

Incidence of antimicrobial-resistance genes and integrons in antibiotic resistance bacteria isolated from eels and aquaculture ponds

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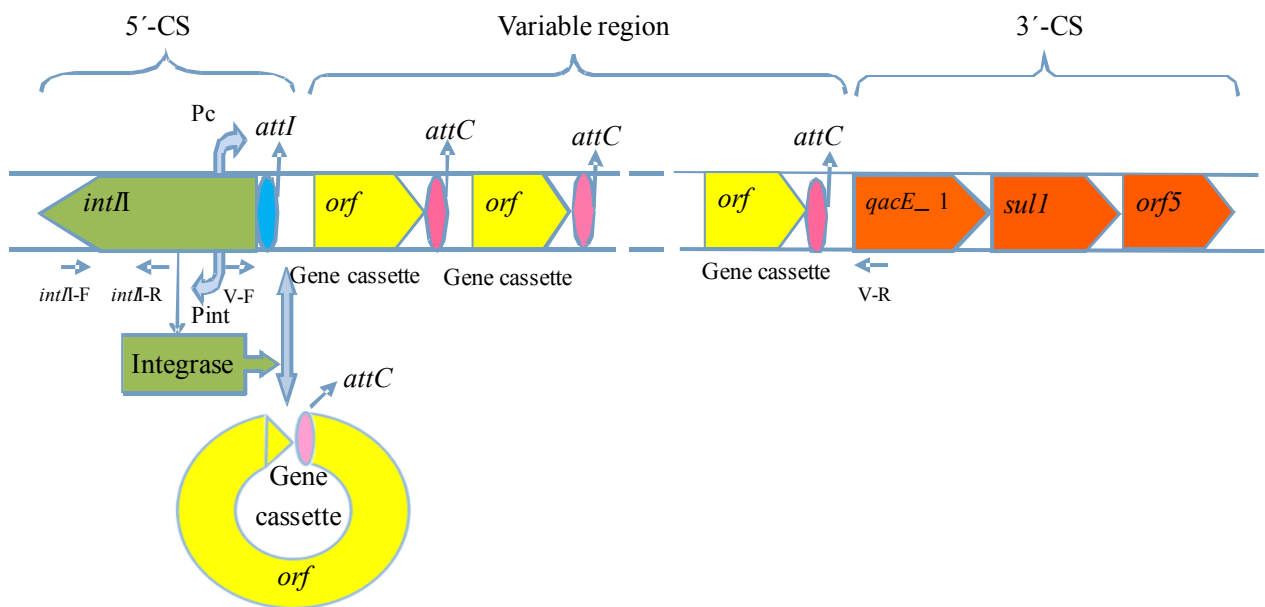


Fig. S1 Typical structure of class I integrons. The 5'-CS contains *intI*, *attI* and *Pc*. The variable region may or may not contain one or more gene cassettes. The 3'-CS consists of *qacEΔ1*, *sulI* and *orf5*. The gene cassette usually consists of a *orf* and an *attC*.

