Citation:
Hughes, L and Roberts, W and Johnson, D (2020) The impact of DNA adenine methyltransferase knockout on the development of triclosan resistance and antibiotic cross-resistance in Escherichia coli. Access Microbiology. ISSN 2516-8290 DOI: https://doi.org/10.1099/acmi.0.000178

Link to Leeds Beckett Repository record:
http://eprints.leedsbeckett.ac.uk/id/eprint/7249/

Document Version:
Article (Published Version)

Creative Commons: Attribution 4.0

This is a published article published by Microbiology Society in Access Microbiology on 18th November 2020, available online: https://doi.org/10.1099/acmi.0.000178

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please contact us and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.
The impact of DNA adenine methyltransferase knockout on the development of triclosan resistance and antibiotic cross-resistance in *Escherichia coli*

Lewis Hughes, Wayne Roberts and Donna Johnson*

**Abstract**

**Background.** DNA adenine methyltransferase (*dam*) has been well documented for its role in regulation of replication, mismatch repair and transposition. Recent studies have also suggested a role for *dam* in protection against antibiotic stress, although this is not yet fully defined. We therefore evaluated the role of *dam* in the development of antibiotic resistance and triclosan-associated cross-resistance.

**Results.** A significant impact on growth rate was seen in the *dam* knockout compared to the parental strain. Known triclosan resistance-associated mutations in *fabI* were seen regardless of *dam* status, with an additional mutation in *lrhA* seen in the *dam* knockout. The expression of multiple antibiotic resistance-associated genes was significantly different between the parent and *dam* knockout post-resistance induction. Reversion rate assays showed that resistance mechanisms were stable.

**Conclusions.** *dam* knockout had a significant effect on growth, but its role in the development of antibiotic resistance is likely confined to those antibiotics using *acrAD*-containing efflux pumps.

**DATA SUMMARY**

The whole-genome sequencing data of the strains used in this study are available from NCBI under the BioProject accession number PRJNA517874.

**BACKGROUND**

In order to maximize survival, clonal bacterial populations (cultured from a single colony) exhibit phenotypic cell–cell variation. While it has generally been assumed that mutation, spread through the population via vertical descent, is the cause of such variation, it is becoming increasingly apparent that epigenetic changes are also involved [1, 2]. Adenine methylation is the most common epigenetic change in prokaryotes [3], and in Gram-negative bacteria it is primarily mediated by DNA adenine methyltransferase (*dam*) [3, 4]. *dam* has been shown to be involved in mismatch repair [3, 4], regulation of replication [5, 6], transposition [7, 8] and control of gene expression [9]. *dam* has also been linked to antibiotic resistance (ABR) – Adam *et al.* saw increased resistance to ampicillin, tetracycline and nalidixic acid in *Escherichia coli* as a result of epigenetically induced changes in the expression of resistance-associated genes [2]. Conversely, a role for *dam* has been suggested in protection against antibiotic stress; *E. coli* lacking *dam* exhibit compromised survival in the presence of ampicillin, likely as a result of a build-up of double strand breaks [10]. The expression of broad-spectrum resistance-associated genes such as the *acrAB/D-tolC* efflux pumps of *E. coli* have been shown to be regulated by *dam* [11–13], adding support to the potential role for *dam* in the development of ABR. Given that *dam* homologues are widespread amongst bacteria [14], a full understanding of the role of adenine methylation in the development of resistance is critical for the identification of potential new targets for drug development.

Triclosan (TCS) is a broad-spectrum biocide that has recently been restricted due to concerns that it may have toxic or carcinogenic effects, in addition to concerns about antibiotic cross-resistance [15–18], but it is still used in a range of products such as soaps and deodorants [19].
Salmonella enterica serovar Typhimurium, TCS selects for increased resistance to ampicillin, tetracycline, ciprofloxacin and kanamycin, and also increased expression of the acrAB efflux pump [20, 21]. Furthermore, TCS has been seen to modulate efflux pump expression directly in Stenotrophomonas maltophilia, by binding to the repressor smeT, allowing expression of the smeDEF efflux pump [22]. While data [23] support the involvement of efflux pumps in TCS-mediated cross-resistance, the specific mechanisms have yet to be fully elucidated. Due to the heavy commercial dependence on TCS, the Scientific Committee in Consumer Safety highlights the need for further in vitro studies to demonstrate if, when used at sub-lethal concentrations, TCS causes the development of antibiotic cross-resistance and to determine the mechanisms behind this [15]. Our hypothesis is that dam is able to regulate efflux pump expression and that this mechanism underpins the development of TCS-induced cross-resistance.

