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Persistence of a wild type *Escherichia coli* and its multiple antibiotic-resistant (MAR) derivatives in the abattoir and on chilled pig carcasses

Anne A. Delsol [a,*], Deborah E. Halfhide [a], Mary C. Bagnall [b], Luke P. Randall [b], Virve I. Enne [c], Martin J. Woodward [b], John M. Roe [a]

[a] Faculty of Medical and Veterinary Sciences, University of Bristol, Langford, BS40 5DU, UK
[b] Department of Food and Environmental Safety, Veterinary Laboratories Agency, New Haw, Surrey, KT15 3NB, UK
[c] Department of Cellular and Molecular Medicine, University of Bristol, Bristol, BS8 1TD, UK

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ABSTRACT

The aim of this study was to evaluate the ability of an *Escherichia coli* with the multiple antibiotic resistance (MAR) phenotype to withstand the stresses of slaughter compared to an isogenic progenitor strain. A wild type *E. coli* isolate (345-2RifC) of porcine origin was used to derive 3 isogenic MAR mutants. *Escherichia coli* 345-2RifC and its MAR derivatives were inoculated into separate groups of pigs. Once colonisation was established, the pigs were slaughtered and persistence of the *E. coli* strains in the abattoir environment and on the pig carcasses was monitored and compared. No significant difference ($P>0.05$) was detected between the shedding of the different *E. coli* strains from the live pigs. Both the parent strain and its MAR derivatives persisted in the abattoir environment, however the parent strain was recovered from 6 of the 13 locations sampled while the MAR derivatives were recovered from 11 of 13 and the number of MAR *E. coli* recovered was 10-fold higher than the parent strain at half of the locations. The parent strain was not recovered from any of the 6 chilled carcasses whereas the MAR derivatives were recovered from 3 out of 5 ($P<0.001$). This study demonstrates that the expression of MAR in 345-2RifC increased its ability to survive the stresses of the slaughter and chilling processes. Therefore in *E. coli*, MAR can give a selective advantage, compared to non-MAR strains, for persistence on chilled carcasses thereby facilitating transit of these strains through the food chain.

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1. Introduction

It is generally accepted that the use of antimicrobial agents in livestock production contributes to the increased incidence of antibiotic resistance in both commensal bacteria and pathogens (Aarestrupp et al., 2008b; Hammerum and Heuer, 2009). In 2008 59% of combined species antimicrobials sold for food animal production in the UK were authorised for pigs and/or poultry and the largest percentage of single species products was sold for use in pigs (http://www.vmd.gov.uk/General/DARC/pubs.htm). A number of studies using a pig model have demonstrated that antimicrobial treatment regimes commonly used in the industry are responsible for increasing the number of antibiotic-resistant enteric and zoonotic bacteria in pigs (Aarestrupp et al., 2008a; Delsol et al., 2003; Delsol et al., 2004a, b). These results provide strong evidence that treated pigs have the potential to enter abattoirs with a higher number of resistant bacteria than untreated pigs, thereby increasing the risk of such bacteria moving through the food chain and infecting man. Enne et al. (2008) showed that of 2480 *Escherichia coli* isolated from pigs at slaughter 92% were resistant to at least one antimicrobial and 62.8% were resistant to 3 or more unrelated antimicrobials from classes used in human medicine.

Multi-drug resistance in bacteria is commonly attributed to mobile genetic elements such as plasmids or transposons (Gold and Moellering, 1996; Jacoby and Archer, 1991). However chromosomal multi-drug resistance systems such as the multiple antibiotic resistance (*mar*) locus of *E. coli* may also be involved (Cohen et al., 1989; George and Levy, 1983). The *mar*-locus of *E. coli* is reported to mediate reduced susceptibility (4 to 8 fold) to a number of unrelated antimicrobials primarily by up-regulating the outflow of antimicrobials via the AcrAB-TolC efflux pump (Okusu and Nikaido, 1996) and down-regulating influx through the Outer Membrane Protein F (OmpF) (Cohen et al., 1989). The up-regulation of the *soxRS* regulon has also been shown to up-regulate *acrAB*, resulting in the MAR phenotype (Miller et al., 1994; White et al., 1997).

The MAR phenotype confers low level resistance to antibiotics such as β-lactams, tetracyclines and fluoroquinolones, resistance to organic solvents such as cyclohexane (White et al., 1997) and decreased susceptibility to disinfectants such as chlorhexidines, acridines and triclosan (Mc Murray et al., 1998; Moken et al., 1997; Russell, 2000,

* Corresponding author. Tel.: +44 117 928 9478; fax: +44 117 928 9612.
E-mail address: a.a.g.delsol@bris.ac.uk (A.A. Delsol).