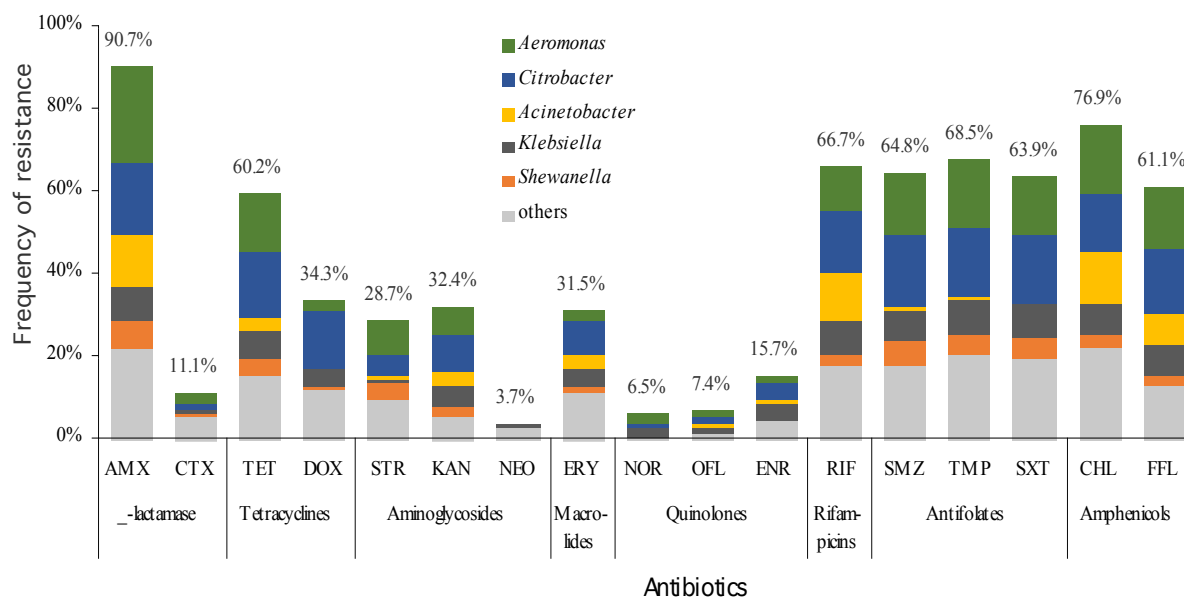


Fig. S2 Frequency of resistance to 17 antimicrobials among the antibiotic resistance bacteria strains isolated from eels and farming water. Amoxicillin: AMX; Cefotaxime: CTX; Tetracycline: TET; Doxycycline: DOX; Streptomycin: STR; Kanamycin: KAN; Neomycin: NEO; Erythromycin: ERY; Norfloxacin: NOR; Ofloxacin: OFL; Enrofloxacin: ENR; Rifampicin: RIF; Sulfamethoxazole: SMZ; Trimethoprim: TMP; Sulfamethoxazole/Trimethoprim: SXT; Chloramphenicol: CHL; Florfenicol: FFL.

Table S1 Primers used for the detection of antimicrobial-resistance genes.

Target gene	Primer sequence forward (5'-3')	Primer sequence reverse (5'-3')	Size (bp)	Annealing temp (°C)	Reference	
β-lactams	<i>bla_{TEM}</i>	GAGTATTCAACATTTTCGT	ACCAATGCTTAATCAGTGA	857	56	(Maynard et al. 2004)
	<i>bla_{SHV}</i>	TCGCCTGTGTATTATCTCCC	CGCAGATAAAATCACCACAATG	768	58	(Maynard et al. 2004)
	<i>bla_{OXA}</i>	GCAGCGCCAGTGCATCAAC	CCGCATCAAATGCCATAAGTG	198	58	(Maynard et al. 2004)
	<i>MOX</i>	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	520	58	(Perez-Perez& Hanson 2002)
	<i>DHA</i>	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	405	59	(Perez-Perez& Hanson 2002)
	<i>EBC</i>	TCGGTAAAGCCGATGTTGCGG	CTTCCACTGCGGCTGCCAGTT	302	62	(Perez-Perez& Hanson 2002)
Aminoglycosides	<i>strA-B</i>	TATCTGCGATTGGACCCCTCTG	CATTGCTCATCATTTGATCGGCT	538	55	(Sunde & Norström 2006)
	<i>aac(3)-I</i>	ACCTACTCCCAACATCAGCC	ATATAGATCTCACTACGCGC	369	58	(Ramos & Velilla 2012)
	<i>aac(3)-IV</i>	CTTCAGGATGGCAAGTTGGT	TCATCTCGTTCTCCGCTCAT	286	58	(Cong 2011)
	<i>aadA</i>	TGATTTGCTGGTTACGGTGAC	CGCTATGTTCTCTTGTCTTTG	284	58	(Cong 2011)
	<i>aphA1</i>	ATGGGCTCGCGATAATGTC	CTCACCGAGCAGTTCCAT	600	58	(Maynard et al. 2004)
Tetracyclines	<i>tetA</i>	GCTACATCCTGCTTGCCTTC	CATAGATCGCCGTGAAGAGG	210	55	(Guo et al. 2013)
	<i>tetB</i>	TTGGTTAGGGCAAGTTTTG	GTAATGGGCCAATAACACCG	659	55	(Guo et al. 2013)
	<i>tetC</i>	CTTGAGAGCCTTCAACCCAG	ATGGTCGTCATCTACCTGCC	418	55	(Schwaiger et al. 2009)
	<i>tetD</i>	AAACCATTACGGCATTCTGC	GACCGGATACACCATCCATC	787	56	(Sandalli et al. 2010)
	<i>tetE</i>	ATGAACCGCACTGTGATGATG	ACCGACCATTACGCCATCC	744	58	(Jun et al. 2010)
Quinolones	<i>qnrA</i>	TCAGCAAGAGGATTTCTCA	GGCAGCACTATGACTCCCA	516	54	(Robicsek et al. 2006)
	<i>qnrB</i>	TCGGCTGTCAGTTCTATGATCG	TCCATGAGCAACGATGCCT	469	58	(Robicsek et al. 2006)
	<i>qnrS</i>	TGATCTCACCTTACCAGCTTG	GAATCAGTTCTTGCTGCCAGG	566	59	(Kehrenberg et al. 2006)
Antifolates	<i>dfpA1</i>	TTGTGAAACTATCACTAATGGT	CTTGTTAACCCTTTTGCCAGA	480	55	(Hu et al. 2011)
	<i>dfpA5</i>	TCCACACATAACCCTGGTCCG	ATCGTCGATATATGGAGCGTA	300	55	(Hu et al. 2011)
	<i>dfpA7</i>	TTGAAAATWTCATTGATTTCT	TTAGCCTTTTTTCCAAATCTGRATGT	474	55	(Hu et al. 2011)
	<i>dfpA12</i>	ATGAACTCGGAATCAGTACGC	TTAGCCGTTTCGACGCGCAT	498	58	(Hu et al. 2011)
	<i>sull</i>	TTCGGCATTCTGAATCTCAC	ATGATCTAACCCTCGGTCTC	822	55	(Maynard et al. 2004)
Amphenicols	<i>catI</i>	AGTTGCTCAATGTACCTATAAC	TTGTAATTCATTAAGCATTCTGCC	547	58	(Maynard et al. 2004)
	<i>cmlA</i>	CCGCCACGGTGTGTTGTTATC	CACCTTGCCTGCCATCATTAG	698	58	(Keyes et al. 2000)
	<i>floR</i>	TATCTCCCTGTGCTTCCAG	AGAACTCGCCGATCAATG	399	58	(Keyes et al. 2000)
Macrolides	<i>ereA</i>	GCCGGTGCTCATGAACTTGAG	CGACTCTATTTCGATCAGAGGC	419	58	(Cong 2011)
Rifampicins	<i>arr2/3</i>	TTGGATTCCCATCTCTCATG	ACTAGTCTCAATGACGTGTAA	443	55	(Zhu et al. 2012)

