macrolides and lincosamides				
Azithromycin (50)	Clarithromycin (50)	Clindamycin (20)	Erythromycin (20)	Anhydroerythromycin (20)
Roxithromycin (50)	Spiramycin (100)	Tylosin (50)		
Tetracyclines				
Chlortetracycline (200)	Doxycycline (200)	Oxytetracycline (200)	Tetracycline (200)	
Fluoroquinolones				
Ciprofloxacin (200)	Enrofloxacin (200)	Moxifloxacin (200)	Ofloxacin (200)	
Sulfonamides				
Sulfachlorpyridazine (50)	Sulfadiazine (100)	Sulfadimethoxine (50)	Sulfadimidine (20)	Sulfadoxine (50)
Sulfaethoxypyridazine (50)	Sulfamerazine (50)	Sulfamethoxazole (20)	N4-Acetylsulfamethoxazole (100)	
Sulfamethoxy-pyridazine (10)	Sulfathiazole (100)	Trimethoprim (20)		
Others				
Linezolid (100)	Vancomycin (100)	Metronidazole (100)		