Host-specific differences in the contribution of an extended spectrum β-lactamase (ESBL) IncI1 plasmid to intestinal colonisation by Escherichia coli O104:H4

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Table S1. Bacterial strains and primers used in this study.

Strain/ plasmid	Genotype/phenotype	Reference
BL211	*E. coli* O104:H4 Δ*stx2AB::*Gent^{R}; Tet^{R}; Ctx^{R}	[5]
BL320	BL211 derivative lacking pESBL	This study
pIMF27	Inc1 curing vector, Kan^{R}	[13]

Primers

Gene	Sequence	Reference
Chromosomal markers		
terD (434 bp)	5'-AGTAAAGCAGCTCCGTCAAT-3' 5'-CCGAACAGCATGGCAGTCT-3'	[3]
rfb$_{O104}$ (351 bp)	5'-TGAACTGATTTTTAGGATGG-3' 5'-AGAACCTCACTCAAATTATG-3'	[3]
fliC$_{H4}$ (201 bp)	5'-GGCGAAACTGACGGCTGCTG-3' 5'-GCACCAACAGTTACCGCCGC-3'	[3]
Plasmid-specific markers		
aggR (254 bp) for pAA	5'-GTATACACAAAAGAAGGAAGC-3' 5'-ACAGAATCGTCAGCATCAGC-3'	[37]
bla$_{CTX-M}$ (593 bp) for pESBL	5'-ATGTGCAGYACCAGTAARGTKATGGC-3' 5'-TGGGTRAARTARGTSACCAGAAYAAGCGG-3'	[38]
alternative bla$_{CTX-M}$ (260 bp)	5'-GCGTGATACCACTTCACCTC-3' 5'-TGAAGTAAGTGACCAGAATC-3'	[39]

PCR reactions were performed using the QIAGEN Multiplex PCR reagents and the following conditions: 95˚C for 15 min, followed by 25 cycles consisting of 95 ˚C for 30s, 54 ˚C for 30s and 72 ˚C for 1 min, with a final extension step of 72 ˚C for 5 min. PCR products were analysed by electrophoresis on 2% agarose gels and visualised using ethidium bromide (5 µg/mL) or Redsafe^{TM}.

Table S2. Diarrhoeal status of infant rabbits infected with the different strains of bacteria.

Strain	BL211	BL320
Diarrhoea (%)	18	11
Disease category		
Diarrhoea	0	0
Intestinal disease	3	2
None	14	17
Total number of animals	17	19
P-value* versus BL211	-	NS

*Fisher's exact test

Table S3. Recovery of *E. coli* O104 strains in weaned sheep at 4 days post infection.

Strain	Animal No.	cfu/g tissue sample [log_{10}]				
		ileum	caecum	colon	rectum	RAJ[a]
BL211	1	0[b]	2.5	2.8	0	0
	2	0	1[c]	0	1	1
	3	5.0	4.2	3.9	1	1
BL320	1	0	2.3	2.3	2.8	1
	2	2.3	1	1	1	2.0
	3	0	1	1	1	2.3