Table S2 Primers used for the identification of integrons and the variable region

Target gene	Primer	Sequence (5'-3')	Size (bp)	Reference
<i>intII</i>	<i>intII</i> F	GTTCGGTCAAGTTCTG	890	(Xu et al. 2007)
	<i>intII</i> R	CGTAGAGACGTTCGGAATG		
<i>intIII</i>	<i>intIII</i> F	CAAGCATCTCTAGGCGTA	1056	(Xu et al. 2007)
	<i>intIII</i> R	AGTAGCATCAGTCCATCC		
<i>intIII</i>	<i>intIII</i> F	CATCAAGCTGCTCGATCA	878	(Xu et al. 2007)
	<i>intIII</i> R	ACAACCTCTTGCAACCGTTC		
<i>intII</i> variable region	<i>hep58</i>	TCATGGCTTGTATGACTGT	variable	(Ndi & Barton 2011)
	<i>hep59</i>	GTAGGCTTATTATGCACGC		
<i>intIII</i> variable region	<i>hep74</i>	CGGGATCCCGACGCATGCACGATTTGTA	variable	(Ahmed et al. 2007)
	<i>hep51</i>	GATGCCATCGCAAGTACGAG		
<i>intII</i> 3'-CS	<i>qacEA1/sulIF</i>	TAGCGAGGGCTTTACTAAGC	300	(Maynard et al. 2004)
	<i>qacEA1/sulIR</i>	ATTCAGAATGCCGAACACCG		

Table S3 Phylogenetic affiliation and characterization of class I integrons whose variable regions were amplified and sequenced from eels and farming water in China.

