water

Prevalence of Antibiotic Resistance Genes in Pharmaceutical Wastewaters

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Supplementary

Table S1: Description of sites and sources of metagenomic DNA of Pharmaceutical wastewater

Environmental Samples	Sites	Source Description	No of composite samples taken
PFI	Pharmaceutical Production Plant	IFD & Holding tanks	8
PFII	Pharmaceutical Production Plant	IFD & Holding tanks	8
PFIII	Pharmaceutical Production Plant	IFD & Holding tanks	8
PFIV	Pharmaceutical Production Plant	Public drainage outlets	8

PFI-IV – Pharmaceutical Facilities I to IV; IFD – Internal Facility Drainages. The untreated wastewater samples were taken from different points within the production facilities.

Table S2: Primers and conditions used to amplify tetracycline resistance genes by the PCR techniques

Target Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)	Reference
tet(A)	tetA-F	GCTACATCCTGCTTGCCTTC	210	55	Ng, L. K. *et al*., 2001
	tetA-R	CATAGATCGCCGTGAAGAGG			
tet(B)	tetB-F	TACGTGAATTTATTGCTTCGG	206	55	Ng, L. K. *et al*., 2001
	tetB-R	ATACAGCATCCAAAGCGCAC			
tet(C)	tetC-F	CTTGAGAGCCTTCAACCCAG	418	55	Ng, L. K. *et al*., 2001
	tetC-R	ATGGTCGTCATCTACCTGCC			
tet(D)	tetD-F	GGAATATCTCCCGGAAGCGG	187	68	Aminov *et al*., 2002
	tetD-R	GGAATATCTCCCGGAAGCGG			
tet(E)	tetE-F	AAACCACATCCTCCATACGC	278	55	Ng, L. K. *et al*., 2001
	tetE-R	AAATAGGCCACAACCGTCAG			
tet(G)	tetG-F	GCTCGGTGGTATCTCTGCTC	468	60	Ng, L. K. *et al*., 2001
	tetG-R	AGCAACAGAATCGGGAACAC			
tet(J)	tetJ-F	CGAAAACAGACTCGCCAATC	184	61	Aminov *et al*., 2002
	tetJ-R	TCCATAATGAGGTGGGGC			
tet(Y)	tetY-F	ATTTGTACCGGCAGAGCAAAC	181	68	Aminov *et al*., 2002
	tetY-R	GGCGCTGCCGCCATTATGC			
tet(Z)	tetZ-F	CCTTCTCGACCAGGTCGG	204	61	Aminov *et al*., 2002
	tetZ-R	ACCCACAGCGTGTCCGTC			
tet(M)	tetM-F	ACAGAAAGCTTATTATATAAC	171	55	Aminov *et al*., 2001
	tetM-R	TGGCGTGTCTATGATGTTCAC			
tet(O)	tetO-F	AACTTAGGCATTCTGGCTCAC	515	55	Ng, L. K. *et al*., 2001

	tetO-R	TCCCACTGTTCCATATCGTCA			
tet(Q)	tetQ-F	AGAATCTGCTGTTTGCCAGTG	169	63	Aminov *et al.,* 2001
	tetQ-R	CGGAGTGTCAATGATATTGCA			
tet(T)	tetT-F	AAGGTTTATTATATAAAAGTG	169	46	Aminov *et al*., 2001
	tetT-R	AGGTGTATCTATGATATTTAC			
tet(W)	tetW-F	GAGAGCCTGCTATATGCCAGC	168	64	Aminov *et al*., 2001
	tetW-R	GGGCGTATCCACAATGTTAAC			
tet(X)	tetX-F	CAATAATTGGTGGTGGACCC	468	55	Ng, L. K. *et al*., 2001
	tetX-R	TTCTTACCTTGGACATCCCG			
tet(BP)	tetBP-F	AAAACTTATTATATTATAGTG	169	46	Aminov *et al*., 2001
	tetBP-R	TGGAGTATCAATAATATTCAC			

Table S3: Primers and conditions used to amplify aminoglycosides resistance genes by PCR techniques

