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# Transfer of Ampicillin resistance from *S. Typhimurium* DT104 to *E. coli* K12 in food

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1 **Transfer of Ampicillin resistance from *S. Typhimurium* DT104 to *E. coli* K12 in**  
2 **food**

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6

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20

21 **Running title:**  $\beta$ -lactamase Transfer in Food

22 **Key Words:** Ampicillin Resistance, *S. Typhimurium* DT104, *E. coli* K12,  $\beta$ -  
23 lactamase, *bla*<sub>TEM</sub>

24

25

26 **Abstract**

27 **Aims:** To investigate the transfer of antibiotic resistance from a donor *S.*

28 Typhimurium DT104 strain to a recipient *E. coli* K12 strain .

29 **Methods and Results:** Mating experiments were conducted in broth, milk and ground  
30 meat (beef) at incubation temperatures of 4, 15, 25 and 37°C for 18 and 36 h.

31 Ampicillin resistance transfer was observed at similar frequencies in all transfer  
32 media at 25 and 37°C ( $10^{-4}$  to  $10^{-5}$  log<sub>10</sub> cfu/ml/g, transconjugants per recipient) for 18

33 h. At 15°C, transfer was observed in ground meat in the recipient strain ( $10^{-6}$ , log<sub>10</sub>  
34 cfu/g, transconjugants per recipient), but not in broth or milk. At 4°C, transfer did not

35 occur in any of the examined mediums. Further analysis of the *E. coli* K12 nal<sup>R</sup>  
36 transconjugant strain revealed the presence of a newly acquired β-lactamase gene

37 *bla*<sub>TEM</sub>. Transconjugants isolated on the basis of resistance to ampicillin did not  
38 acquire any other resistant markers.

39 **Conclusion:** This study demonstrates the transfer of antibiotic resistance in food  
40 matrices at mid-range temperatures.

41 **Significance and Impact of the Study:** It highlights the involvement of food  
42 matrices in the dissemination of antibiotic resistant genes and the evolution of  
43 antibiotic resistant bacteria.

44

45

46 **Introduction**

47 *Salmonella* Typhimurium DT104 is a major cause of enteric infections world-wide  
48 (Beaudin *et al.* 2002). This serovar typically expresses resistance to ampicillin,  
49 chloramphenicol/florfenicol, streptomycin/spectinomycin, sulfonamides and  
50 tetracycline (ACSSuT) (Golding *et al.* 2007). Such multi-drug resistant (MDR) *S.*  
51 Typhimurium DT104 are of concern especially in immuno-compromised patients, as it  
52 restricts clinical options in the treatment of salmonellosis. In more general terms, it  
53 has potential consequences in relation to transfer and dissemination of antibiotic  
54 resistant material to other bacteria including pathogens.

55

56 Class A  $\beta$ -lactamases are the most widespread enzymes in Gram-negative bacteria  
57 (Chochani *et al.* 2006, Bonnet, 2004). These enzymes are widely distributed in  
58 *Salmonella* spp. and are frequently associated with the above (ACSSuT) penta  
59 resistance profile (Guerra *et al.* 2004). In *Salmonella*,  $\beta$ -lactamase resistance is often  
60 conferred by the presence of the *bla*<sub>TEM</sub> or *bla*<sub>PSE</sub> gene (Randall *et al.* 2004). Of these,  
61 the *bla*<sub>TEM-1</sub> gene which is generally plasmid-mediated (Larson and Ramphal, 2002) is  
62 considered the most frequently expressed  $\beta$ -lactamase in *Salmonella* in Europe  
63 (Guerra *et al.* 2004; Tzouveleki *et al.* 2003).

64

65 A number of studies have investigated the transfer of antibiotic resistance in food-  
66 borne bacterial pathogens such as *Salmonella*, but few studies have examined gene  
67 transfer between bacteria *in-situ* in food. Reports of gene transfer (by transformation)  
68 of kanamycin in *Bacillus subtilis* in milk (Kharazmi *et al.*, 2002) and the transfer of  
69 vancomycin resistant genes among enterococcal strains by conjugation during cheese

70 and sausage fermentation (Cocconelli *et al.*, 2003) have been cited. However, the  
71 majority of studies on the transfer of antibiotic resistance have been carried out in  
72 liquid systems (McMahon *et al.* 2007; Wilcks *et al.*, 2005; Chen *et al.*, 2004; Allen  
73 and Poppe, 2002) or on filters (Hummel *et. al.*, 2007; Gevers *et al.*, 2003; Pourshaban  
74 *et al.*, 2002) and thus do not reflect the *in-situ* dynamics of food matrices. This  
75 balance of activity is unfortunate, as liquid or filter based systems have been reported  
76 to underestimate the rates of genes transfer compared to more complex matrices  
77 (Netherwood *et al.*, 1999). Moreover, concern has been raised about food being an  
78 important and under-estimated avenue for antibiotic resistance dissemination and  
79 evolution (Wang *et al.* 2005).

