

**Diversity of antibiotic-resistance genes in Canadian isolates of *Aeromonas salmonicida*
subsp. *salmonicida*: dominance of pSN254b and discovery of pAsa8**

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Running title: *A. salmonicida*: pSN254b dominance and pAsa8 discovery

Supplementary Table S1. Primers used in this study

Primer	Sequence (5'-3')	Reference
PCR positive control (<i>tapA</i> gene on the chromosome)		
<i>tapA</i> F	ACATGAAGAAGCAATCAGGC	1
<i>tapA</i> R	AGAGGTCATGCGTTAGCAG	1
PCR for the detection of pAB5S9		
ORF101-strB_F1 ^a	CTCGTGAATGTCGGTATCCTGTCT	2
ORF101-strB_R1 ^a	CTCGGTCGTGAGAACAATCTGATG	2
parA-resA_F1	CGTCAATGATGATGTGGTCGTACC	2
parA-resA_R1	CTGATCGACCAGGAAGGCTATAGC	2
ORF397-tnp_F1	TGTTAGCACTCGGTTGCCTTCGAT	2
ORF397-tnp_R1	GACATACACACCGTCGAGAAACAG	2
PCR for the detection of pSN254		
tnp-urf2_F1 ^b	CATTCGGTCGACCATACGACGATC	2
tnp-urf2_R1 ^b	CTGCGTGTGCTGCAAGGAAATCC	2
traC_F1	CTACGACAATGGCTTGATCCAAGAC	2
traC_R1	GTACAGAATGGCAGGCAGTTGAGG	2
ORF1417_F1	CGAGACCTTCCCTTTTACTGATGC	2
ORF1417_R1	CAGACAGAGGACGAGTTGTAGAAC	2
PCR for the detection of pAsa4		
DD1-pAsa4-traG-F1	AGGTTGCTCTGGAAAGCCTCTGAT	2
DD2-pAsa4-traG-R1	TGTGGATGCCTGTGCTCTCCATTA	2
PCR for the characterization of pRAS3		
Reg_1_F2	GGATAGTCGATCTGCTGGATACG	2

Reg_1_R2	CTACCCTGTGGAACACCTACATCT	²
Reg_2_F1	CGTTGTGGATGTGCTTCAGCAATC	²
Reg_2_R1	GAGTGGTTGAGCAATACAGGATGC	²
PCR for the characterization of pAsa8		
Geno_pAsa8_F1b	CATGATGGTCACACCTCGATACTC	This study
Geno_pAsa8_R1	GCAATCCACTACTCAGTAGGTGAG	This study
Geno_pAsa8_F2b	GGAATCTGGTGGATGCGCATCTTC	This study
Geno_pAsa8_R2	CGTCTGATCCAAGAACAACCATCC	This study
Geno_pAsa8_F4	GTGATGATGTGAAATCTGCGTGGG	This study
Geno_pAsa8_R4	GCACTATCGCTTGCTTTCACCAGG	This study
Geno_pAsa8_F5	GCTTGCGGTCTACAGTGATATCTC	This study
Geno_pAsa8_R5	CGGTACTCAAATGCAACGCTACTAC	This study

a. The ORF101-strB primers were designed to give different amplicon sizes in pAB5S9 and pAB5S9b.

b. The tnp-urf2 primers were specific to pSN254b.

References

- 1 Ebanks, R. O. *et al.* Expression of and secretion through the *Aeromonas salmonicida* type III secretion system. *Microbiology* **152**, 1275-1286, doi:152/5/1275 [pii]10.1099/mic.0.28485-0 (2006).
- 2 Vincent, A. T. *et al.* Detection of variants of the pRAS3, pAB5S9, and pSN254 plasmids in *Aeromonas salmonicida* subsp. *salmonicida*: multidrug resistance, interspecies exchanges, and plasmid reshaping. *Antimicrob Agents Chemother* **58**, 7367-7374, doi:10.1128/AAC.03730-14 (2014).