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2002) despite these products having diverse intracellular targets. Whilst the level of resistance is low, data suggests that it acts as an intermediate step towards higher levels of antimicrobial resistance (Alekshun and Levy, 1997; Levy, 2000) possibly providing the organisms with a competitive advantage in environments where antimicrobials and disinfectants are used. Other studies have also identified an association between fluoroquinolone resistance and organic solvent tolerance both in human clinical *E. coli* isolates (Oethinger et al., 1998) and in pig *Salmonella enterica sv*. Typhimurium DT104 isolates (Delsol et al., 2004a, b). In fact the latter study also demonstrated that pigs treated with a fluoroquinolone were entering abattoirs with an increased number of organic solvent-resistant bacteria compared to untreated pigs.

In order for antimicrobial-resistant bacteria to pass through the food chain, these bacteria must survive as contaminants during slaughter, food processing and retailing. Data generated during the course of previous studies indicates that following antimicrobial treatment, bacteria expressing the MAR phenotype may be better able to survive certain stresses, such as disinfection, that occur during food processing (Moken et al., 1997; Randall and Woodward, 2001; Russell, 2000, 2002). If so, MAR strains may have a greater potential to pass along the food chain to humans. In this study we evaluated the ability of *E. coli* expressing the MAR phenotype to withstand the stresses of slaughter and food processing compared to an otherwise isogenic parent strain and hence whether such MAR mutants are more likely to be transmitted through the food chain than wild type bacteria.

2. Materials and methods

2.1. E. coli strains

A wild type *E. coli* (345-2RifC) isolate of porcine origin marked with a no-cost chromosomal rifampicin-resistance mutation, as determined by assays to measure competitive fitness, was used for this study (Enne et al., 2005). This isolate was chosen as it had previously been used in the same animal model and had shown excellent colonisation characteristics (Enne et al., 2005).

MAR mutants were derived by passaging 345-2RifC in the presence of tetracycline (5 mg/L) at 30 °C as previously described (George and Levy, 1983; Randall and Woodward, 2001). Colonies were selected and passaged a second time in the presence of tetracycline (5 mg/L) to ensure purity, before storing on cryogenic beads at −80 °C. A total of 20 mutants were tested for their suitability in animal studies.

The minimum inhibitory concentration (MIC) values of ampicillin, chloramphenicol, nalidixic acid and tetracycline were determined for the parent strain (345-2RifC) and its MAR derivatives using the method of the British Society for Antimicrobial Chemotherapy (Andrews, 2001). Resistance to cyclohexane was tested as previously described (Randall et al., 2001). The susceptibility of the MAR mutants to ampicillin and chloramphenicol and their tolerance to cyclohexane were re-determined after five passages in Luria–Bertani (LB) broth (VWR, Pennsylvania, USA; Becton-Dickinson and Company, New Jersey, USA) to establish the stability of mutants.

Growth curves were performed in LB broth and minimal medium over a 24-h period for the parent strain and stable mutants. Inocula of the strains were grown overnight at 37 °C in the respective media (LB or minimal medium) and then the main culture inoculated at 10^5 CFU/mL in 4 replicate wells of a 96 well micro-titre plate and incubated at 37 °C. The optical density was recorded at intervals of 15 min for the first 4 h and then every 30 min up to 24 h. The optical density was recorded using a FLUROstar OPTIMA plate reader (BMG Labtech, Aylesbury, UK) set to read in absorbance mode at 600 nm.

To identify mutations in the mar-locus of MAR mutants, PCR amplification of the *acrR*, *marR* and *soxR* genes and their respective promoter regions was performed using the primers listed in Table 1 and an "Expand High Fidelity PCR System" (Roche Molecular Diagnostics, California, USA) according to the manufacturer's instructions. The PCR thermocycler conditions were as follows; 94 °C for 5 min; 30 cycles of 30 s denaturing at 94 °C, 1 min annealing at 50 °C and 1 min extension at 72 °C; followed by a final extension of 72 °C for 10 min, and then holding at 4 °C. PCR amplicons were sequenced using an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, California, USA). The DNAstar LaserGene SeqMan program was used to assemble sequences and to compare them to the relevant accession numbers and parent strains.