METHODS

TCS resistance

The minimum inhibitory concentration (MIC) of the parental and dam (ECK3374) knockout (E. coli BW25113 strain and isogenic knockout strain, Keio Knock-out Collection, Dharmacon) was determined using broth microdilution. Parallel dam knockout and parental cultures were serially sub-cultured in nutrient broth with increasing TCS concentrations for 7 consecutive days. TCS was used at 1 µg ml⁻¹ until day 5 and 10 µg ml⁻¹ between days 5 and 7. The growth rates of initial cultures and TCS-resistant mutants, obtained from single colonies cultured on nutrient agar (10 µg ml⁻¹ TCS), were assessed over 24 h using spectrophotometry and antibiotic cross-resistance using disc diffusion (MASTRING-S systemic Gram negative M14 multi-disc, MAST, UK). The sensitivity of each strain was determined according to the guidelines in the BSAC Methods for Antimicrobial Susceptibility Testing [24]. Fitness costs were calculated from relative growth rates.

RT-qPCR

RNA was extracted from starting cultures and resistant mutants, using the PureLink RNA Mini kit (Thermo Fisher Scientific, UK) following the standard protocol. RNA concentration and 260/230 and 260/280 ratios were determined through microvolume spectrophotometry (Denovix). RNA integrity was assessed via gel electrophoresis. Non-degraded samples (260/230~2.2 and 260/280~2.0) were accepted for cDNA synthesis using the Verso cDNA Synthesis kit (Thermo Scientific) following the standard protocol. RT-qPCR was performed with iTaq universal SYBR Green supermix (Thermo Fisher, UK) using a CFX96 Touch Real-Time PCR Detection System. The primer sequences were as indicated in Table 1. Cycling conditions were as follows: 95 °C for 5 min; 40 cycles of 95 °C for 5 s; 60 °C for 30 s. hcaT was shown to be a suitable reference gene by Normfinder, as determined experimentally from three genes (hcaT, cysG and rpoS) [25]. Fold change was calculated using ΔΔCt and was relative to the starting parent strain. Differences in mean fold changes were assessed using Welch’s analysis of variance (ANOVA) with a significance level 0.05 in SPSS (V.25).

Reversion rate assay and ability to grow at high TCS concentrations

Initial strains and TCS-resistant mutants were cultured in TCS-free nutrient broth for 24 h at 37 °C. Ability to grow in the presence of TCS was assessed by plating 100 µl of the overnight culture on nutrient agar/TCS plates (10 µg ml⁻¹). Cultures were then propagated in TCS-free media every 24 h for 10 days with a sample being plated on nutrient agar/TCS plates alongside. The reversion rate was determined as the time in days until the loss of TCS resistance. As reversion

| Gene | Primer sequence | Gene | Primer sequence |
|------|-----------------|------|-----------------|
| acrA | F GAGTACGATCAGGCTCTGGA | CysG | F TTGTCCGGGTTGTTGGTGATCT |
| R AGGAAGCTGTCTGAGCTG | R ATGCGGTGAAGCTTTGGAGAT |
| acrB | F CAGGATCAACGGCACCAGTA | rpoS | F TATGAGTCAAGATACGCT |
| R AGGAAGCTGTCTGAGCTG | R GGAACAGCCTGGTTGGATTT |
| acrR | F AAGAAAGCGGCGAAACACATC | HcaT | F GGCACCTGCTGACCTCTCT |
| R CAGCGAGTGTGATGATACCA | R TAGTGACCAGTTTGCCG |
| tolC | F CGTTTTCGGCTTCTTTCAG | brhA | F GGCAGTAACCGACTTACTCC |
| R TTTTAAGGGGCTGTTG | R CCTGCGAACGACGTGTTACT |
| marA | F CATAGCATTGGACTGGAT | Fabl | F CCGCGTAAAGGACACAGT |
| R TACTTTCTCTAGCTTTG | R GATCGGACCACAGGAGAT |
| marR | F AGGCATCGTTCGAATG | |
| R TACGTTCACCGGAGATA | |

Table 1. RT-qPCR primer sequences
was not noted after 10 days, we continued to assay the upper limit of resistance. Therefore, at days 10, 15, 20, 25 and 30 TCS concentration in the plates was increased to 0.1, 0.15, 0.2, 0.5 and 1 μg ml⁻¹, respectively.

