*J Antimicrob Chemother* 2012; **67**: 2069–2089
doi:10.1093/jac/dks196 Advance Access publication 22 May 2012

**Journal of Antimicrobial Chemotherapy**

# Bacterial stress responses as determinants of antimicrobial resistance

**Keith Poole***

*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada K7L 3N6*

*Tel: +1-613-533-2458; Fax: +1-613-533-2022; E-mail: poolek@queensu.ca

Bacteria encounter a myriad of stresses in their natural environments, including, for pathogens, their hosts. These stresses elicit a variety of specific and highly regulated adaptive responses that not only protect bacteria from the offending stress, but also manifest changes in the cell that impact innate antimicrobial susceptibility. Thus exposure to nutrient starvation/limitation (nutrient stress), reactive oxygen and nitrogen species (oxidative/nitrosative stress), membrane damage (envelope stress), elevated temperature (heat stress) and ribosome disruption (ribosomal stress) all impact bacterial susceptibility to a variety of antimicrobials through their initiation of stress responses that positively impact recruitment of resistance determinants or promote physiological changes that compromise antimicrobial activity. As de facto determinants of antimicrobial, even multidrug, resistance, stress responses may be worthy of consideration as therapeutic targets.

**Keywords:** oxidative stress, two-component systems, ECF sigma factors, stringent response, stress-induced mutagenesis

## Introduction

It is well known that *in vivo* susceptibility of bacteria to antimicrobials does not always match *in vitro* efficacy[1] and that mutational expression of antimicrobial resistance mechanisms can be selected or mutants expressing them enriched in the absence of antimicrobial exposure,[2,3] indications that environmental conditions can impact bacterial susceptibility to antimicrobials. A significant environmental impact on bacteria is stress, which, in effecting a myriad of adaptive and protective responses, alters gene expression patterns and cell physiology in ways that can and do influence antimicrobial susceptibility. This occurs indirectly, as a result of stress-induced growth cessation or dormancy,[4–6] since antimicrobials typically act on growing cells,[7] or directly as a result of the stress-dependent recruitment of resistance determinants (e.g. antimicrobial efflux),[8] changes to antimicrobial targets,[9–11] amelioration of the adverse consequences of antimicrobial action,[12–14] alterations to the membrane barrier functions,[9,15,16] generation of resistance mutations[17] or promotion of resistant growth modes (biofilms).[18] Moreover, since antimicrobials themselves are growth-inhibiting stressors that often elicit protective responses in bacteria, these agents can provoke their own resistance-promoting responses.[19–21] This review highlights a variety of bacterial stress responses that have been linked to antimicrobial resistance (Table 1), providing support for stress responses themselves being resistance determinants.

## Nutritional stress

Antibiotics generally preferentially kill rapidly replicating bacteria[7] and it has been suggested that reduced growth and metabolic activity associated with non-optimal (e.g. nutrient-limited) growth environments might explain resistance in these instances. Certainly at least some of the resistance attributable to the biofilm mode of growth in *Pseudomonas aeruginosa* arises from established biofilm cells being largely oxygen starved and occurring in an anaerobic stationary phase/non-growing state that renders them resistant to antimicrobials; by comparison, young biofilm cells are more aerobic, are growing and thus are antimicrobial susceptible.[22] Similarly, screening of active and dormant cells within a *P. aeruginosa* biofilm reveals that the latter are less susceptible to the aminoglycoside tobramycin.[23] A contributing factor to the antibiotic tolerance of anoxic *P. aeruginosa* biofilm cells appears to be their reduced metabolic activity—the addition of arginine (a fermentable nutrient) or nitrate/nitrite (to stimulate anaerobic respiration) enhanced antimicrobial killing of mature anaerobic (but not aerobic) biofilm cells.[24] Oxygen limitation also leads to a cessation of replication and the adoption of an antibiotic-tolerant state in *Mycobacterium tuberculosis*,[25] and it has been suggested that reduced growth and metabolic activity of quiescent *M. tuberculosis* infections is responsible for the observed *in vivo* antibiotic tolerance of this organism in the face of effective antibiotic activity *in vitro*.[26] The hypoxia-induced cessation of *M. tuberculosis* growth is an active process and is dependent upon hypoxia-promoted synthesis of triacylglycerol (TAG) by the hypoxia-inducible TAG synthase TgsI—under hypoxia, *tsgI* mutants are antibiotic susceptible.[27] TAG inhibition of *M. tuberculosis* growth results from a reduced TCA cycle flux and attendant decrease in metabolic activity. Interestingly, iron (Fe)-limiting growth conditions also induce TAG synthesis and antibiotic tolerance.[27]