2.2. Pig model

For each investigation, six 5-month old pigs from one litter (Large White cross) were housed as 2 groups of 3 in separate pens with HEPA filtration. The pigs were fed, *ad libitum*, standard grower rations on which they had been fed since weaning. All reached slaughter weight within 2 weeks of their inclusion in the study.

All animal procedures complied with Animal (Scientific Procedures) Act 1986 and were performed under Home Office Licence.

For the inoculation in the pig model, the parent strain and the MAR derivatives were grown separately overnight in nutrient broth (Oxoid Limited, Basingstoke, UK) at 37 °C. The cells were centrifuged at 5000 g for 15 min, washed in saline and re-suspended in antacid composed of $4MgCO_3.Mg(OH)_2.4H_2O$ (50 g/L); $Mg_2Si_3O_8$ (50 g/L) and $NaHCO_3$ (50 g/L). Bacterial/antacid suspension (50 mL) containing 10^9 CFU of culture was administered to each pig by oral gavage within 30 min of preparation. The suspension for the MAR derivatives was composed of

Table 1
Primers used to produce PCR amplicons for initial and subsequent sequencing.[a]

Forward primer	Sequence (5′ to 3′)	Reverse primer	Sequence (5′ to 3′)	Amplified base pairs
Accession number U00734				
*acrRO*F	GAACCTGAAGAACGACCTGA	*acrRO*R	CATCAGAACGACCGCCAGAG	197–1140
*AcrRO*1F	GATTACGTTGTGCCTGTTGC	*AcrRO*1R	ACATCCTCGATGTGGCTCTA	
*AcrRO*2F	GACGTAGAGCCACATCGAGG			
Accession number M96235				
*marRO*F	CCAGCCCCAGGCCAATTGC	*marRO*R	CGCTTGTCATTCGGGTTCG	1200–1726
*marRO*1F	CGTGGCATCGGTCAATTCAT	*marRO*1R	GGATAGAGCAGAGCACTTA	
*marRO*2F	TGTCTCCGCTGGATATTACC	*marRO*2R	CTGGACATCGTCATACCTCT	
Accession number X59593				
*soxRO*F	TAAGCGGCTGGTCAATATGC	*soxRO*R	AATGAGGTGTGTTGACGTCG	614–1329
*soxRO*1F	GGCAATCAGCGGCGATATAA	*SoxRO*1R	CGGAATGCCAATACGCTGAG	
*soxRO*2F	CAATGGCGAGAAGAGTTGGA	*SoxRO*2R	AATGCGCCGATCCAACTCTT	

[a] Primers underlined are primers to amplify the entire region. These primers and other primers were subsequently used in sequencing reactions.

equal numbers of each mutant selected. The investigation of the parent strain was run at a different time from its MAR derivatives but for each investigation, the pigs were inoculated on days 1, 3, 7, 9 and 12 (day 0 was pig arrival) to ensure that the marked strains had colonised the intestinal tract in high enough numbers to be re-isolated in all pigs immediately before slaughter.

Faecal samples were collected by digital manipulation (whereby for each pig a gloved finger was inserted into the rectum and faeces removed directly into a sample pot) on day 0 (prior to inoculation), and on days 2, 7, 9 and 13 (slaughter day). Faecal samples (1 g) from each pig were then emulsified in 9 mL of phosphate buffered saline (PBS) and a 10-fold serial dilution series prepared.

2.3. Sampling in abattoir

The University's private abattoir was used, in which the throughput is extremely low with less than 10 animals on slaughter days, which is only 2 days per week.

A wet–dry swab sampling technique was used to sample selected sites within the abattoir. Jumbo swabs (MW&E, Corsham, UK) and a metal template with a sampling area of 50 cm^2 were used throughout. At each site the template was sterilised with an azo wipe, the first swab was wetted in Maximum Recovery Diluent (MRD, Oxoid) (10 mL) and used to scrub the sample area vertically, horizontally and across both diagonals. The swab was then broken off into the MRD (10 mL) and the process repeated with a dry swab, which was broken off into the same pot of MRD.

Sites within the abattoir were sampled on 3 occasions: before the pigs entered, during processing and the following morning more than 18 h after routine cleaning procedures. These sites included lairage (100 cm^2 surface), stick knife, scald tank water (10 mL), scraper (total surface), de-hairing scrapers, gambrelling table (2×50 cm^2 of surface), all knives for manual de-hairing (swabs were pooled to make one sample), all knives used for gutting (swabs were pooled as one sample), saw used to cut the carcass in half (total surface), knives used to trim the fat, apron washer (2×50 cm^2 of surface), aprons (2×50 cm^2 of surface of each apron, all samples were then pooled as one), and the hands of each abattoir worker (entire surface swabbed once immediately after slaughter).