Target Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)	Reference
aac(3)-IV	*aac*(3)-IV-F	TGCTGGTCCACAGCTCCTTC	653	56	Heuer *et al*., 2002
	aac(3)-IV-R	CGGATGCAGGAAGATCAA			
aac(6')-Ib(*aac*A4)	*aac*(6')-Ib(*aac*A4)-F	TGACCTTGCGATGCTCTATG	509	-	Heuer *et al*., 2002
	aac(6')-Ib(*aac*A4)-R	TTAGGCATCACTGCGTGTTC			
aac(3)-I	*aac*(3)-I-F	ACCTACTCCCAACATCAGCC	169	60	Heuer *et al*., 2002
	aac(3)-I-R	ATATAGATCTCACTACGCGC			
aac(3)-II	*aac*(3)-II-F	ACTGTGATGGGATACGCGTC	237	60	Heuer *et al*., 2002
	aac(3)-II-R	CTCCGTCAGCGTTTCAGCTA			
aac(3)-III	*aac*(3)-III-F	GAAGTACGCAGAAGAGA	491	58	Heuer *et al*., 2002
	aac(3)-III-R	ACATGGCAAGCTCTAGGA			
*aph*A1**(***aph*(3')-Ia**)**	*aph*A1**(***aph*(3')-Ia**)-**F	ATGGGCTCGCGATAATGTC	600	58	Vakulenko and Mobashery, 2003
	*aph*A1**(***aph*(3')-Ia**)-**R	CTCACCGAGGCAGTTCCAT			
aph(3'')-I (*str*A)	*aph*(3'')-I (*str*A)-F	CCTGGTGATAACGGCAATTC	546	55	Vakulenko and Mobashery, 2003
	aph(3'')-I (*str*A)-R	CCAATCGCAGATAGAAGGC			
aph(6)-Id (*str*B)	*aph*(6)-Id (*str*B)-F	ATCGTCAAGGGATTGAAACC	509	56	Vakulenko and Mobashery, 2003
	aph(6)-Id (*str*B)-R	GGATCGTAGAACATATTGGC			
*aad*A(ANT(3'')-Ia)	*aad*A(ANT(3'')-Ia)-F	GTGGATGGCGGCCTGAAGCC	529	68	Vakulenko and Mobashery,

					2003
	*aad*A(ANT(3'')-Ia)-R	AATGCCCAGTCGGCAGCG			
ant(6)-I(*aad*E)	*ant*(6)-I(*aad*E)-F	ACTGGCTTAATCAATTTGGG	577	58	Vakulenko and Mobashery, 2003
	ant(6)-I(*aad*E)-R	GCCTTTCCGCCACCTCACCG			
*aad*B(*ant*(2'')-Ia)	*aad*B(*ant*(2'')-Ia)-F	ATGGACACAACGCAGGTCAC	534	59	Vakulenko and Mobashery, 2003
	*aad*B(*ant*(2'')-Ia)-R	TTAGGCCGCATATCGCGACC			

Table S4: Primers and conditions used to amplify β-Lactam resistance genes by PCR techniques

Target Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)	Reference
bla-TEM	*bla*-TEM-F	ATAAAATTCTTGAAGACGAAA	1076	50	Mabilat and Goussard, 1993
	bla-TEM-R	GACAGTTACCAATGCTTAATCA			
bla-NDM-1	*bla*-NDM-1-F	GTAAAACGACGGCCAG	1705	55	Walsh *et al*., 2011
	bla-NDM-1-R	CAGGAAACAGCTATGAC			
bla-OXA	*bla*-OXA-F	GTCTTTCGAGTACGGCATTA	720	55	Vahaboglu *et al*., 1998
	bla-OXA-R	ATTTTCTTAGCGGCAACTTAC			
bla-IMP	*bla*-IMP-F	CTACCGCAGCAGAGTCTTTG	587	50	Senda *et al*., 1996
	bla-IMP-R	AACCAGTTTTGCCTTACCAT			
bla-CTX	*bla*-CTX-F	TTTGCGATGTGCAGTACCAGTAA	544	55	Edelstein *et al*., 2003
	bla-CTX-R	CGATATCGTTGGTGGTGCCATA			

Table S5: Primers and conditions used to amplify sulphonamide and chloramphenicol resistance genes by PCR technique

Target Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)	Reference
*cat*A1	*cat*A1-F	CGCCTGATGAATGCTCATCCG	750	60	National Food Institute Denmark
	*cat*A1-R	CCTGCCACTCATCGCAGTAC			
*cml*A	*cml*A-F	TACTCGGATCCATGCTGGCC	578	50	National Food Institute Denmark
	*cml*A-R	TCCTCGAAGAGCGCCATTGG			
*sul*1	*sul*1-F	ATCGCAATAGTTGGCGAAGT	798	55	National Food Institute, Denmark
	*sul*1-R	GCAAGGCGGAAACCCGCGCC			
*sul*2	*sul*2-F	GCGCTCAAGGCAGATGGCATT	284	70	Aarestrup *et al*., 2003
	*sul*2-R	GCGTTTGATACCGGCACCCGT			

Table S6: Primers and conditions used to amplify some genetic elements by PCR technique

Target Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)	Reference
*intI*1	*intI*1-F	CCTCCCGCACGATGATC	280	55	Goldstein *et al*., 2001
	*intI*1-R	TCCACGCATCGTCAGGC			
*intI*2	*intI*2-F	TTATTGCTGGGATTAGGC	233	50	Goldstein *et al*., 2001
	*intI*2-R	ACGGCTACCCTCTGTTATC			
*Tn*916/1545	*Tn*916/1545-F	CTCTATCCTACAGCGACAGC	949	55	Roberts *et al*., 2001
	*Tn*916/1545-R	ATATACGAGTTTGTGCTTGT			

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