

Supplementary Information

Table S1 Determination of LD₅₀ of LM EGD-e, LM- $\Delta rli43$ and LM- $\Delta rli43-rli43$ in mice

Group	LM EGD-e			LM- $\Delta rli43$			LM- $\Delta rli43-rli43$		
	Dose/cfu	Mortality	LD ₅₀	Dose/cfu	Mortality	LD ₅₀	Dose/cfu	Mortality	LD ₅₀
1	1.68×10 ⁶	(10/10)		2.1×10 ⁹	100 (10/10)		2.68×10 ⁶	100 (10/10)	
2	8.40×10 ⁵	(9/10)	10 ^{5.56}	2.1×10 ⁸	80 (8/10)	10 ^{7.32}	1.34×10 ⁶	80 (8/10)	10 ^{5.76}
3	4.20×10 ⁵	(6/10)		2.1×10 ⁷	50 (5/10)		6.20×10 ⁵	70 (7/10)	
4	2.10×10 ⁵	(2/10)		2.1×10 ⁶	20 (2/10)		3.10×10 ⁵	20 (2/10)	
5	1.05×10 ⁵	(0/10)		2.1×10 ⁵	0 (0/10)		1.52×10 ⁵	0 (0/10)	

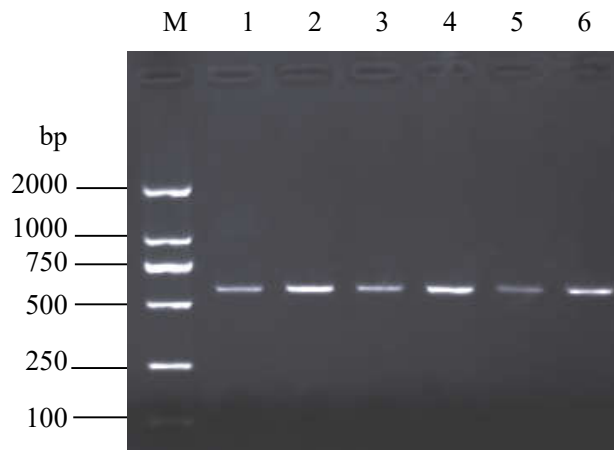


Figure S1 Amplification of LM *rli43* gene by RT-PCR. M: DNA marker DL-2000 (2000, 1000, 750, 500, 250, 100 bp); 1-6: Amplified products of *rli43* gene by RT-PCR.