© The Author 2012. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**Table 1.** Stress-inducible antimicrobial resistance mechanisms

| Stress | Resistance mechanism | Stress-responsive regulator[a] | Organism | Reference(s) |
|---|---|---|---|---|
| Oxidative | AcrAB-TolC | SoxRS | *E. coli, S. enterica, K. pneumoniae* | 74–79 |
| | WaaYZ | SoxRS | *E. coli* | 82 |
| | MexXY-OprM | PA5471 | *P. aeruginosa* | 3 |
| | MexAB-OprM | MexR | *P. aeruginosa* | 83,100 |
| | CmeABC[b] | ?[c] | *C. jejuni* | 97 |
| | NorA | MgrA | *S. aureus* | 84 |
| | NorB | MgrA, SarZ | *S. aureus* | 84,85 |
| | Tet38 | MgrA, SarZ | *S. aureus* | 84,85 |
| | bNOS[d] | ?[c] | *B. subtilis* | 117 |
| | $H_2S$[d] | ?[c] | *B. anthracis, S. aureus, P. aeruginosa* | 118 |
| | polyamines | ?[c] | *E. coli* | 13,119 |
| | indole | ?[c] | *E. coli* | 12,112,113 |
| | persisters | ?[c] | *E. coli* | 219 |
| | biofilm | MqsA | *E. coli* | 286 |
| Ribosomal | MexXY-OprM | PA5471 | *P. aeruginosa* | 19 |
| | biofilm | ?[c] | *E. coli* | 284 |
| Nitrosative | MexEF-OprN | MexT | *P. aeruginosa* | 103 |
| Envelope[e] | MexCD-OprJ | AlgU | *P. aeruginosa* | 181 |
| | Pmr/Arn | PhoPQ, ParRS | *S. enterica, K. pneumoniae, P. aeruginosa* | 15,145–147 |
| | drug-induced aberrant polypeptide turnover | AmgRS | *P. aeruginosa* | 138,140 |
| | MprF, DltABCD | GraRS | *S. aureus* | 16,171 |
| | MdtABC | BaeRS | *E. coli* | 142,143 |
| Nutrient limitation/ growth impairment | TAG-mediated decrease in TCA cycle flux and growth | ?[c] | *M. tuberculosis* | 27 |
| | ppGpp-mediated inhibition of peptidoglycan biosynthesis | ?[c] | *E. coli* | 11,33,36 |
| | ppGpp-mediated up-regulation of antioxidant processes | ?[c] | *P. aeruginosa* | 14 |
| | Pmr/Arn | PhoPQ, PmrAB | *S. enterica* (Typhimurium), *P. aeruginosa, K. pneumoniae, Y. pestis* | 15,47,51,53–56 |
| | stress-induced mutagenesis | SOS/LexA[f], RpoS, RpoE | *E. coli, B. subtilis, P. putida, M. tuberculosis* | 17,230–232,251 |
| | biofilm | ?[b] | *P. aeruginosa, S. aureus, L. pneumophila, A. actinomycetemcomitans* | 278–281 |
| | persisters | SOS/LexA[f], TisAB, RpoS | *E. coli* | 5,219,269 |
| | SCVs | SigB | *S. aureus* | 4,270,272 |
| Heat | drug-induced aberrant polypeptide turnover | RpoH, AsrA | *P. aeruginosa* | 208 |
| | PBP2X-mediated thickening of cell wall | ClpL, PBP2X | *K. pneumoniae* | 209 |
| Antibiotic/SOS | QnrB | LexA | Enterobacteriaceae | 254,255 |
| | STX | LexA | *V. cholerae* | 258 |
| | IntI[g] | LexA | *Vibrio* sp., *E. coli* | 259,261 |

[a]Proteins that sense the indicated stress and/or play a role in recruiting the indicated resistance mechanism in response to the stress.
[b]*cmeABC* is peroxide inducible, although the impact of peroxide on antimicrobial resistance in *C. jejuni* has not been assessed.
[c]Regulatory protein/system responsible has not been identified.
[d]A link to environmental stress has yet to be demonstrated.
[e]Known cell envelope stress-inducible antimicrobial resistance mechanisms are highlighted together with their regulatory proteins. A more detailed listing of envelope stress regulators that impact antimicrobial resistance can be found in Table 2.
[f]LexA is a repressor that regulates SOS response genes.
[g]Antibiotic activation of the SOS response stimulates integron-encoded IntI integrase activity promoting recombinational events that lead to integron capture of new antimicrobial resistance genes or expression of integron-resident resistance genes.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