**Genome sequencing**

DNA was extracted using a PureLink Genomic DNA Extraction kit (Invitrogen) with the standard protocol. A microvolume spectrophotometer (Denovix) was used to quantify the concentration and 260/230 and 260/280 ratios. Initial cultures and resistant mutants of the parent and dam knockout strains were sequenced using Illumina MiSeq chemistry and 2×250 bp paired end reads (MicrobesNG, UK). Raw reads were processed using the Comprehensive Genome Analysis pipeline in PATRIC and variants identified using the Variation Analysis service [26]. The BioProject accession number for the sequences is PRJNA517874.

**RESULTS**

**Dam loss had a significant effect on the generation time of E. coli**

Dam has been linked to alterations in the growth of E. coli [27] and, given this, we initially sought to confirm these findings by assessing the baseline effect of dam on growth and in doing so confirm that alterations post-TCS resistance were not linked to significant differences pre-induction. Both strains were able to grow, confirming the non-essential status of dam in E. coli, but the mean generation time for the dam knockout was significantly higher compared to the parent pre-TCS exposure. The absence of dam was seen to equate to a fitness cost of −7.4% (Table 2). There was an increased generation time for the parental strain, and this is believed to be related to the culture volume (100 μl) and slightly decreased aeration from the shaking of the spectrophotometer.

**Dam knockout induced resistance to TCS and altered global antibiotic resistance patterns**

Since methylation of GATC sites by dam mediates survival of E. coli in the presence of antibiotics [10], we assessed the impact of dam knockout on a range of antibiotics (ampicillin, cephalothin, colistin sulphate, gentamicin, streptomycin, sulphatriad, tetracycline, cotrimoxazole).

Prior to the induction of TCS resistance, and with the exception of streptomycin (Fig. 1b), the resistance profiles of the dam knockout and the parent were not significantly different. Within the parental strain, cross-resistance to cephalothin developed alongside TCS resistance (Fig. 1a). However, within the dam knockout the development of TCS resistance led to increased resistance to streptomycin and gentamicin (Fig. 1b, c), suggesting that the loss of dam may have a role in resistance to aminoglycoside antibiotics. In contrast, the increase in resistance to cotrimoxazole (Fig. 1e) for both the TCS-resistant parent and the dam knockout suggests a non-dam-dependent mechanism of cross-resistance. Additionally, the dam knockout was also more resistant to TCS, with an MIC of 0.9 μg ml⁻¹ compared to 0.4 μg ml⁻¹ for the parent, suggesting a further role for dam in resistance to TCS.

**The dam knockout showed no difference in the mechanism of TCS resistance or in the development of resistance-associated mutations**

Loss of dam has been associated with an increase in mutation rate through the partial induction of the SOS regulon and loss of mismatch repair capability, suggesting the possibility of increased genomic instability [28]. We hypothesized that this increase in mutation rates could result in global antibiotic resistance-associated mutations within the TCS-resistant dam knockout. In order to assess this, we sequenced parental and dam knockout strains pre- and post-TCS exposure (Table 3). Sequencing showed that there were few mutations, with the most significant being a substitution present in the fabI gene, resulting in a change at amino acid 93 (glycine to valine). This mutation has been widely associated with TCS resistance and confers altered binding properties to enoyl-acyl carrier protein reductase (ENR) [29].

In the TCS-resistant dam knockout there was an additional mutation upstream of fabI as well as upstream of 5 s rRNA, and an insertion in lrhA, a transcriptional repressor of the lysR family. Within the resistant parent there were no other mutations commonly associated with broad-spectrum AMR, and TCS resistance was therefore attributed to those mutations seen within fabI. For both the TCS-resistant parent and the dam knockout, these mutations were seen to be highly stable, as neither the parent nor the dam knockout TCS-resistant mutants reverted to sensitivity after 30 days of growth in the presence of TCS at concentrations up to 100 times greater than the pre-TCS resistance MIC.