The wet–dry technique was also used to swab each carcass at 3 sampling points (leg, belly, and back). Each carcass was sampled once immediately before chilling and again (immediately adjacent to the first area sampled) after 48 h at 1 °C, the earliest time point at which they could enter the food chain as a meat product.

From each MRD suspension, an aliquot was used to prepare a 10-fold dilution series.

2.4. Re-isolation of marked E. coli

For faecal samples taken from pigs prior to slaughter, aliquots (20 µL) of the 10^{-1} to 10^{-4} dilutions were spread on MacConkey (MAC) agar supplemented with rifampicin (50 mg/L) and incubated at 37 °C overnight.

For samples taken in the abattoir or from a carcass, 10 aliquots (0.5 mL) of the neat dilution, 100 µL of the neat dilution and 20 µL of the neat to 10^{-3} dilutions were spread on 10 MAC agar supplemented with rifampicin (50 mg/L). The plates and the MRD suspensions were incubated at 37 °C overnight.

Colonies that grew on the MAC agar were then replica plated onto Isosensitest (ISO) agar (Oxoid) in glass Petri dishes, overlayed with cyclohexane (>99%) and incubated overnight at 30 °C. Any colonies which grew on the cyclohexane-covered plates were scored as multiple-antibiotic-resistant (MAR) strains. Three colonies from each sample were purified from a MAC agar plate and stored on cryobeads for further analysis.

2.5. Random amplification of polymorphic DNA

Random amplification of polymorphic DNA (RAPD) was carried out to determine relatedness among *E. coli* isolates recovered from abattoir and faecal samples, and the profiles compared to those of the parent strain and its MAR derivatives. To prepare template DNA for RAPD PCR isolates were grown overnight on blood agar and one colony was emulsified in 100 µl of molecular biology grade water and boiled for 5 min. RAPD PCR was then performed as described previously (Nowrouzian et al., 2003) using Hi-Fidelity PCR master mix (Abgene, Epsom, UK) and 1 µL of template. RAPD PCR products were visualised by UV illumination after gel electrophoresis on 1% agarose gels in Tris boric acid/EDTA buffer incorporating ethidium bromide.

2.6. Statistical methods

The counts (CFU/g faeces and CFU/100 cm^2) were transformed to log10 for all comparisons of the parent and MAR strains. The mean counts prior to slaughter were compared by a 2 sample *t*-test assuming equal variances. The carcass samples were analysed using an interval censored regression model fitted by the 'intreg' procedure in STATA (Stata/IC 10.0 for Windows (2007); StataCorp LP, 4905 Lakeway Drive, College Station, TX 77845 USA 2007), because some of them were recorded as a range or as an upper limit. Separate models were fitted to the pre-chill counts for the leg, belly and back samples and a single model to the 33 post-chill counts from all the areas with robust standard errors to allow for the clustering by animal. The strains were compared by Wald tests with a significance level of 5% ($P=0.05$).

3. Results

3.1. Phenotypic and genotypic characteristics of the MAR mutants

All 20 MAR mutants showed a 2- to 4-fold reduction in sensitivity to each of ampicillin, chloramphenicol, nalidixic acid and tetracycline and were cyclohexane-tolerant (Table 2).

Of the 20 MAR mutants, 16 lost their cyclohexane tolerance after 5 passages in LB broth and 1 failed to grow in LB broth after storage. Three mutants: EC51, EC48 and EC35 retained their cyclohexane tolerance and their growth profiles in LB broth and minimal medium compared to the parent strain (data not shown). Based on these results EC 51, EC 48 and EC 35 were selected for genotypic analysis and for use in the animal studies.

The three MAR mutants differed by one base-pair from the parent strain in the *marR* gene but they did not differ in the *acrR* or *soxR* genes or the promoter regions of *marR*, *acrR* or *soxR*. The nucleotide changes identified were the following: for EC 35 and EC 51, the nucleotide C was replaced by T at position 292 (compared to the parent strain) resulting in a stop codon after glycine 97 of MarR; for EC 48 the nucleotide G was replaced by A at position 191 resulting in a stop codon following glycine 63. We hypothesise that these mutations result in a truncated MarR repressor protein thereby reducing MarR functionality and giving rise to mutants with the MAR phenotype. The truncated repressor protein would be at least 28 amino acids shorter than the full-length protein of 125 amino acids.