### *Nutrient limitation and the stringent response*

A classic example of nutritional stress is amino acid deprivation, which activates what has long been known as the stringent response.[28] Associated with increased production of the alarmone guanosine 5′-(tri)diphosphate 3′-diphosphate [(p)ppGpp],[29] the stringent response is characterized by reduced expression of genes typically associated with growth and increased expression of survival genes that economize the use of scarce nutrients via a process known as transcriptional switching (i.e. starvation modulation of the housekeeping sigma factor σ-70, such that its activity is diminished in favour of alternative sigma factors).[30] Activated by a variety of nutritional stresses [depletion of Fe, phosphate (Pi), carbon source or fatty acids],[30] the stringent response-mediated increase in ppGpp has a myriad of effects on bacterial cell physiology and, perhaps not surprisingly, impacts antimicrobial susceptibility.[10] For example, it has been known for some time that amino acid deprivation and the stringent response are linked to reduced penicillin susceptibility in *Escherichia coli*—*relA* mutants unable to synthesize ppGpp are more susceptible to penicillin-dependent lysis during amino acid deprivation[31] and the penicillin resistance of a mutant *E. coli* strain was similarly lost in the absence of *relA*.[32] Increases in ppGpp levels provided by the cloned *relA* gene in an otherwise wild-type *E. coli* are also associated with penicillin[33] as well as mecillinam[34] resistance, and the mecillinam resistance of *in vitro*-selected mecillinam-resistant mutants is associated with and dependent upon elevated ppGpp.[34] Fe limitation of *E. coli* has also been shown to increase ppGpp levels and mecillinam resistance.[35] Accumulation of ppGpp inhibits peptidoglycan[11] as well as phospholipid and fatty acid[36] synthesis, and this appears to be central to the ppGpp-promoted reduction in penicillin susceptibility in *E. coli*. Indeed, substrates of the phospholipid biosynthetic enzyme sn-glycerol-3-phosphate acyltransferase (PlsB) accumulate in response to elevated ppGpp, and overexpression of *plsB* overcomes this, relieving the fatty acid/phospholipid synthetic block[36] and restoring the penicillin susceptibility[33] of ppGpp-producing cells. Presumably *plsB* is a ppGpp target and ppGpp-dependent inhibition of phospholipid biosynthesis interferes with membrane-associated steps in peptidoglycan biosynthesis, thereby promoting penicillin resistance. ppGpp inhibition of cell wall synthesis has been described in other bacteria (*Streptomyces coelicolor*, *Bacillus subtilis* and *M. tuberculosis*) where it is also linked to reduced β-lactam susceptibility.[10] ppGpp is also linked to resistance to other antimicrobials—*E. coli* mutants deficient in ppGpp production are more susceptible to trimethoprim, gentamicin and polymyxin B.[37] Increased ppGpp accumulation in mutant *E. coli* is also linked to increased resistance to the peptide antibiotic microcin J25[38] and increased survivability in the presence of fluoroquinolones (FQs).[39] Antimicrobials (mupirocin,[40] vancomycin[40] and penicillin[33]) themselves have been reported to stimulate ppGpp accumulation, but this has not been correlated with any increases in antimicrobial resistance save in *Enterococcus faecalis*, where a mutant unable to synthesize ppGpp showed increased susceptibility to vancomycin.[40] ppGpp production is also linked to vancomycin and bacitracin resistance in *Streptomyces coelicolor*.[41]