80

81 The objective of this study was to ascertain if antibiotic resistance gene transfer can  
82 occur between a donor *S. Typhimurium* DT104 and *E. coli* K12, in broth, milk and  
83 ground meat under different temperature and time conditions encountered in food  
84 processing.

85

86

## 87 **Material and Methods**

### 88 **Bacterial strains**

89 A previously characterised *S. Typhimurium* DT104 strain resistant to ampicillin,  
90 chloramphenicol, streptomycin, sulfonamides and tetracycline (R-type ACSSuT) was  
91 obtained from the culture collection at Ashtown Food Research Centre, Dublin. An  
92 antibiotic susceptible strain of *E. coli* K12 (NC10538:06) was obtained from the  
93 Health Protection Agency (HPA), London. All strains were stored on cryoprotect  
94 beads (Technical Consultant Services Ltd., Heywood, Lancashire, UK) at  $-20^{\circ}\text{C}$ .

95

### 96 **Preparation of nalidixic acid resistant mutants and antibiogram profiling**

97 *E. coli* K12 were rendered chromosomally resistant to 50  $\mu\text{g}/\text{ml}$  of nalidixic acid, by  
98 the method of Blackburn and Davies (1994). In brief, this involved growing each  
99 strain separately in nutrient broth (Oxoid) containing nalidixic acid 50  $\mu\text{g}/\text{ml}$  and  
100 plating directly onto nutrient agar plates containing the same drug concentration.  
101 Resultant colonies were serially subcultured to confirm mutant stability. The above  
102 procedures produced nalidixic acid resistant *E. coli* K12 recipient isolates, which  
103 could be easily differentiated and recovered during subsequent studies. This strain is  
104 referred to as *E. coli* nal<sup>R</sup> in our study.

105

106 The antibiogram profiles of all donor and recipient strains were established by the  
107 Bauer-Kirby Disc Diffusion method, following the Clinical and Laboratory Standards  
108 Institute recommended method (Anon., 2004) as described by Walsh *et al.* (2001).  
109 Donor and recipient strains were maintained on cryoprotect beads, as described  
110 above.

111

112 **Preparation of inoculum**

113 Protect beads coated with *S. Typhimurium* DT104 and *E. coli* K12 nal<sup>R</sup> were  
114 incubated in 30 ml volumes of LB Broth (Miller, Germany) at 37°C for 18 h, to form  
115 stationary phase cultures containing approximately 10<sup>9</sup> cfu/ml. A 1.0 ml aliquot from  
116 each stationary phase culture was serially diluted in 9 ml volumes of Maximum  
117 Recovery Diluent (MRD, Oxoid) to form inocula containing approximately 10<sup>6</sup> cfu/  
118 ml<sup>-1</sup> culture.

119

120 **Control experiments**

121 The donor and recipient strains were grown independently, inoculated and recovered  
122 from each food matrix during each experiment, by plating on to TSA-ampicillin (50  
123 µg/ml) (to recover the no. of donor strains) and TSA-nalidixic acid (50 µg/ml) (to  
124 recover the no. of transconjugant strains). The number of colonies recovered from  
125 these plates was then used to calculate the frequency of transfer of antibiotic  
126 resistance.

127

128 **Antibiotic resistance transfer experiment in broth or milk**

129 Nine ml volumes of LB Broth (Miller) or retail pasteurised milk were inoculated with  
130 1 ml volumes of the above inocula to form suspensions containing approx. 10<sup>5</sup> cfu/ml  
131 of donor (*S. Typhimurium* DT104) or recipient (*E. coli* K12 nal<sup>R</sup>) suspensions. Donor  
132 and recipient cell suspensions were mixed in 1:1 ratios, incubated for 18 h at 4, 15, 25  
133 or 37°C and then plated directly on TSA containing 50 µg/ml of ampicillin and 50  
134 µg/ml of nalidixic acid and incubated at 37°C for 24 h, to recover potential  
135 transconjugants. Single strain suspensions of each strain were incubated at the test

136 temperatures for 18 h in broth and milk ( $10^5$  cfu/ml) and were used as controls in this  
137 experiment.