**There were significant differences in the expression of resistance-associated regulatory genes in the dam knockout**

Several resistance mechanisms are mediated through changes in efflux pump expression, and TCS-associated cross-resistance has been suggested to act via efflux pumps [23, 30]. In order to assess both these observations, we investigated the expression levels of several efflux components. We also looked at the expression levels of genes

| Table 2. Mean generation times and fitness costs associated with dam loss and TCS resistance |
|---------------------------------|-----------------|-----------------|
| Mean generation time/–se min    | Fitness cost relative to parent start/–se % |
| Parent start                    | 66.2/–0.4       | –               |
| TCS-resistant parent            | 81.9/–1.2       | −23.5/–1.9      |
| dam knockout start              | 72.0/–0.6       | −7.4/–1.1       |
| TCS-resistant dam knockout      | 81.5/–2.0       | −22.9/–3.2      |
whose sequences were mutated (Fig. 2). Interestingly, post-resistance induction, we found no significant differences between the parent and dam knockout in expression of acrAB-tolC (Fig. 2a, c and d), or within the multiple-antibiotic resistance protein marA (Fig. 2h), or the transcriptional regulator acrR (Fig. 2e), suggesting that the action of these pathways is not dam-dependent. In contrast, expression levels differed for crd, marR, rpoS, fabI and lrhA (Fig. 2b, f, g, i and j), suggesting that dam affects the regulation of some efflux pump genes and that TCS-associated antibiotic cross-resistance may be more predominant for antibiotics whose mechanisms of resistance are related to efflux by the acrAD-tolC efflux pump, such as some aminoglycosides [31], an observation supported by increased resistance to streptomycin and gentamicin in the TCS-resistant dam knockout. The elevated expression levels of rpoS seen within the dam knockout (Fig. 2g may account for the relatively few mutations seen within the start and the TCS-resistant dam knockout, as rpoS has a protective role in DNA damage due to its ability to upregulate both the SOS response and DNA polymerase Pol II.

**DISCUSSION**

Methylation of the adenine within 5‘-GATC-3‘ sites of double-stranded DNA following replication is a key process within DNA mismatch repair [32], alterations in gene expression [12] and the initiation of chromosome replication [33] and, as such, loss of dam has potentially wide-reaching effects. Here we have shown that the loss of dam results in a significantly decreased mean generation time and that its absence contributes to differences in the ABR profile compared to a wild-type...
parental strain and confers a measure of resistance to the antimicrobial TCS. The increased generation time (Table 2) seen with the dam knockout matches observations of dam-deficient strains of uropathogenic E. coli [34]. This increase is likely a consequence of the role of dam in replication [32, 34]. We also observed a longer lag period in the dam knockout, which may be a consequence of the lack of replication initiation coordination. Whilst a clear fitness cost is seen within the dam knockout (Table 2), this value is significantly less than that for the TCS resistance dam knockout and parent. We also did not detect any inherent mutations, expression values or antibiotic susceptibilities within the starting dam knockout that would indicate that it is inherently unable to develop resistance. A comparison of the ABR profiles (Fig. 1) between the parent and dam knockout strains prior to resistance induction showed that the dam knockout is marginally more resistant to the tested antibiotics than the parent strain, although the difference is only significant for streptomycin. We attribute this increase in resistance to the observed increased expression of rpoS, a general stress response regulator [35], which is significantly higher in the starting dam knockout (Fig. 2g). A recent study has shown that 100 genes are regulated by rpoS in E. coli, including penicillin-binding proteins (PBPs) [36]. rpoS has been associated with antibiotic resistance to β-lactams in E. coli [37] and tolerance to carbapenems in Pseudomonas aeruginosa via regulation of PBP expression [38], and been demonstrated to have a significant effect in single- and double-strand DNA break repair and tolerance [39]. rpoS has also been shown to be protective against the type of DNA damage caused by aminoglycosides in E. coli [40]. This mechanism was not exacerbated by TCS resistance within either the parent or DAM knockout (Fig. 1b), demonstrating that this is not a mechanism of cross-resistance and nor is it DAM-dependent here. The increased rpoS expression may also explain the low number of mutations seen within the TCS-resistant dam knockout, as rpoS has a protective role in DNA damage due to its ability to upregulate the ada response [37]. Cross-resistance to cephalothin was seen within the TCS-resistant parent but not the TCS-resistant knockout, suggesting a role for dam. Broadly speaking, β-lactam resistance occurs via one of two mechanisms, either through the production of β-lactamase, which is most common in Gram-negative species, or via the production of an altered penicillin-binding protein [41]. As neither of these pathways would derive from TCS resistance, we suggest that cephalothin cross-resistance developed from upregulation of marA and the concurrent decrease of marR (Fig. 2f, h), whereby upregulation of the resistance-nodulation-cell division (RND) family efflux systems (acrAB, acrAD, acrEF, mdtEF and mdtABC) results in resistance. Significantly, each of the five listed RND family drug exporters have been shown to confer resistance to β-lactam antibiotics within E. coli [42]. Whilst significant differences in the expression of the acrAB-tolC system were not seen, it is possible that mdtEF and mdtABC expression levels were elevated.