Very recently ppGpp and the stringent response have been linked to resistance to several antimicrobials in nutrient-limited and biofilm cells of *P. aeruginosa* and the protective mechanism responsible elucidated.[14] Nguyen *et al*.[14] show that nutrient-limited planktonic and biofilm cells that are defective in the genes for ppGpp production (*relA spoT*) are markedly less resistant to antimicrobials than their wild-type counterparts, with ppGpp-deficient biofilm cells showing increased susceptibility to several classes of antimicrobials, including an aminoglycoside (gentamicin), a β-lactam (meropenem), a cationic antimicrobial peptide (CAP; colistin) and an FQ (ofloxacin). Interestingly, the mechanism of cell killing common to these agents relates to production of hydroxyl radicals (·OH)[42,43] and, indeed, ppGpp-deficient biofilm cells of *P. aeruginosa* showed elevated ·OH formation and enhanced killing by oxidants such as paraquat, indicating that the increased antimicrobial susceptibility of *relA spoT P. aeruginosa* relates to increased oxidative stress.[14] The increase in ·OH/oxidative stress in ppGpp-deficient cells was linked to increased production of 4-hydroxy-2-alkylquinolines (HAQs) previously implicated in intracellular signaling,[44] but also known to be pro-oxidant molecules[45]—deletion of HAQ synthetic genes obviated the increase in ·OH formation in *relA spoT* mutants and restored resistance to antimicrobials.[14] Interestingly, increasing HAQ synthesis in otherwise wild-type cells had no impact on ·OH levels or antimicrobial resistance, indicating that its pro-oxidant effects likely required a deficiency in antioxidant defences. In agreement with this, ppGpp-deficient cells showed reduced levels of catalase and superoxide dismutase activity that was independent of HAQ synthesis.[14] Apparently the stringent response promotes antimicrobial resistance by curtailing HAQ synthesis (thus avoiding its destructive pro-oxidant properties) and increases antioxidant defences, both of which serve to ameliorate the oxidative killing of cells upon exposure to antimicrobials. These results suggest that starved, non-growing cells may be at greater risk from oxidative stress/killing and so take steps to avoid it, and in so doing provide protection from the oxidative killing that is promoted by bactericidal antimicrobials. Of note, ppGpp-deficient *E. coli* also exhibited enhanced ·OH formation and antimicrobial susceptibility relative to wild-type cells,[14] suggesting that the stringent response curtailing of oxidative stress in its promotion of antimicrobial tolerance may be a general feature of, at least, Gram-negative bacteria.

A recent detailed study of the effects of nutrient deprivation on antibiotic resistance in *E. coli* showed that phosphate starvation promoted a transient increase in resistance to the FQ ofloxacin, while amino acid starvation was linked to transient resistance to ofloxacin and ampicillin, and the combination of amino acid and glucose starvation led to prolonged resistance to ofloxacin and ampicillin.[46] While the mechanisms responsible were undefined, it appears that a number of stress responses were involved, with the SOS and stringent responses implicated for certain agents and/or starvation conditions.[46]

### *Nutrient limitation and outer membrane remodelling*

Nutrient limitation in the form of low environmental divalent cation (e.g. $Mg^{2+}$) levels is a well-known and somewhat unique cue for a cellular response that impacts antimicrobial susceptibility in a number of bacteria.[47–49] Perhaps best studied in *Salmonella*, the response to low $Mg^{2+}$ is mediated

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

by the PhoPQ two-component system (TCS), where PhoQ, the sensor kinase, senses the nutrient limitation and activates the PhoP response regulator to up-regulate a variety of target genes that ultimately promote adaptation to this nutrient stress.[15] A subset of PhoP target genes has a role in the modification of lipopolysaccharide (LPS), divalent cations such as $Mg^{2+}$ playing a central stabilizing role in the barrier function of the outer membrane (OM) via an interaction with the LPS constituent of this membrane.[50] Significantly, a number of these PhoPQ-related modifications impact the activity of the CAP family of antimicrobials, which include the polymyxins [polymyxin B (PXB) and colistin] as well as the CAPs of innate immunity.[51] Thus loss-of-function *phoPQ* mutants show enhanced susceptibility to CAPs[47,52] and $Mg^{2+}$ limitation promotes CAP resistance.[47–49] Two of the more significant PhoPQ-stimulated loci from a resistance perspective are *pagP* and *pmrAB*.[51,53] *pagB* encodes a palmitoyltransferase that adds a palmitate chain to lipid A, thereby reducing OM fluidity and CAP entry while *pmrAB* encodes a TCS that controls a locus [*pmrE* (*ugd*)-*pmrHFIJKLM*; also known as *arn*] responsible for the synthesis and addition of 4-amini-arabinose (4AA) to lipid A, with the resulting increase in positive surface charge serving to reduce CAP binding to LPS and thus entry into cells.[51,53] *pmrAB* is also activated by low environmental $Fe^{3+}$ and low pH, providing for 4AA modification of LPS and CAP resistance independently of PhoPQ and low $Mg^{2+}$.[51] The PhoPQ- and PmrAB-controlled phosphoethanolamine (pEtN) transferases encoded by *pmrC* and *cptA*, respectively, also contribute modestly to CAP resistance under low $Mg^{2+}$ conditions, via their modification of LPS core heptose and lipid A, respectively, with positively charged pEtN.[51] Low $Mg^{2+}$-responsive PhoPQ[47,54,55] and PmrAB[47,56] homologues have been described in other bacteria, including *Yersinia pestis*,[54] *P. aeruginosa*[55,56] and *Klebsiella pneumoniae*,[47] where they similarly regulate LPS modification loci and contribute to CAP resistance.[47,54,56] In *P. aeruginosa*, too, there are several reports of *phoQ*[57,58] and *pmrB*[57,59,60] mutations responsible for PXB[57,59] and colistin[58,60] resistance in clinical isolates dependent on Arn-mediated LPS modification.[57,58,60] A *phoP* knockout in *E. coli* increases the susceptibility of the organism to ampicillin,[61] suggesting a link between this $Mg^{2+}$-responsive TCS and β-lactam resistance, although a mechanism was not studied.