138

### 139 **Antibiotic resistance transfer experiment in ground meat**

140 Beef trimmings (70% w/w visible lean), obtained from a beef abattoir in the Dublin  
141 area were minced (Crypto Ltd., London) divided into 30 g portions, blast frozen at –  
142  $30^{\circ}\text{C}$  for 2 h (Woods M3C<sub>3</sub>, Avon Refrig. Co. Ltd. U.K.) and stored at  $-20^{\circ}\text{C}$ .

143 Samples from each batch of ground meat were confirmed as *Salmonella* free (ISO  
144 method 6579) and *E. coli* free (ISO method 6649-2). Prior to use, ground meat  
145 samples were defrosted overnight at  $4^{\circ}\text{C}$ . Duplicate 25 ml volumes of the donor  
146 strain inoculum ( $10^7$  cfu/ml) (*S. Typhimurium* DT104) and 25 ml of the respective  
147 recipient strain inocula ( $10^7$  cfu/ml) (*E. coli* K12 nal<sup>R</sup>), were added to 450 ml volumes  
148 of MRD (1:10 dilution), to form combined inoculating suspensions containing  $10^6$   
149 cfu/ml. Single strain inoculating suspensions were prepared by adding 50 ml of donor  
150 or recipient inocula to 450 ml volumes of MRD to give a final suspension of  $10^6$   
151 cfu/ml of donor or recipient cells. The single strain suspensions of the donor and  
152 recipient cultures were also incubated at  $37^{\circ}\text{C}$  for 18 or 36 h and were used as controls  
153 in this experiment.

154

155 Thirty gram ground meat samples (retained within a sterile sieve) were immersed in  
156 each of the above single or combined inoculating suspensions for 1 min and recovered  
157 by removal of the sieve from the inoculation suspensions, allowed to drain and then  
158 reminced (in a sterilise mincer). Preliminary studies established that this process  
159 resulted in ground meat samples with an inoculum of approximately  $\log_{10} 10^5$  cfu/g  
160 (data not shown). Samples (25 g) of inoculated ground meat were placed in



161 individual sterile bags, incubated at temperatures 4, 15, 25 and 37°C for 18 h (and also  
162 36 h for ground meat). At these time intervals, bags for each different temperature  
163 were retrieved and microbiologically examined. Following incubation the contents of  
164 each bag were stomached for 2 min with 225 ml Maximum Recovery Diluent (MRD,  
165 Oxoid) in a stomacher bag fitted with an integral filter (Seward Ltd., London). The  
166 resultant filtrate was serially-diluted in 9 ml aliquots of MRD, plated onto TSA  
167 containing ampicillin (50 µg/ml) and nalidixic acid (50 µg/ml) and incubated at 37°C  
168 for 24 h. Plates from samples co-inoculated or singly inoculated with *E. coli* K12  
169 nal<sup>R</sup> (as potential recipient), were overlaid with Mac Conkey No. 3 (Oxoid). All  
170 plates were then incubated at 37°C for 24 h and the numbers of colonies per plate was  
171 counted.

172

173 The antibiotic resistance profiles of all recovered recipient, donor and presumptive  
174 transconjugant strains of *S. Typhimurium* DT104 and *E. coli* K12 nal<sup>R</sup> were  
175 confirmed using the Bauer-Kirby disc diffusion method as described above.

176

177 This experiment was replicated on three different occasions using separate batches of  
178 broth, milk and ground meat, fresh inocula and all of the antibiotic resistance profiles  
179 were rechecked (by disc diffusion) on each occasions. The average of these three  
180 replicate experiments was used to calculate the frequency of antibiotic resistance  
181 transfer.

182

183 **Stability of transconjugants**

184 The stability of the *E. coli* K12 nal<sup>R</sup> transconjugant was assessed by daily sequential  
185 subculture on TSA containing ampicillin (50 µg/ml) and nalidixic acid (50 µg/ml) for  
186 14 days.

187

188 **Molecular detection of β-lactamase genes**

189 **DNA isolation**

190 DNA was purified from donor, recipient and transconjugant strains using the Wizard  
191 Genomic DNA purification kit (Promega, Madison, WI), according to the  
192 manufacturers recommendations. In each case, the amount of recovered (template)  
193 DNA was spectrophotometrically determined (O'Mahony *et al.*, 2005). The integrity  
194 of each DNA sample was assessed by conventional agarose gel [1.5%, (w/v)]  
195 electrophoresis in 1 X tris-EDTA-acetic acid (TEA) buffer containing 0.5 µg/ ml  
196 ethidium bromide (EtBr). DNA preparations were stored at 4°C.