Supplementary Table S2. *A. salmonicida* subsp. *salmonicida* isolates used in this study

Isolate	Origin			Date of isolation	Plasmids found by PCR genotyping	Genes found by multiplex PCR	Plasmids ^b					Prophages ^c			<i>AsaGEI</i> ^c	Phenotypic assay ^d		
	Fish	Region ^a	Quebec subregion				pAsa1	pAsa2	pAsa3	pAsa11	pAsa5	1	2	3		A-layer	Lipases	Proteases
A449	Brown trout	France		1975	pAsa4	<i>cat, sul1, tetA(E)</i>	+	+	+	None	+	+	+	None	+	+	-	
01-B522	Brook trout	Quebec (NW)	NW-B	2001	pAsa4-like (<i>traG</i> +)	<i>sul1, tetA(E)</i>	+	+	+	+	+	+	+	1a	+	+	+	
01-B526	Brook trout	Quebec (NW)	NW-C	2001	-	-	+	+	+	+	+	+	+	1a	+	+	+	
01-B516	Brook trout	Quebec (NW)	NW-H	2001	-	-	+	+	+	+	+	+	+	2a	+	+	+	
07-9324	Brook trout	Quebec (NW)	NW-B	2007	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
07-7817	N/A ^e	Quebec (NW)	NW-B	2007	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
07-7346	Atlantic salmon	Quebec (NE)	NE-D	2007	-	-	+	+	+	+	+	+	None	2a	+	+	+	
07-5957	Atlantic salmon	Quebec (NE)	NE-D	2007	-	-	+	+	+	+	+	+	None	2a	+	+	+	
07-7287	Brook trout	Quebec (SW)	SW-C	2007	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
08-2647	Brook trout	Quebec (NW)	NW-B	2008	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
08-2783	Brook trout	Quebec (SW)	SW-C	2008	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
08-4188	Brook trout	Quebec (NW)	NW-B	2008	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	
09-0167	Atlantic salmon	Quebec (NE)	NE-D	2009	-	-	+	+	+	+	+	+	None	2a	+	+	+	
m17524-09	Brook trout	Quebec (NW)	NW-B	7-Sep-09	-	-	+	+	+	+	+	+	+	2a	+	+	+	
m14349-09	Atlantic salmon	Quebec (NW)	NW-B	04-aout-09	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	1a	+	+	+	

m23281-09	Brook trout	Quebec (NW)	NW-G	03-dec-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m23067-09	Brook trout	Quebec (NE)	NE-C	01-dec-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m19438-09	Brook trout	Quebec (NW)	NW-B	13-Oct-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m16583-09	Brook trout	Quebec (NW)	NW-B	2-Sep-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m14231-09	Atlantic salmon	Quebec (NE)	NE-D	13-Jul-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m11743-09	Brook trout	Quebec (SW)	SW-C	2-Jul-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m11431-09	Brook trout	Quebec (SW)	SW-C	30-Jun-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m10419-09	Brook trout	Quebec (SW)	SW-C	17-Jun-09	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m9906-09	Brook trout	Quebec (SW)	SW-C	11-Jun-09	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
m9954-10	Brook trout	Quebec (NW)	NW-B	20-May-10	-	-	+	+	+	+	+	+	+	+	+	1a	+	+	+
m8029-10	Brook trout	Quebec (NW)	NW-B	28-Apr-10	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m11603-10	Brook trout	Quebec (NW)	NW-B	10-Jun-10	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m6363-10	Brook trout	Quebec (NE)	NE-C	7-Apr-10	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
m9221-10	Brook trout	Quebec (NE)	NE-C	11-May-10	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M10935-11	Brook trout	Quebec (NW)	NW-B	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M15448-11	Brook trout	Quebec (NW)	NW-B	2011	pAsa8	<i>floR, sul1, tetG, tetA</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
M16474-11	Brook trout	Quebec (NW)	NW-B	2011	pAsa8	<i>floR, sul1, tetG, tetA</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
M19878-11	Brook trout	Quebec (NW)	NW-B	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M11500-11	Brook trout	Quebec (NW)	NW-H	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+