CAP (PXB) resistance has also been linked to Pi limitation in *Pseudomonas fluorescens*[62] and *P. aeruginosa*,[63] in parallel with an increase in the levels of a positively charged ornithine lipid (OL) in the membrane. While the overall increase in membrane positive charge might be expected to limit PXB binding, thus explaining resistance, and, indeed, reduced PXB binding to whole cells and isolated membranes was observed for Pi-limited *P. fluorescens*,[62] a study of a *P. aeruginosa* mutant unable to synthesize OL revealed the dispensability of this modification for PXB resistance under Pi-limiting conditions.[63] PXB binding to OMs of Pi-limited *P. aeruginosa* was nonetheless compromised, and this is likely explained by a reduced Pi content of the LPS, producing a less anionic surface macromolecule that binds cationic drugs less well.

Polyamines have also been linked to antimicrobial (quinolones, aminoglycosides and cationic peptides) resistance in *P. aeruginosa*,[64] and recently polyamine-mediated resistance to polycationic antimicrobials (aminoglycosides and CAPs) was shown to result from polyamine binding to OM/LPS, where it protected cells from the OM-disrupting effects of these agents.[65] Significantly, expression of polyamine synthetic genes in *P. aeruginosa* was induced in response to low $Mg^{2+}$ levels and exposure to CAPs,[65] confirming polyamine-mediated antimicrobial resistance as a stress response.

## Oxidative stress

Organisms that grow aerobically are routinely exposed to oxidative stress in the form of reactive oxygen species (ROSs) [e.g. peroxide, superoxide (SO)] that are the unavoidable by-products of aerobic respiration. ROSs damage a variety of cellular macromolecules and thus elicit adaptive oxidative stress responses in bacteria intended to permit survival in the presence of this stressor.[66] Expression of a number of multidrug efflux systems is positively impacted by agents of oxidative stress, these efflux systems possibly playing a role in ameliorating the effects of this stress. Similarly, antioxidant mechanisms are recruited in response to antimicrobial exposure, antimicrobials being known to generate ROSs that are key to the often lethal effects of these agents.[42,67] As such, oxidative stress responses have the potential to contribute to antimicrobial resistance in a variety of ways.

### *Redox-responsive regulators of multidrug efflux system*

SoxRS, originally defined as an SO-responsive TCS that controls an adaptive SO stress response, since it was activated by redox-cycling agents (e.g. paraquat) that generated SO inside aerobically growing cells,[68] is now known to respond directly to these redox-cycling agents.[69] As univalent oxidants, these agents can, however, directly oxidize SoxR (to activate the SoxRS regulon), although their major toxic activities may well have to do with depletion of cellular NADPH, and thus interference with NADPH-requiring biosynthetic processes (i.e. SoxRS may be responding to a form of metabolic stress).[69] Nonetheless, an important component of the protective SoxRS-mediated response to redox-cycling agents is the AcrAB-TolC multidrug efflux system, with *acrAB* expression positively regulated by SoxS[70] in response to redox-cycling agents in *E. coli*[71] and *Salmonella enterica* serovar Typhimurium.[72,73] Not surprisingly, then, mutations leading to constitutive *soxS* expression and elevated *acrAB* expression and antimicrobial resistance have been described in laboratory and/or clinical isolates of *E. coli*[74,75] and *Salmonella* Typhimurium,[76] as well as *S. enterica* serovar Virchow,[77] *S. enterica* serovar Enteritidis[78] and *K. pneumoniae*[79] often as a result of mutations in *soxR*.[74,76,77,79] SoxS also controls expression of the siRNA *micF*, whose expression reduces OmpF translation, with reduction of this OM porin serving to promote antimicrobial resistance by limiting antimicrobial uptake.[70,80] Although few studies have examined the impact of SoxRS-responsive stresses on antimicrobial resistance, paraquat has been shown to enhance resistance of *E. coli* to enoxacin, dependent upon SoxRS,[81] although the contribution of AcrAB-TolC to this was not examined.