197

198 **Amplification of β-lactamase-encoding *bla*<sub>TEM</sub> gene by PCR**

199 DNA samples were examined for the presence of the *bla*<sub>TEM</sub> encoding β-lactamase  
200 genes, using the PCR primers and cycle conditions, previously reported by Arlet and  
201 Philippon (1991) as shown in Table 1.

202

203

204 **Calculation of frequency of antibiotic resistance transfer**

205 The frequency of antibiotic resistance transfer was calculated as follows;

$$\frac{\text{No. of transconjugants } \log_{10} \text{ cfu per ml/ g}}{\text{No. of recipient cells } \log_{10} \text{ cfu per ml/g}}$$

206

207 (McMahon *et al.* 2007; Ohlsen *et al.* 2003; Netherwood *et al.* 1999).

208

209 **Results**

210 **Ampicillin resistance transfer experiment in broth, milk and ground meat.**

211 The frequencies of transfer of ampicillin resistance from the donor strain (*S.*

212 Typhimurium DT104) into the recipient strain (*E. coli* K12 nal<sup>R</sup>) in each of the

213 matrices (broth, milk or ground meat), at a range of incubation temperatures (4, 15,

214 25, or 37°C) and times (18 or 36 h) are presented in Table 2. When transfer was

215 found to occur under defined conditions, it was reproducible for each of three

216 independent replicate experiments. Newly acquired antibiotic resistance profiles in

217 the transconjugant strains (as detected by disc diffusion analysis), were found to be

218 consistent for each independent replicate experiment.

219

220 At 25 and 37°C, ampicillin resistance transfer was observed in all matrices at a rate of

221 between 10<sup>-2</sup> to 10<sup>-4</sup> cfu/ml/g transconjugants per recipient at 37°C and 10<sup>-4</sup> to 10<sup>-5</sup>

222 cfu/ml/g transconjugants per recipient at 25°C (Table 2). The highest frequency of

223 transfer observed in this study was observed at 37°C, from *S.* Typhimurium DT104 to

224 *E. coli* K12 nal<sup>R</sup> (10<sup>-2</sup> cfu/ g<sup>-1</sup>transconjugants per recipient) in ground meat stored for

225 36 h.

226 At 15°C, ampicillin resistance transfer was observed in ground meat, but was not  
227 observed in milk or broth mating experiments. At 4°C, ampicillin resistance transfer  
228 was not observed in any the examined matrices.

229

### 230 **Antibiotic Resistance transfer and stability of transconjugants**

231 Disc diffusion analysis confirmed that the transconjugant isolates of *E. coli* K12 nal<sup>R</sup>  
232 possessed (newly acquired) resistance to ampicillin. Transconjugants isolated on the  
233 basis of resistance to ampicillin did not acquire any other resistance markers  
234 (resistance to chloramphenicol, streptomycin, sulphonamides and tetracycline) present  
235 in the donor strain. The *E. coli* K12 nal<sup>R</sup> transconjugant isolates continued to express  
236 ampicillin resistance (as determined by continuous culture in the presence of  
237 ampicillin and nalidixic acid) for 14 consecutive days.

238

### 239 **Molecular detection of β-lactamase genes.**

240 PCR analysis demonstrated the presence of the β-lactamase gene *bla*<sub>TEM</sub> in the donor  
241 strain/*S. Typhimurium* DT104, but not in original recipient strain of *E. coli* K12 nal<sup>R</sup>.  
242 After mating, PCR analysis revealed the newly acquired *bla*<sub>TEM</sub> gene in the *E. coli*  
243 K12 nal<sup>R</sup> transconjugant.

244

245 **Discussion**

246 This study found that ampicillin resistance could be transferred among bacterial  
247 species in meat systems, at temperatures which occur within the food processing and  
248 the distribution chain. The current study detected ampicillin resistance gene transfer  
249 at temperatures as low as 15°C, suggesting that conditions/components of the meat  
250 matrix are favourable for gene transfer at low temperatures. It is not yet clear, which  
251 aspects of the meat matrix are important to facilitate gene transfer. However, ground  
252 meat provides a large (non-motile) area for bacterial attachment in contrast to mating  
253 experiments in liquids. The advantages of solid matrix for mating experiments have  
254 been previously reported (Lagido *et al.* 2003; Molin and Tolker-Nielsen, 2003). Hirt  
255 *et al.* (2002), also reported that the presence of plasma increased tetracycline  
256 resistance transfer in *Enterococcus faecalis* in a rabbit endocarditis model, suggesting  
257 that the composition of the meat matrix may present a favourable environment for  
258 gene transfer. All of these factors may have contributed to ground meat being the  
259 most suitable matrix for ampicillin resistance gene transfer in this study.