Table 2
Minimum inhibitory concentration (MIC) values of antibiotics and cyclohexane tolerance for *E. coli* 345-2RifC and MAR mutants derived from it.

E. coli strains	Number tested	MIC (mg/L)				
		AMP	CHLOR	NAL	TET	CYCLO
345-2RifC	1	4	8	4	2	Sensitive
MAR mutants	20	8	32	8	4	Resistant

AMP, ampicillin; CHLOR, chloramphenicol; NAL, nalidixic acid; TET, tetracycline; CYCLO, cyclohexane.

3.2. Colonisation by rifampicin-marked E. coli

Both the parent strain and its MAR derivatives colonised the intestinal tract of the pigs. On the day of slaughter the pigs inoculated with the parent strain were shedding this strain at an average of 2.43 $\log_{10}$ CFU/g faeces (±1.074) and the pigs inoculated with the 3 MAR derivatives (EC 48, EC 51 and EC 35) were shedding them at an average of 3.90 $\log_{10}$ CFU/g (±1.284). This difference was not statistically significant ($P>0.05$). A representative number of *E. coli* (20 recovered parent strain and 20 recovered MAR derivatives) from the live pigs were confirmed as the study strains by RAPD PCR.

3.3. Recovery of rifampicin-marked E. coli from abattoir and pig carcasses

Both the parent strain and its MAR derivatives persisted in the abattoir environment (Table 3). The parent strain was recovered from 6 of the 13 sampling locations and the MAR derivatives from 11 of the 13 locations. The number of MAR *E. coli* recovered was 10-fold higher than the parent strain at half of the locations, namely the bleeding knives, scraper, gambrelling table, tools for degutting, saw (to split the carcasses) and aprons worn by workers. RAPD PCR was performed on all of the rifampicin-marked *E. coli* recovered and confirmed that they were the inoculated strains. None of the parent strains recovered had become cyclohexane-resistant and all of the MAR derivatives recovered were still cyclohexane-resistant. Before slaughter and following cleanup all samples were negative for rifampicin-marked *E. coli*.

The parent strain and MAR derivatives were recovered from all carcasses pre-chilling at each of the 3 sampling locations: leg, belly and back (Table 4). For both strains the highest number recovered was from the leg and the lowest number was from the back. The MAR derivatives were recovered in significantly higher numbers than the parent strain from all 3 sampling sites ($P<0.01$), with at least a 10-fold difference at each sampling sites (Table 4).

The parent strain was not recovered from any of the 6 carcasses post-chilling, whereas the MAR derivatives were detected on 3 out of 5 chilled carcasses from the leg and back only. No MAR derivatives were re-isolated from the belly and recovery of the MAR derivatives post-chill was not limited to the carcasses with the highest number of MAR derivative CFU/cm^2 pre-chill. From the 3 carcasses with MAR survival post-chill, the percentage-survival was 0%, 0.9% and 10.3% on the backs and 0.3%, 0.1% and 1% respectively on the legs.

On average the MAR strains was present in significantly higher numbers than the parent strain ($P=0.001$).

Table 3
Numbers of parent and MAR derivative *E. coli* re-isolated from the abattoir during processing.

	Parent	MAR
Log_{10} CFU/item		
Scald tank	<0.3 per 10 mL	<0.3 per 10 mL
Bleeding knives	<0.3	1.9
Scraper	<0.3	1.5
Dehairing knives	0.4	0.4
Worker hand	2.9	2.6
Tools (degutting)	<0.3	1.8
Saw (split carcass)	<0.3	2.3
Knives to trim fat	<0.3	0.4
Log_{10} CFU/100 cm^2 of surface		
Gambrelling table	1.6	2.7
Dehairer	1	1.9
Lairage	4.8	2.55
Apron washer	<0.3	<0.3
Apron	1.5	2.6

The detection limit was 0.3 $\log_{10}$ CFU/item or 0.3 $\log_{10}$ CFU/100 cm^2. Anything below the detection limit was scored as <0.3 $\log_{10}$ CFU. All values are single data points.

Table 4
Numbers of *Escherichia coli* ($\log_{10}$ CFU/100 cm^2) recovered from the surfaces of dressed pig carcasses.