Intriguingly, redox-cycling agents have also been shown to induce SoxRS-dependent expression of the LPS core biosynthetic locus *waaYZ* in *E. coli*, where WaaY functions in the phosphorylation of core heptose residues.[82] *waaYZ* mutants are more susceptible to several antimicrobials, including norfloxacin, certain

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

macrolides and β-lactams, likely because anionic Pi strengthens the OM permeability barrier by facilitating divalent cation-mediated cross-bridging of LPS molecules, and its loss in the mutant compromises the OM barrier.[82] SoxRS thus appears to regulate LPS modification in response to redox-cycling agents, one that is intended to protect cells from these agents and the stresses they impart, and in so doing enhances resistance to other antimicrobials.

A number of additional regulators of multidrug efflux systems respond to oxidative stress, including MexR, the repressor of the *mexAB-oprM* multidrug efflux operon of *P. aeruginosa*;[83] MgrA, the global regulator in *S. aureus* that regulates >300 genes, including the major facilitator (MF) family antimicrobial efflux genes *norA*, *norB* and *tet38*;[84] and SarZ, an MgrA functional homologue that regulates several genes in *S. aureus*, including the *norB* and *tet38* efflux genes.[85] In the case of MexR, *in vitro* oxidation of the protein with peroxide or cumene hydroperoxide caused the protein to dissociate from its target *mexAB-oprM* promoter DNA, thus *in vivo* exposure of *P. aeruginosa* to these ROSs produced a modest increase in *mexAB-oprM* expression.[83] Still, there was no indication that oxidative stress enhanced antimicrobial resistance in *P. aeruginosa*,[83] and transcriptomic studies of *P. aeruginosa* genes responsive to oxidative stress did not identify *mexAB-oprM* as being significantly (>2-fold) ROS inducible.[86,87] The MgrA protein, like MexR, is sensitive to thiol-based oxidation (by peroxide or organic hydroperoxide), which also causes its dissociation from target DNA.[84] MgrA negatively regulates *norA*, encoding an FQ-exporting multidrug transporter,[88] *norB*, which encodes a multidrug transporter linked to norfloxacin and biocide resistance,[89] and *tet38*, which encodes a tetracycline exporter,[89] and an *mgrA* mutant shows elevated resistance to norfloxacin, biocides and tetracycline.[89] Thus oxidative stress might be expected to enhance efflux gene expression, and thus multidrug resistance in *S. aureus*, although this has yet to be tested. Low pH has also been shown to promote an MgrA-dependent increase in *norB* expression, leading to reduced susceptibility to norfloxacin.[90] Like MexR and MgrA, SarZ is sensitive to thiol-based oxidation, its binding to target DNA also being compromised upon oxidation.[85] While it negatively regulates known determinants of antimicrobial resistance such as *norB* and *tet38*, and a *sarZ* mutant shows reduced susceptibility to several antimicrobials,[85] the target genes whose derepression is responsible for this have not been confirmed and no evidence has been presented for oxidative stress promoting a SarZ-dependent increase in antimicrobial resistance.