260

261 Resistance to ampicillin was found to be transferable from *S. Typhimurium* DT104 to  
262 *E. coli* K12 nal<sup>R</sup> in broth, milk and ground meat, at 25 and 37°C. At these  
263 temperatures, similar rates of transfer ( $10^{-4}$  to  $10^{-5}$  cfu/ml/g transconjugants per  
264 recipient) were observed in all three matrices after 18 h. These results suggest that at  
265 non-stress (optimal 37°C and sub-optimal 25°C) temperatures, the transfer frequency  
266 of the organisms studied were not significantly affected by the nature of the transfer  
267 environment (broth, milk or ground meat). This is agreement with a study by  
268 Cocconelli *et al.* (2003), who found similar frequencies of transfer during sausages  
269 and cheese fermentation ( $10^{-7}$  to  $10^{-8}$  cfu/ml/g, transconjugants per recipient) at 30°C,

270 again suggesting that the nature of the transfer environment may not significantly  
271 impact on the frequency of antibiotic resistance transfer at non-stress temperatures.  
272  
273 Our study observed lower frequencies of ampicillin resistance transfer ( $10^6 \log_{10}$  cfu/g  
274 tranconjugants per recipient) at stressed temperatures such as 15°C in ground meat  
275 and no transfer in broth or milk. Lower rates of antibiotic resistant transfer at non-  
276 optimal temperatures have been previously reported (McMahon *et al.* 2007). At  
277 15°C, the nature of the transfer environment was found to influence the frequency of  
278 ampicillin resistance from the donor to the recipient strains. However, while reduced  
279 rates of transfer were expected, it is interesting that ampicillin resistance transfer  
280 occurred at 15°C in ground meat, but not in broth or milk. Cocconcelli *et al.* (2003)  
281 reported the transfer of antibiotic resistance by conjugation at 10°C in sausage and  
282 cheese. However, unlike the current study, Cocconcelli *et al.* (2003) did not try to  
283 transfer antibiotic resistance within a liquid system at this temperature (10°C) making  
284 it difficult to compare these data. A knowledge gap exists in area of transfer of  
285 antibiotic resistance in food making comparison with other studies difficult.  
286  
287 No ampicillin resistance transfer was found to occur in any matrices examined at 4°C.  
288 This appears to be due to the overall reduction in the metabolic rates of the  
289 (mesophilic) donor and recipient strains used. However, McMahon *et al.* (2007)  
290 reported antibiotic resistance transfer in broth between *E. coli* at 5°C, suggesting that  
291 the occurrence or absence of such transfer may be related to the characteristics of the  
292 donor and/or recipient organisms (and possible mating criteria), rather than simple  
293 thermodynamic ( $Q_{10}$ ) factors. This is underpinned by Frischer *et al* (1993) who  
294 reported transfer at temperatures between 4 and 33°C while working with a marine

295 *Vibrio* spp., but significantly reduced rates of gene transfer at 37°C. It would therefore  
296 be unwise to assume that effective maintenance of correct chill-chain conditions  
297 during food production will prevent gene transfer among all bacterial species in food  
298 and the food chain.

299

300 PCR analysis confirmed the transfer of the  $\beta$ -lactamase gene *bla*<sub>TEM</sub> (conferring  
301 ampicillin resistance) from *S. Typhimurium* DT104 to *E. coli* K12 nal<sup>R</sup>. It also  
302 confirmed that the acquired resistance persisted in the transconjugants for some  
303 considerable time. This observation significantly increases the potential importance  
304 of such transfers, in terms of the general persistence and dissemination of antibiotic  
305 resistance in food and food environments and in more specific terms, confirming the  
306 exchange of clinically significant antibiotic resistance within the food chain. Such  
307 processes mean that antibiotic resistance genes entering the human food chain may be  
308 spread among other bacteria, allowing the persistence and dissemination of antibiotic  
309 resistance to other more directly significant pathogens in food production and the  
310 processing chain.