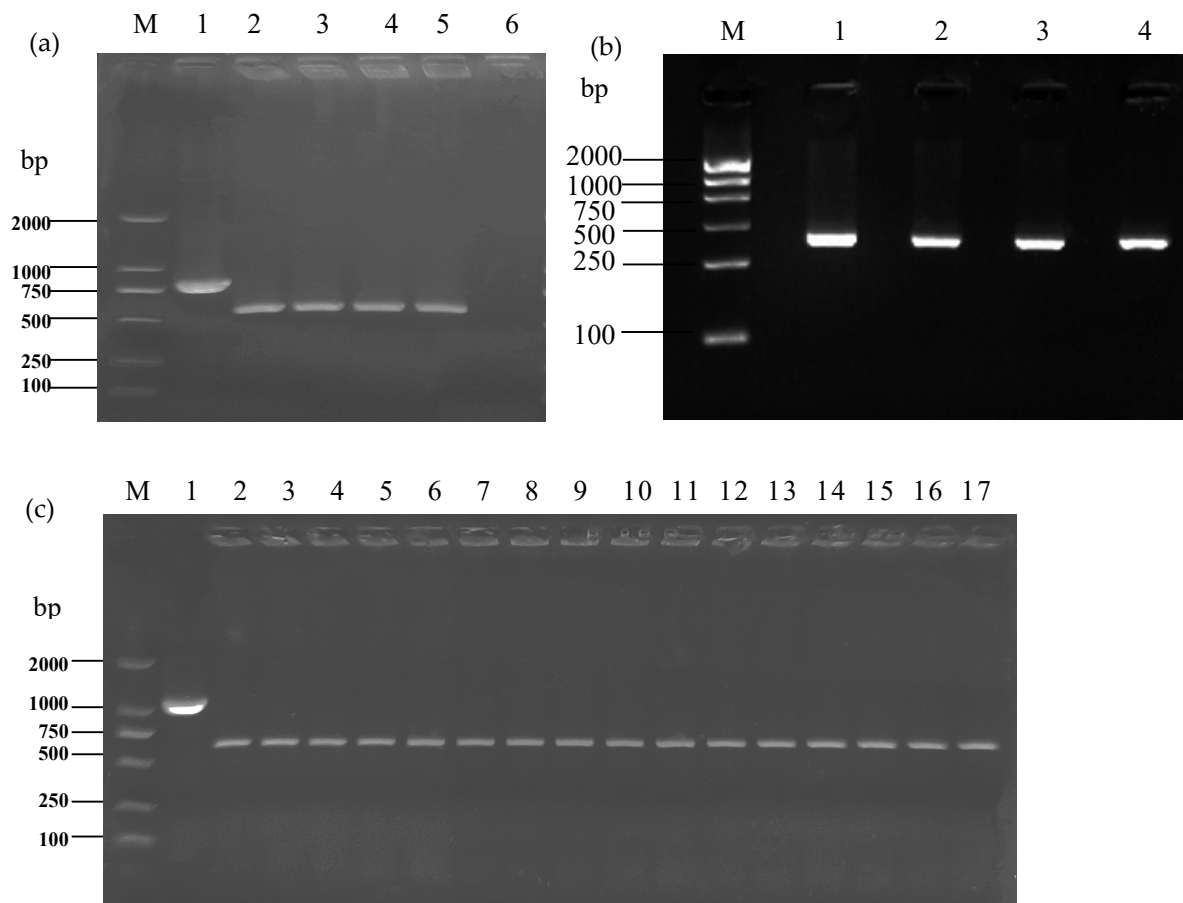


Figure S2 Construction of LM- $\Delta rli43$ deletion and complementary strains. (a) Screening and identification of recombinant LM- $\Delta rli43$ by PCR. M: DNA marker DL-2000 (2000, 1000, 750, 500, 250, 100 bp); 1: LM EGD-e strain (positive control); 2-5: Amplified products of recombinant LM- $\Delta rli43$ by PCR, 6: Negative control. (b) Screening and verification of complement strain LM- $\Delta rli43-rli43$ by PCR. M: DNA marker DL-2000 (2000, 1000, 750, 500, 250, 100 bp); 1-4: Amplified products of complement strain LM- $\Delta rli43-rli43$ by PCR. (c) Analysis of genetic stability of LM- $\Delta rli43$ by PCR. M: DNA marker DL-2000 (2000, 1000, 750, 500, 250, 100 bp); 1: LM EGD-e strain (positive control); 2-17: PCR Identification of LM- $\Delta rli43$ of 1, 2, 4, 5, 7, 10, 12, 13, 16, 19, 20, 22, 25, 26, 27, 30 generation.