## *ROS-mediated induction of multidrug efflux*

Multidrug efflux systems of the Resistance-Nodulation-Division (RND) family, which are major determinants of intrinsic and acquired antimicrobial resistance in Gram-negative bacteria, are increasingly recognized as components of bacterial stress responses, including oxidative and nitrosative stress responses.[8] The *mexXY* genes of the *P. aeruginosa* MexXY-OprM multidrug efflux system that accommodates various antimicrobials (e.g. FQs, β-lactams, macrolides, tetracycline and aminoglycosides) are induced by oxidative stress,[3] mediated by the oxidative stress-inducible[86,87] PA5471 gene product.[3] This efflux system is commonly associated with aminoglycoside resistance in clinical isolates, particularly cystic fibrosis (CF) lung isolates, where it is the primary determinant of aminoglycoside resistance.[91] The latter is an intriguing observation given that the CF lung is known to be rich in ROSs,[92] which may thus be promoting the development of PA5471-/MexXY-mediated aminoglycoside resistance in CF lung isolates. In agreement with this, *in vitro* exposure of *P. aeruginosa* to peroxide enhanced the frequency with which MexXY-dependent aminoglycoside-resistant mutants could be recovered,[3] in this way demonstrating a positive association between oxidative stress and antimicrobial resistance. Intriguingly, the *mexXY* operon is also induced by antimicrobials that target the ribosome (e.g. tetracycline, chloramphenicol, erythromycin, aminoglycosides),[19] as a result of their disruption of the ribosome,[93] and this is similarly mediated by the PA5471 gene product, which is also induced by these agents.[19] Consistent with translation disruption being the trigger for induction of PA5471 and *mexXY*, mutations that disrupt protein synthesis (*fmt*, *folD*) increase expression of PA5471 and *mexXY*.[94] In reconciling *mexXY* induction by both oxidative stress and ribosome disruption, one possibility is that oxidative stress also disrupts ribosome function or in some way perturbs translation. In this vein, ROSs have been shown to reduce translational fidelity in *E. coli* by interfering with the editing activity of an aminoacyl-tRNA synthetase.[95] Alternatively, since ribosome disruption via mutation or antibiotic exposure leads to the production of aberrant polypeptides that are prone to oxidative modification in *E. coli*,[96] it may be that oxidatively modified/damaged proteins are the common trigger for PA5471 and *mexXY* induction, with MexXY-OprM possibly playing some role in ridding the cells of these damaged/aberrant polypeptides. The RND family *cmeABC* multidrug efflux operon of *Campylobacter jejuni* was also shown to be peroxide inducible, although the influence of ROS on CmeABC-mediated antimicrobial resistance was not examined.[97]

The *mexAB-oprM* locus, the major efflux determinant of intrinsic and acquired resistance to a variety of clinically relevant antimicrobials, particularly FQs and β-lactams, in lab and clinical isolates of *P. aeruginosa*, is also stress inducible—by chlorinated phenols such as pentachlorophenol (PCP).[98,99] While PCP is a known uncoupler of oxidative phosphorylation, and thus will adversely impact energy production in the cell, the observation that PCP is a MexAB-OprM substrate[98] and is recognized by a regulator of *mexAB-oprM* expression, NalC,[99] suggests that it likely resembles a natural substrate for this efflux system. Still, PCP induction of *mexAB-oprM* can occur independently of NalC, apparently mediated by MexR, although this latter *mexAB-oprM* regulator does not bind PCP.[100] One possibility is that PCP promotes oxidative stress that impacts the redox-sensitive MexR[83] directly. PCP has, for example, been shown to dramatically increase $O_2$ flux in *P. aeruginosa*, generating an oxidative stress that could impact MexR activity.[101]

## *Reactive nitrogen species-mediated induction of multidrug efflux*

The *P. aeruginosa mexEF-oprN* multidrug efflux locus linked to FQ, trimethoprim and chloramphenicol resistance[102] is also stress inducible, expression of this otherwise quiescent operon being activated in response to nitrosative stress [e.g. in the presence of nitrosating or nitric oxide (NO)-generating agents].[103] This induction is dependent upon the MexT transcriptional activator[104,105] previously shown to be required for *mexEF-oprN* expression in

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

multidrug-resistant *nfxC*[106] and *mexS*[107] mutants.[103] Moreover, several of the MexT targets identified in an array study of the MexT regulon[108] were also shown to be induced in response to nitrosative stress,[103] suggesting that MexT controls a regulon with some function in a nitrosative stress response. Interestingly, chloramphenicol but not the related florfenicol, which lacks a nitro group, induces *mexEF-oprN* expression, again dependent upon MexT.[103] This highlights the importance of the nitro moiety of chloramphenicol for this induction, an interesting observation given that some common products of nitrosative stress in bacteria are nitrated amino acids (i.e. chloramphenicol may resemble a nitrated nitrosative stress product that is an intended signal for MexT and substrate for MexEF-OprN). The observation that XenB, implicated in removal of nitro groups from nitroglycerine and trinitrotoluene in *Pseudomonas*,[109,110] is co-regulated with *mexEF-oprN* by nitrosative stress is consistent with these playing a role in 'detoxifying' nitrated products of nitrosative stress.