M16486-11	Brook trout	Quebec (NW)	NW-H	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13460-11	Brook trout	Quebec (SE)	SE-A	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13729-11	Brook trout	Quebec (SE)	SE-A	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M14481-11	Brook trout	Quebec (SE)	SE-B	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M15879-11	Brook trout	Quebec (SE)	SE-B	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M17739-11	Brook trout	Quebec (SE)	SE-B	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M13732-11	Brook trout	Quebec (SW)	SW-C	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M17053-11	Brook trout	Quebec (SW)	SW-C	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M15878-11	Rainbow trout	Quebec (SE)	SE-B	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13182-11	Atlantic salmon	Quebec (NE)	NE-C	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M17735-11	Brook trout	Quebec (NE)	NE-C	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M15576-11	Brown trout	Quebec (NW)	NW-B	2011	-	-	+	+	+	+	+	+	+	None	2a	+	+	+	
M15469-11	Brook trout	Quebec (NW)	NW-B	2011	pSN254b	<i>floR, sul1, sul2, tetA</i>	N/A	N/A	N/A	N/A	N/A	+	+	+	+	1a	+	+	+
M22710-11	Brook trout	Quebec (SW)	SW-C	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13764-11	Brook trout	Quebec (NW)	NW-G	2011	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M18076-11	Lumpfish	Quebec (SE)	SE-A	2011	-	-	+	+	+	+	+	+	+	+	+	None	+	+	+
M23911-11	Brook trout	Quebec (NW)	NW-B	2011	-	-	+	+	+	+	+	+	+	+	+	1a	+	+	+
M12357-12	Brook trout	Quebec (NW)	NW-B	12-Jun-12	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M21375-12	Brook trout	Quebec (NE)	NE-C	4-Oct-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+

M16237-12	Brook trout	Quebec (NE)	NE-C	1-Aug-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M12976-12	Brook trout	Quebec (NW)	NW-H	19-Jun-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M22895-12	Brook trout	Quebec (NW)	NW-G	25-Oct-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M10745-12	Brown trout	Quebec (NW)	NW-B	4-May-12	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M9754-12	Brook trout	Quebec (NW)	NW-G	11-May-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M17930-12	Brook trout	Quebec (SW)	SW-C	23-Aug-12	-	-	+	+	+	+	+	+	+	+	+	1a	+	+	+
M12418-12	Brook trout	Quebec (NW)	NW-B	13-Jun-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M21368-12	Brook trout	Quebec (NW)	NW-C	4-Oct-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13050-12	Brook trout	Quebec (NW)	NW-H	27-Jun-12	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
M13566-12	Brook trout	Quebec (SE)	SE-B	27-Jun-12	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
M14404-12	Atlantic salmon	Quebec (NE)	NE-C	10-Jul-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M16671-12	Brook trout	Quebec (NW)	NW-H	7-Aug-12	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
M16042-12	Brook trout	Quebec (SE)	SE-B	30-Jul-12	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
SHY13-162	Brook trout	Quebec (NE)	NE-C	25-Jan-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-574	Brook trout	Quebec (NE)	NE-C	25-Feb-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	(+)	+
SHY13-1470	Brook trout	Quebec (NW)	NW-H	9-May-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-2188	Brook trout	Quebec (NW)	NW-B	28-Jun-13	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
SHY13-2222	Brook trout	Quebec (NE)	NE-C	3-Jul-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	-	+
SHY13-2257	Brook trout	Quebec (NW)	NW-G	8-Jul-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+