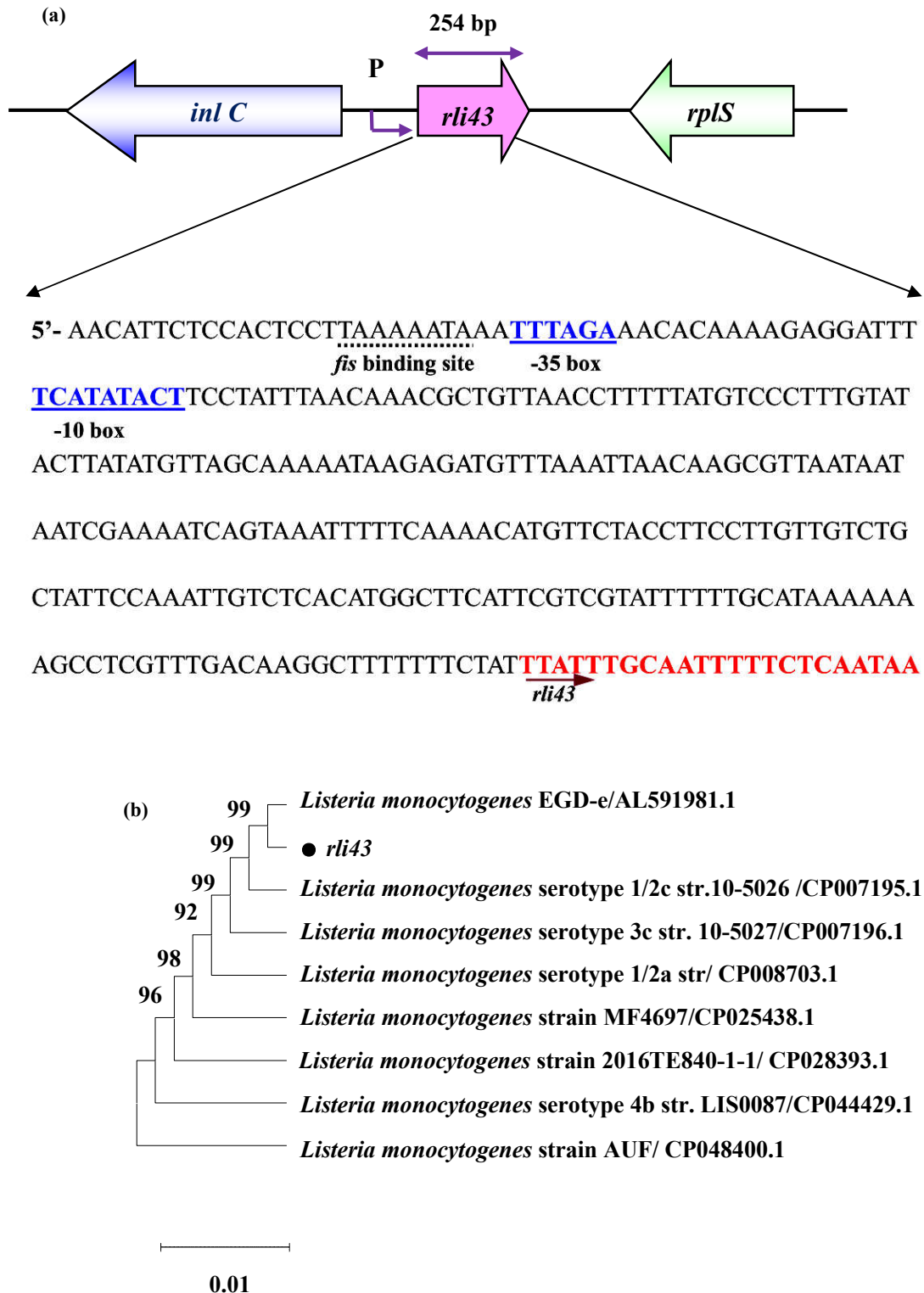


Figure S3 Molecular characterization of *rli43* gene and its phylogenetic analysis. (a) Genetic location of *rli43* gene in the genome of LM EGD-e; (b) Phylogenetic analysis of *rli43* gene of different serotypes of LM.

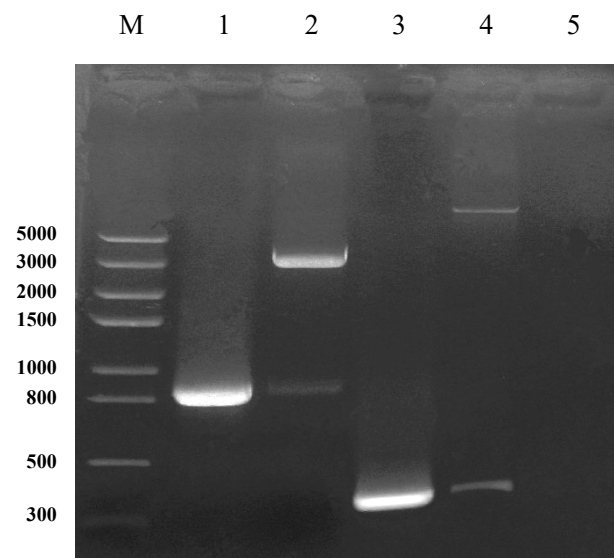


Figure S4 Screening and identification of pMR-LacZ-*htrA* and pUT18C-*rli43* using PCR and restriction enzyme digestion. M: DNA marker DL-5000 (5000, 3000, 2000, 1500, 1000, 800, 500, 300 bp); 1: Identification of pUT18C-*rli43* by PCR; 2: pUT18C-*rli43* digested by restriction enzyme; 3: Verification of pMR-LacZ-*htrA* by PCR; 4: pMR-LacZ-*htrA* digested by restriction enzyme; 5: Negative control.