Cross-resistance to tetracycline was increased in the TCS-resistant parent and dam knockout. Within the TCS-resistant parent we saw no mutations within the ribosomal-binding site, or chromosomal mutations leading to increased expression of the intrinsic resistance tetracycline tet-on tet-off system (Table 3), suggesting that the mechanism of resistance is broad-spectrum efflux by an unobserved mechanism such as that highlighted above. Additionally, we observed the development of cross-resistance to cotrimoxazole for both the TCS-resistant parent and dam knockout. In E. coli, cotrimoxazole resistance is primarily via mutations in the target sites of the two composite drugs trimethoprim

| Mutation | Gene | Mutation | Amino acid change | Position | Fraction of sequences |
|----------|------|----------|-------------------|----------|-----------------------|
| TCS-resistant parent | Nonsyn | fabl | 278G>T | Gly93Val | 1345019 | 1 |
| TCS-resistant parent | Nonsyn | tfaD | CAGCGAC>TACGGAT | GlySerAsp2GlyAsnAsp | 577016 | 0.57 |
| Synon | tfaD | 18C>A | Ile6Ile | 577004 | 0.53 |
| Synon | yecE | 54G>T | Gly18Gly | 1945705 | 0.52 |
| Intergenic | tfa-mu1 | AC>G | – | 576968 | 0.52 |
| Intergenic | tfa-mu1 | C>G | – | 576974 | 0.56 |
| Intergenic | tfa-mu1 | GCGGGCC>ACGCGCG | – | 576980 | 0.6 |
| dam knockout start | Intergenic | kgtP-5SrRNA (rrnG operon) | T>C | – | 2719426 | 1 |
| TCS-resistant dam knockout | Nonsyn | fabl | 278G>T | Gly93Val | 1345019 | 1 |
| Intergenic | fabl | G>A | – | 1345411 | 0.51 |
| Synon | pcnB | 243G>A | Val81Val | 155338 | 0.95 |
| Insertion | lrhA | 28_29insACCTCG | Asn10_Leu11insLeuAsp | 2400079 | 0.86 |
| Intergenic | kgtP-5SrRNA (rrnG operon) | T>C | – | 2719426 | 1 |
Fig. 2. Expression of the components of the acrAB-tolC efflux pump was not significantly different in the dam knockout compared to the parent, but expression was increased in both TCS-resistant strains. There were significant differences between the dam knockout and parent pre-resistance induction in rpoS, fabI and marR. In TCS-resistant strains, there were only significant differences in the expression of acrD, lrrA, fabI and marR, although there were significant differences between pre-post-induction levels of marA for both the parent and dam knockout. Expression was calculated from three repeats of three lines (three technical repeats of three biological repeats, n=9), error bars show standard error,* denotes t-test P<0.05.
[dihydrofolate reductase (dfr) and sulphonamides [dihydractone synthase (folP)]] [43, 44]. However, we did not detect any point mutations in either of these genes (Table 3). Increased expression of E. coli acrAB-tolC and mexAB-oprM systems have been shown to confer resistance to sulphonamides. Efflux of sulphonamides would inevitably reduce the overall effectiveness of chlorimoxazole, as sulphonamides and trimethoprim work bactericidally in combination to reduce cellular tetrahydrofolic acid levels. As discussed previously, expression of the acrAB-tolC genes did not increase post-TCS resistance, and resistance may therefore be mediated by an alternative efflux system.

An interesting observation from this work is the identification of the insertion mutation in lhrA in the dam knockout (Table 3). This mutation is also seen in chloramphenicol-resistant dam knockouts (Hughes, et al., unpublished data). This mutation causes an insert frame of leucine and aspartic acid. While the effect of the mutation is unknown, it is predicted to be deleterious by the Protein Variation Effect Analyzer (Provean) [45]. LhrA belongs to the lys family and contains a helix–turn–helix (HTH) DNA-binding domain (amino acids 11–68), which overlaps with the insertion mutation (between amino acids 10 and 11). Mutations in such domains in other HTH-containing DNA-binding proteins have been shown to decrease DNA-binding capability [46, 47]. If the DNA-binding ability of lhrA is decreased as a consequence of this mutation it would no longer be able to repress rpoS to the same extent as the wild-type, which may contribute to the increased rpoS expression seen here. Within the TCS-resistant dam knockout a synonymous mutation was seen in pcnB. While deletion mutations of pcnB have been shown to confer resistance to high concentrations of chloramphenicol, ampicillin and kanamycin, the significance of this mutation, apart from a general contribution to altered fitness costs, is unknown [48].