	Parent	MAR derivatives	*P*-values
Leg	1.5 (0.92–2.08)[a]	3.16 (2.52–3.80)	<0.001
Belly	1.71 (1.20–2.22)	3.0 (2.16–3.31)	0.009
Back	1.42 (0.99–1.84)	2.6 (2.10–3.06)	<0.001

[a] 95% confidence intervals.

All of the *E. coli* recovered from the carcasses were confirmed as the study strains by their resistance profiles (summarised in Table 2) and RAPD PCR.

4. Discussion

This study has demonstrated that MAR derivatives of wild type *E. coli* 345-2RifC were not only fit *in vivo*, as shown by their colonisation and amplification in the host, but they also had an increased ability to survive the stresses of slaughter and chilling, thereby increasing the likelihood of them passing along the food chain. Our results showed that there was no statistically significant difference between the parent and MAR derivatives' colonisation of the intestinal tract, yet the MAR derivatives were recovered from 11 of the 13 abattoir locations sampled, the number of MAR derivatives recovered was 10-fold higher than the parent strain at half of these locations and the MAR derivatives persisted on 3 out of 5 chilled carcasses; in contrast, the parent strain was recovered from only half as many sampling locations (6 out of the 13) and it was not recovered from any of the 6 carcasses post-chilling.

All MAR mutants selected for further analysis showed a typical MAR phenotype, as evidenced by reduced susceptibility to certain antimicrobials and cyclohexane tolerance (Table 2). For isolates EC35, EC48 and EC51 selected for use in the animal studies, sequencing suggested that the MAR phenotype was due to mutations in the *marR* gene that resulted in truncated MarR repressor products, which we deduce reduced MarR activity.

Stable and persistent MAR derivatives were selected by exposing *E. coli* 345-2RifC to tetracycline, the most commonly used antimicrobial in the pig industry (http://www.vmd.gov.uk/General/DARC/pubs.htm; VMD, 2009). The ease with which this was achieved suggests that such mutants may readily arise in pigs and therefore the application of appropriate surveillance strategies for such isolates would seem prudent, especially as such isolates could cause clinical problems in humans. There is currently little information on the prevalence of the MAR phenotype in veterinary enteric bacteria, however at least two studies do show that MAR mutants of both *Salmonella* and *E. coli* occur in veterinary isolates (Randall et al., 2001).

The over-expression of efflux pumps alone is unlikely to give rise to clinical levels of resistance leading to therapeutic failure (Gibb et al., 1991; Howard et al., 1990; Pers et al., 1996; Piddock et al., 2000). In fact in many cases the increases in antibiotic MIC do not exceed recommended breakpoint concentrations, consequently bacteria with the MAR phenotype can easily remain unnoticed until they have acquired further resistance mechanisms. An association between organic solvents such as cyclohexane and up-regulation of efflux has been reported, making them useful markers for MAR in *E. coli* (White et al., 1997). However, it is possible for MAR strains with up-regulated *acrAB*, *marRAB* and *soxRS* expression to remain cyclohexane-sensitive (Webber and Piddock, 2001). In addition strains selected as cyclohexane-tolerant lost their tolerance whilst being investigated following passage in disinfectant-free broth, despite retaining reduced susceptibility to antibiotics (Randall et al., 2005). It is therefore possible that screening for cyclohexane tolerance alone may not be sufficient to identify bacteria expressing the MAR phenotype. Currently the use of efflux pump inhibitors (EPIs) is also being investigated as a way of tackling MAR mutants, however to date none have been successfully licensed for use in bacterial infections in humans (Lomovskaya and Bostian, 2006).

Although bacteria respond to temperature shifts by stress response mechanisms, the time for chilling in our experiments was very swift (a matter of minutes), and consequently the survival of MAR mutants was likely to be due to innate resistance to chilling rather than an adaptive response. The three strains that were used in the study were stably MAR and the causative mutations we assume were in *marR*. A line for further investigation is the contribution of the *mar* regulon in response to temperature stress (Fontaine et al., 2008). Alternatively, the selection process selected strains that had acquired a number of changes other than mutation in *marR* such as altered lipid structures that render organisms less sensitive to cold (Harwood, 2007).

In conclusion, this study has looked at the effects of MAR for a single wild type *E. coli* strain and further studies are needed to determine how widespread this phenomenon is within the wild type *E. coli* population. Despite this, the results clearly show that in, at least some, enteric bacteria the MAR phenotype gives a selective advantage to persist in an abattoir environment and on chilled carcasses, thereby facilitating transit of these stains through the food chain.

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