SHY13-2263	Brook trout	Quebec (NW)	NW-H	8-Jul-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-2317	Brook trout	Quebec (SE)	SE-B	12-Jul-13	-	-	+	+	-	+	+	+	+	+	+	None	+	+	+
SHY13-2425	Brook trout	Quebec (SW)	SW-C	22-Jul-13	-	-	+	+	-	+	+	+	+	+	+	None	+	+	+
SHY13-2458	Brook trout	Quebec (NW)	NW-B	22-Jul-13	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
SHY13-2534	Atlantic salmon	Quebec (NE)	NE-D	31-Jul-13	-	-	+	+	+	+	+	+	+	+	None	2a	+	+	+
SHY13-2627	Brook trout	Quebec (NW)	NW-H	9-Aug-13	pAsa4-like (<i>traG</i> +))	<i>sul1, tetA(E)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-2630	Brook trout	Quebec (NW)	NW-B	9-Aug-13	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	1a	+	+	+
SHY13-2825	Brook trout	Quebec (SE)	SE-B	20-Aug-13	-	-	+	+	+	+	+	+	+	+	+	None	+	-	+
SHY13-2873	Brook trout	Quebec (SW)	SW-C	22-Aug-13	-	-	+	+	+	+	+	+	+	+	+	None	+	+	+
SHY13-2909	Brook trout	Quebec (NW)	NW-H	27-Aug-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-3101	Brook trout	Quebec (SW)	SW-C	12-Sep-13	-	-	+	+	+	+	+	+	+	+	+	1a	+	+	+
SHY13-3127	Brook trout	Quebec (NW)	NW-B	12-Sep-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-3795	Brook trout	Quebec (SW)	SW-C	23-Oct-13	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	-	+	+	+	+	+	1a	+	+	+
SHY13-3798	Brook trout	Quebec (NW)	NW-H	23-Oct-13	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
SHY13-3799	Brook trout	Quebec (NW)	NW-H	23-Oct-13	pAsa4-like (<i>traG</i> +))	<i>sul1, tetA(E)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2004-05 MF26	N/A	New Brunswick		2004	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2005-175 K2	Brook trout	New Brunswick		2005	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
2009-178 K9	Atlantic salmon	New Brunswick		2009	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+

2009-157 K5	Brook trout	New Brunswick		2009	pRAS3	<i>tetA(C)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2009-195 K29	Brook trout	New Brunswick		2009	pRAS3	<i>tetA(C)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2009-144 K3	Brook trout	New Brunswick		2009	pAB5S9b, pRAS3	<i>floR, sul2, tetH,</i> <i>tetA(C)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2010-47 K18	Brook trout	New Brunswick		2010	pAB5S9b, pRAS3	<i>floR, sul2, tetH,</i> <i>tetA(C)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
2004-68 K52	Atlantic salmon	Nova Scotia		2004	pSN254b	<i>floR, sul1, sul2, tetA</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
RS 1458	Rainbow trout	Ontario		1993	pAsa4-like (<i>traG+</i>)	<i>tetA(E)</i>	+	+	+	+	+	+	+	+	+	2a	+	+	+
RS 1706	Chinook salmon	Ontario		2000	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
RS 1705	Brook trout	Ontario		2000	-	-	+	+	+	+	+	+	+	+	+	1a	+	+	+
RS 1744	Coho salmon	Ontario		2000	-	-	+	+	+	+	+	+	+	+	+	2a	+	+	+
RS 1752	Pumpkinseed	Ontario		2001	-	-	+	+	+	+	+	+	+	+	+	1b	+	+	+
RS 1835	Coho salmon	Ontario		2011	-	-	+	+	+	-	+	+	+	+	+	2a	+	+	+

- a. NW = northwest, SW = southwest, NE = northeast, SE = southeast (see Figure 4).
- b. See : Attère SA, et al. Diversity and Homogeneity among Small Plasmids of *Aeromonas salmonicida* subsp. *salmonicida* Linked with Geographical Origin. *Front Microbiol* 6: 1274. (2015)
- c. See: Emond-Rheault JG, et al. Variants of a genomic island in *Aeromonas salmonicida* subsp. *salmonicida* link isolates with their geographical origins. *Vet Microbiol* 175: 68-76. (2015)
- d. See: Daher, R. K. et al. Alteration of virulence factors and rearrangement of pAsa5 plasmid caused by the growth of *Aeromonas salmonicida* in stressful conditions. *Vet Microbiol* 152, 353-360 (2011).
- e. N/A: Not available