Marginally increased resistance to TCS was seen in the dam knockout strain compared to the parental strain prior to resistance induction, with MICs of 0.9 μg ml⁻¹ v 0.4 μg ml⁻¹, respectively. Post-TCS resistance, TCS-exposed knockout and parent strains were able to grow at concentrations up to 100 times greater than the initial concentration. TCS acts by disrupting the synthesis of fatty acids by competitive inhibition of ENR. TCS interaction increases the affinity of ENR for nicotinamide adenine dinucleotide (NAD⁺), resulting in the development of the stable ternary complex ENR/NAD/TCS. In this form, ENR is unable to synthesize fatty acids [47]. In the dam knockout, the level of fabI is significantly increased (Fig. 2.1), and this may contribute to a higher tolerance for TCS through the increased availability of ENR. There is an additional mutation (G>A) upstream of fabI, (Table 3); this base is the first site of the fadr-binding site located in the fabI promoter [49]. Fadr is a transcriptional activator of fatty acid synthesis and its loss has been shown to significantly decrease fabI expression [50]. While the functional effect of this mutation is unknown, it may be that it results in increased binding of fadr to the fabI promoter and so contributes to the increased expression seen here. Increased fabI expression is also seen in the resistant dam knockout and parent, with a significantly greater level of expression seen in the dam knockout, which may explain the shorter time to resistance observed here – 3 and 5 days for the dam knockout and parent, respectively. The resistance-associated mutation, Gly93Val, was seen in all sequences for both the resistant parent and dam knockout. This mutation is associated with changes to the protein structure and altered interactions with TCS leading to significant increases in resistance [29]. This is reflected by the ability of the post-resistance induction strains to grow in the presence of a 500-fold greater concentration of TCS (450 μg ml⁻¹).

While dam plays an important role in a range of key physiological processes, and loss of its activity confers a measure of inherent resistance to TCS, the loss of dam does not appear to enhance the development of cross-resistance in most cases, either through an increase in the number of mutations or in the expression level of efflux associated. These findings match the assertion of Cohen et al. [10] that dam provides structural support during exposure to antibiotics. This may, however, depend on the specific mechanism of the agent investigated (e.g. antibiotics whose resistance mechanisms rely on non-acrAB-tolC efflux or antibiotics that target DNA replication, such as quinolones) [10].

Funding information
This work was funded by Leeds Beckett University.

Author contributions
D. J. conceptualized the work and along with W. R. supervised and administered the project. D. J. and L. H. planned, executed and analysed the work. All authors contributed to draft production and writing.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References
1. Ni M, Decrulle AL, Fontaine F, Demarez A, Taddei F et al. Pre-disposition and epigenetics govern variation in bacterial survival upon stress. PLoS Genet 2012;8:e1003148.
2. Adam M, Murabi B, Glenn NO, Potter SS. Epigenetic inheritance based evolution of antibiotic resistance in bacteria. BMC Evol Biol 2008;8:52.
3. Robbins-Manke JL, Zdraveski Z, Marinus M, Essigmann JM. Analysis of global gene expression and double-strand-break formation in DNA adenine methyltransferase- and mismatch repair-deficient Escherichia coli. J Bacteriol 2005;187:7027–7037.
4. Wyrzykowski J, Volkert MR. The Escherichia coli methyl-directed mismatch repair system repairs base pairs containing oxidative lesions. J Bacteriol 2003;185:1701–1704.
5. Raghunathan N, Goswami S, Leela JK, Pandian A, Gowrishankar K. A new role for Escherichia coli dam DNA methylase in prevention of aberrant chromosomal replication. Nucleic Acids Res 2019:47:5698–5711.
6. Messer W, Belieux E, Lother H. Effect of dam methyla- tion on the activity of the E. coli replication origin. OriC. Embo J 1985;4:1327–1332.
7. Roberts D, Hoopes BC, McClure WR, Kleckner N. IS10 transposition is regulated by DNA adenine methylation. Cell 1985;43:117–130.
8. Yin JC, Krebs MP, Reznikoff WS. Effect of dam methylation on Tn5 transposition. J Mol Biol 1988;199:35–45.
9. Westphal LL, Sauvey P, Champion MM, Ehrenreich IM, Finkel SE. Genomewide dam methylation in Escherichia coli during long-term stationary phase. mSystems 2016;1:1.
10. Cohen NR, Ross CA, Jain S, Shapiro RS, Gutierrez A et al. A role for the bacterial GATC methylome in antibiotic stress survival. _Nat Genet_ 2016;48:581–586.