RAJ[a] = recto-anal junction

0[b] = No colonies were recovered even after enrichment

1[c] = Colonies were recovered after enrichment

Figure S1

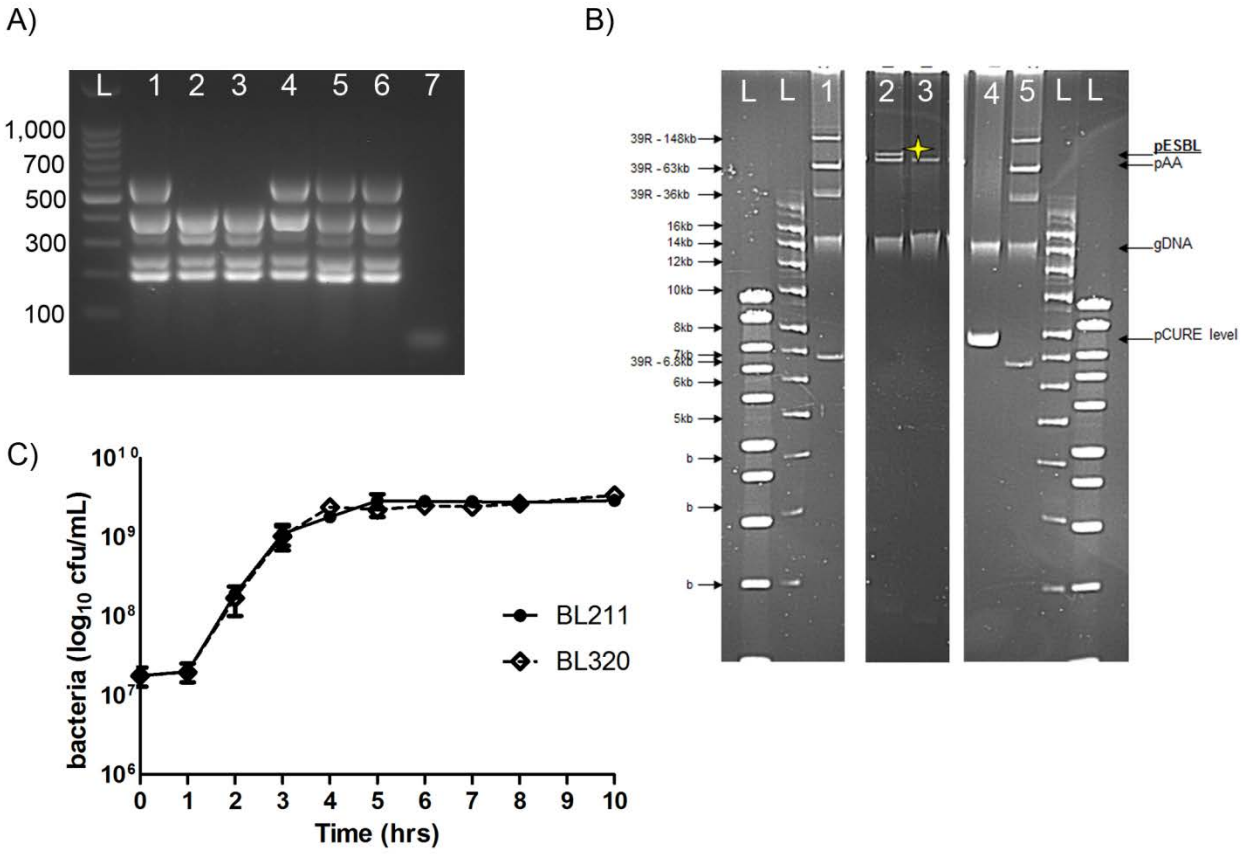

Figure S1. Confirmation of pESBL loss from *E. coli* O104:H4. Multiplex PCR was used to amplify chromosomal (*terD* (434bp), rfb_{O104} (351 bp), *fliC* (201 bp) and plasmid-borne ($bla_{CTX\text{-}M}$ (593 bp), *aggR* (254 bp)) genes (A). Products were visualised on a 2% agarose gel. Lanes: L - 100 bp DNA ladder, 1- parent strain BL211; 2 - pESBL-cured strain BL320; 3 – cefotaxime-sudceptible output colony; 4 to 6 – representative cefotaxime-resistant colonies and 7 – negative control (ddH_2O). Plasmid profile of strains (B). Plasmid DNA and visualised on 0.8% agarose gel. Lanes: L-DNA ladders; 1 – *E. coli* 39R reference strain; 2 – wild type strain BL211; 3 – pESBL-cured strain BL320; 4 – pIFM27 plasmid; 5 – *E. coli* 39R reference strain. All lanes are from the same gel but extra lanes have been removed. Yellow star indicates missing band corresponding to pESBL plasmid. Growth of wild-type and pESBL-cured strain in LB media over time (C). Bacterial numbers were determined by serial dilution and plating

on LB agar supplemented with antibiotics. Data represent means +/- standard deviation of 3 biological replicates.