311

312 Examination of the wider antibiotic resistant profile of the *E. coli* K12 nal<sup>R</sup>  
313 transconjugants revealed that the recipient bacteria had acquired stable resistance to  
314 ampicillin *via* (the generally plasmid mediated) *bla*<sub>TEM</sub> gene. Transconjugants isolated  
315 on the basis of resistance to ampicillin did not acquire any other resistant markers  
316 (chloramphenicol, streptomycin, sulphonamides and tetracycline) of the donor strain,  
317 suggesting no movement of the *Salmonella* Genomic Island 1 (SGI-1) from the donor  
318 *S. Typhimurium* DT104 strain. Similarly a study by Guerri *et al.* (2004),  
319 demonstrated the transfer of ampicillin resistance (only) *via* the *bla*<sub>TEM</sub> gene from *S.*

320 Typhimurium DT104 (SGI-1 containing) to *E. coli* during conjugation. However,  
321 Doublet *et al.* (2005) and Mulvey *et al.* (2006) report that the SGI-1 can be mobilized  
322 from *S. Typhimurium* DT104 to non-SGI-1 containing *Salmonella* and *E. coli* via a  
323 helper IncC plasmid R55, highlighting the possibility of such transfer in food.

324

325 In conclusion, this study has established that antibiotic resistance genes can be  
326 transferred between bacteria in common food systems, suggesting that food matrices  
327 can play a role in gene transfer, dissemination and persistence. Antibiotic resistance  
328 transfer was not observed at 4°C, suggesting that effective chill chain conditions  
329 would reduce the rate and significance of gene transfer in refrigerated foods for the  
330 bacteria examined in this study. However, gene transfer can occur at relatively low  
331 temperatures i.e. 15°C in some food matrices (ground meat) and more rapidly in a  
332 wider range of food matrices at/or above room temperature. The transfer of antibiotic  
333 resistance among Gram-negative bacteria including commensals and clinically  
334 significant pathogens is a matter of public health concern. Such transfers, the factors  
335 governing their rates, stability and the linkages between virulence and antibiotic  
336 resistance, deserve greater research attention in terms of reducing clinical risks and as  
337 an emerging element in the wider dissemination and persistence of antibiotic resistant  
338 genes in the human environment.

339



340

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**Table 1** Thermocycler amplification conditions for the  $\beta$ -lactamase gene: *bla*<sub>TEM</sub> (Arlet and Philippon, 1991)

Gene	Denaturation	Annealing	Extensions	No. of Cycles	Primers
<i>bla</i> <sub>TEM</sub>	94°C-2 min	54°C-1 min	72°C-30 sec	30	Fwd 5`-TTG GGT GCA CGA GTG GGT TA-3` Rev 5`-TAA TTG TTG CCG GGA AGC TA-3`

**Table 2** Frequency of transfer from *S. Typhimurium* DT104 to *E. coli* K12 nal<sup>R</sup> in broth (log<sub>10</sub> cfu/ml), milk (log<sub>10</sub> cfu/ml) for 18 h, and ground meat (log<sub>10</sub> cfu/g) for 18 and 36 h.

	Trans	Trans/Recpt		Trans	Trans/Recpt
<b>Broth (18 h)</b>			<b>Meat (18 h)</b>		
4 °C	0.00	0.00	4 °C	0.00	0.00
15 °C	0.00	0.00	15 °C	0.22 x 10 <sup>0</sup>	2.0 x 10 <sup>-6</sup>
25 °C	4.13 x 10 <sup>0</sup>	2.9 x 10 <sup>-4</sup>	25 °C	1.93 x 10 <sup>0</sup>	4.7 x 10 <sup>-5</sup>
37 °C	4.45 x 10 <sup>0</sup>	5.8 x 10 <sup>-4</sup>	37 °C	3.49 x 10 <sup>0</sup>	4.7 x 10 <sup>-4</sup>
<b>Milk (18 h)</b>			<b>Meat (36 h)</b>		
4 °C	0.00	0.00	4 °C	0.00	0.00
15 °C	0.00	0.00	15 °C	0.52 x 10 <sup>0</sup>	8.1 x 10 <sup>-6</sup>
25 °C	3.17 x 10 <sup>0</sup>	2.6 x 10 <sup>-4</sup>	25 °C	3.08 x 10 <sup>0</sup>	5.8 x 10 <sup>-4</sup>
37 °C	4.07 x 10 <sup>0</sup>	2.6 x 10 <sup>-4</sup>	37 °C	4.23 x 10 <sup>0</sup>	3.3 x 10 <sup>-2</sup>

\*Trans: Transconjugants, Trans/Recpt: Transconjugants per recipient cells