11. X-Z L, Nikaido H. Antimicrobial drug efflux pumps in _Escherichia coli_. In: X-Z Li, Elkins CA, Zgurskaya HI (editors). _Efflux-Mediated Antimicrobial Resistance in Bacteria: Mechanisms, Regulation and Clinical Implications_. Cham: Springer International Publishing; 2016. pp. 219–259.

12. Casadesús J, Low D. Epigenetic gene regulation in the bacterial world. _Microbiol Mol Biol Rev_ 2006;70:830–856.

13. Motta SS, Cluzel P, Aldana M. Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps. _PLoS One_ 2015;10:e011844.

14. Low DA, Weyand NJ, Mahan MJ. Roles of DNA adenine methylation in regulating bacterial gene expression and virulence. _Infect Immun_ 2001;69:7197–7204.

15. Food and Drug Administration, HHS. Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use. Final rule. _Fed Regist_ 2016;81:61106–61130.

16. Triclosan and Antibiotics resistance. What is the biocide triclosan? Internet. [cited 2019 Feb 6]. http://ec.europa.eu/health/scientific_committees/opinions_lyman/triclosan/en/l-3/biocides.htm

17. Braoudaki M, Hilton AC. Adaptive resistance to biocides in _Salmonella enterica_ and _Escherichia coli_ 0157 and cross-resistance to antimicrobial agents. _J Clin Microbiol_ 2004;42:73–78.

18. Carey DE, McNamara PJ. The impact of triclosan on the spread of antibiotic resistance in the environment. _Front Microbiol_ 2014;5:780.

19. Alfhil MI, Lee M-H. Triclosan: an update on biochemical and molecular mechanisms. _Oxid Med Cell Longev_ 2019.

20. Karatzas KAG, Webber MA, Jorgensen F, Woodward MJ, Piddock LJV et al. Prolonged treatment of _Salmonella enterica_ serovar Typhimurium with commercial disinfectants selects for multiple antibiotic resistance, increased efflux and reduced invasiveness. _J Antimicrob Chemother_ 2007;60:947–955.

21. Biroslová L, Mikulášová M. Development of triclosan and antibiotic resistance in _Salmonella enterica_ serovar Typhimurium. _J Med Microbiol_ 2009;58:436–441.

22. Hernández A, Ruiz FM, Romero A, Martínez JL. The binding of triclosan to SME, the repressor of the multidrug efflux pump Smedef, induces antibiotic resistance in _Stenotrophomonas maltophilia_. _PLoS Pathog_ 2011;7:e1002103.

23. Chuanchuen R, Beinlich K, Hoang TT, Becher A, Karkhoff-Schweizer RR et al. Cross-Resistance between triclosan and antibiotics in _Pseudomonas aeruginosa_ is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects nfxB mutants overexpressing MexCD-OprJ. _Antimicrob Agents Chemother_ 2001;45:428–432.

24. Brown DFJ, Wootton M, Howe RA. Antimicrobial susceptibility testing breakpoints and methods from BSAC to EUCAST. _J Antimicrob Chemother_ 2016;71:3–5.

25. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. _Cancer Res_ 2004;64:5245–5250.

26. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T et al. PATRIC, the bacterial bioinformatics database and analysis resource. _Nucleic Acids Res_ 2014;42:D581–591.

27. Kossykh VG, Lloyd RS. A DNA adenine methyltransferase of _Escherichia coli_ that is cell cycle regulated and essential for viability. _J Bacteriol_ 2004;186:2061–2067.

28. Lebner-Olesen A, Skovgaard O, Marinus MG. Dam methylation: coordinating cellular processes. _Curr Opin Microbiol_ 2005;8:154–160.