Isolate	Genetic identification	<i>intII</i> cassette array (accession number)	<i>intII</i> 3'-CS
A1	<i>Klebsiella pneumoniae</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR699626)	+
C3	<i>Klebsiella pneumoniae</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR259314)	+
M1	<i>Klebsiella pneumoniae</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR699627)	+
M3	<i>Aeromonas jandaei</i>	<i>dfrA1-orfC</i> (KR270484)	+
M5	<i>Vogesella perlucida</i>	<i>ereA</i> (KM595287)	+
M6	<i>Edwardsiella tarda</i>	<i>dfrA1-orfC</i> (KR259317)	+
T4	<i>Citrobacter freundii</i>	<i>dfrA12-orfF-aadA2</i> (KM595296)	+
S1	<i>Aeromonas veronii</i>	<i>dfrA1-orfC</i> (KR259327)	-
S2	<i>Aeromonas veronii</i>	<i>dfrA1-orfC</i> (KP901094)	-
S3	<i>Escherichia fergusonii</i>	<i>dfrA17-aadA5</i> (KM595301)	+
S4	<i>Vogesella perlucida</i>	<i>aacA4 - ereA - bla_{OXA-21}-dfrA1</i> (KM595304)	-
S7	<i>Aeromonas veronii</i>	<i>catB3-aadA1</i> (KP942842)	+
S9	<i>Aeromonas veronii</i>	<i>catB3-aadA1</i> (KP942844)	+
A11	<i>Aeromonas veronii</i>	<i>dfrB4-catB3-aadA1</i> (KM595285)	+
A15	<i>Acinetobacter nosocomialis</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR080529)	+
A22	<i>Aeromonas veronii</i>	<i>dfrA12-orfF-aadA2</i> (KR259318)	+
C18	<i>Citrobacter werkmanii</i>	<i>dfrA12-orfF-aadA2</i> (KM595286)	-
S12	<i>Citrobacter freundii</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR259319)	+
S16	<i>Zobellella denitrificans</i>	<i>aadA1</i> (KM595302)	+
S18	<i>Aeromonas caviae</i>	<i>dfrB4-catB3-aadA1</i> (KM595303)	+
S20	<i>Citrobacter freundii</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR699628)	+
T12	<i>Citrobacter braakii</i>	<i>aac(6)-IIc - ereA - tnpA - aac3 - arr - ereA</i> (KR259328)	+
T16	<i>Citrobacter werkmanii</i>	<i>dfrA17-aadA5</i> (KP901090)	+
T17	<i>Citrobacter werkmanii</i>	<i>dfrA17-aadA5</i> (KP901091)	+
T18	<i>Citrobacter werkmanii</i>	<i>dfrA17-aadA5</i> (KP901092)	+
T19	<i>Pantoea agglomerans</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR699629)	+
T20	<i>Shewanella seohaensis</i>	<i>dfrA12-orfF-aadA2</i> (KM595297)	+
T22	<i>Pseudomonas mosselii</i>	<i>aadA2</i> (KP942843) and <i>dfrA17-aadA5</i> (KR259320)	+
T23	<i>Citrobacter freundii</i>	<i>dfrA12-orfF-aadA2</i> (KM595298)	+
M13	<i>Plesiomonas shigelloides</i>	<i>arr2-aacA4-dfrA1-orfC</i> (KM595288)	+
M14	<i>Aeromonas caviae</i>	<i>dfrB4-catB3 - bla_{OXA-10} - aadA1</i> (KM595289)	+
M15	<i>Citrobacter freundii</i>	<i>dfrA12-orfF-aadA2</i> (KM595290)	+
M16	<i>Stenotrophomonas maltophilia</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR259315)	+
M17	<i>Staphylococcus xylosus</i>	<i>aac(6)-Ib-cr-arr-3-dfrA27</i> (KR259316)	+
M18	<i>Shewanella seohaensis</i>	<i>dfrA12-orfF-aadA2</i> (KM595291)	+
M21	<i>Shewanella xiamenensis</i>	<i>dfrA12-orfF-aadA2</i> (KM595292)	+
M23	<i>Aeromonas veronii</i>	<i>dfrA12-orfF-aadA2</i> (KM595293)	+
M24	<i>Shewanella xiamenensis</i>	<i>dfrA12-orfF-aadA2</i> (KM595294)	+
M26	<i>Shewanella seohaensis</i>	<i>dfrA12-orfF-aadA2</i> (KM595295)	+
M27	<i>Aeromonas veronii</i>	<i>catB8</i> (KM595299)	+
M28	<i>Aeromonas veronii</i>	<i>catB8</i> (KM595300)	+

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