29. Singh NJ, Shin D, Lee HM, Kim HT, Chang H-J et al. Structural basis of triclosan resistance. _J Struct Biol_ 2011;174:173–179.

30. Khan R, Roy N, Choi K, Lee S-W. Distribution of triclosan-resistant genes in major pathogenic microorganisms revealed by metagenome and genome-wide analysis. _PLoS One_ 2018;13:e019227.

31. Rosenberg EY, Ma D, Nikaido H. Acrd of _Escherichia coli_ is an aminoglycoside efflux pump. _J Bacteriol_ 2000;182:1754–1756.

32. Marinus MG, Lebner-Olesen A. DNA methylation. _EcoSal Plus_ 2014;6.

33. Reisenauer A, Kahng LS, McCollum S, Shapiro L. Bacterial DNA methylation: a cell cycle regulator? _J Bacteriol_ 1999;181:5135–5139.

34. Stephenson SA-M, Brown PD. Epigenetic influence of dam methylation on gene expression and attachment in uropathogenic _Escherichia coli_. _Front Public Health_ 2016;4:131.

35. Huang Y-M, Kan B, Lu Y, Szeto S. The effect of osmotic shock on rpoS expression and antibiotic resistance in _Escherichia coli_. _Journal of Experimental Microbiology and Immunology_ 2009;13:17–13.

36. Dougherty TJ, Pucci MJ. Penicillin-Binding proteins are regulated by rpoS during transitions in growth states of _Escherichia coli_. _Antimicrob Agents Chemother_ 1994;38:205–210.

37. Gutierrez A, Laureti L, Crusard S, Abida H, Rodriguez-Rojas A et al. β-Lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. _Nat Commun_ 2013;4:1610.

38. Murakami K, Ono T, Viducic D, Kayama S, Mori M et al. Role for rpoS gene of _Pseudomonas aeruginosa_ in antibiotic tolerance. _FEBS Microbiol Lett_ 2005;242:161–167.

39. Merrikh H, Ferrazzoli AE, Bougdour A, Olivier-Mason A, Lovett ST. A DNA damage response in _Escherichia coli_ involving the alternative sigma factor, rpoS. _Proc Natl Acad Sci U S A_ 2009;106:611–616.

40. Baharouglu Z, Krin E, Mazel D. Rpos plays a central role in the SOS induction by sub-lethal aminoglycoside concentrations in _Vibrio cholerae_. _PLoS Genet_ 2013;9:e1003421.

41. Worthington RJ, Melander C. Combination approaches to combat multidrug-resistant bacteria. _Trends Biotechnol_ 2013;31:177–184.

42. Nishino K, Honda T, Yamaguchi A. Genome-wide analyses of _Escherichia coli_ gene expression response to the BaeSR two-component regulatory system. _J Bacteriol_ 2005;187:1763–1772.

43. Sköld O. Sulphonamide resistance: mechanisms and trends. _Drug Resist Updat_ 2000;3:155–160.

44. Huovinen P. Resistance to trimethoprim-sulphamethoxazole. _Clin Infect Dis_ 2001;32:1608–1614.

45. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. _PLoS One_ 2012;7:e46688.

46. Hart BR, Mishra PK, Lintner RE, Hinerman JM, Herr AB et al. Recognition of DNA by the helix-turn-helix global regulatory protein LRP is modulated by the amino terminus. _J Bacteriol_ 2011;193:3794–3803.

47. Escalada MG, Harwood J, Maillard JY, Ochs D. Triclosan inhibition of fatty acid synthesis and its effect on growth of _Escherichia coli_ and _Pseudomonas aeruginosa_. _J Antimicrob Chemother_ 2005;55:879–882.

48. Molshanski-Mor S, Yosef I, Kiro R, Edgar R, Manor M et al. Revealing bacterial targets of growth inhibitors encoded by bacteriophage T7. _Proc Natl Acad Sci U S A_ 2014;111:18715–18720.

49. RegulonDB. fab operon and associated TUs in _Escherichia coli_ K-12 genome. [Internet]. [cited 2020 Jan 28]. http://regulondb. cgg.unam.mx/operon?term=ECK120029041&organism=ECK12&format=jsp&type=operon

50. My L, Ghandour Achkar N, Viala JP, Bouveret E. Reassessment of the genetic regulation of fatty acid synthesis in _Escherichia coli_ global positive control by the dual functional regulator FadR. _J Bacteriol_ 2015;197:1862–1872.