### *Antibiotic-dependent oxidative stress-protective mechanisms*

Given the observation that ROSs (i.e. ·OH) and the oxidative stress that results from antimicrobial exposure are generally associated with the lethal effects of bactericidal antimicrobials,[42,43,67] it stands to reason that antioxidant/oxidative stress-protective responses in bacteria would promote antimicrobial resistance. Certainly elimination of ROS defence genes in *E. coli* has been correlated with increased antimicrobial susceptibility.[111] A possible oxidative stress protective mechanism that promotes antimicrobial resistance in *E. coli* involves indole, an extracellular signalling molecule whose production is promoted by exposure to antimicrobials[12] and, possibly, oxidative stress—ROSs induce the *tnaA* tryptophanase gene whose product catalyses the synthesis of indole.[112] Interestingly, addition of the ·OH-scavenging compound DMSO reduces antimicrobial-dependent indole production,[113] consistent with indole being inducible by the ROSs formed as a result of antimicrobial exposure and providing an explanation for antimicrobial induction of indole production. Most importantly, endogenous indole production as well as exogenous indole addition promotes antimicrobial resistance.[12] Indole production by highly antibiotic-resistant strains of *E. coli* has also been shown to promote resistance in otherwise susceptible members of a heterogeneous population.[114] Its promotion of resistance in the latter study was apparently due to its up-regulation of resistance mechanisms that included, interestingly, oxidative stress-protective mechanisms.[114] Indole has also been shown to enhance the antimicrobial resistance of *P. aeruginosa*.[115]

Despite the known toxicity of NO for bacteria, endogenous NO production by bacteria is known, catalysed by bacterial nitric oxide synthases (bNOSs), where it protects bacteria (*B. subtilis*) from the damaging effects of ROSs.[116] bNOSs have also been shown to protect *Bacillus* sp. against a variety of antimicrobials—mutants lacking bNOS activity show enhanced antimicrobial susceptibility[117]—in part by alleviating the lethal effects of oxidative stress that result from antimicrobial exposure.[42,43,67] While bNOS gene inducibility by oxidative stress in *Bacillus* has not been examined and antibiotic exposure fails to induce bNOS gene expression, preliminary evidence suggests that the β-lactam antimicrobial cefuroxime stimulates bNOS activity in *B. subtilis*.[117] Thus environmental stress conditions can impact NO production, and thus antimicrobial resistance in this organism. Bacterial production of another gas, $H_2S$, has also been linked recently to antimicrobial resistance—deletion of genes for $H_2S$ synthesis in *Bacillus anthracis*, *S. aureus*, *P. aeruginosa* and *E. coli* rendered cells susceptible to multiple antimicrobials.[118] Again, the protective effect of $H_2S$ was due, in part, to its mitigation of antibiotic-dependent oxidative stress-promoted lethal damage to these organisms.[118] Regulation of $H_2S$ production and/or activity/expression of $H_2S$ synthetic proteins/genes was not assessed, so it is not yet clear whether this antimicrobial resistance mechanism will be impacted by environmental growth (i.e. stress) conditions. Antioxidant strategies are, nonetheless, clearly important for antimicrobial resistance in bacteria, and thus environmental conditions that activate protective antioxidant genes/enzymes will promote antimicrobial resistance.

Polyamines also contribute to resistance to ROSs in *E. coli*, both in functioning as antioxidants[119] and in promoting expression of antioxidant/oxidative stress-protective genes.[119–121] Recently a modest positive impact of antibiotics on polyamine production was seen in *E. coli*, and this appeared to alleviate, to some extent, antimicrobial-dependent ROS production and render cells less susceptible to antimicrobials.[13]

A recent report of *in vitro*-selected FQ-resistant *Proteus mirabilis* lacking prototypical target site mutations in *gyrA*, *gyrB* or *parC* but showing an antioxidant phenotype (reduced lipid and protein oxidation compared with a wild-type strain)[122] highlights a possible link between oxidative stress and FQ resistance in this organism. One possibility is that an antioxidant phenotype provides for protection against the FQ-dependent oxidative damage that is key to the lethal activity of these agents.

## Envelope stress

Environmental affronts to cell envelope structure and function are met with adaptive responses in bacteria intended to permit survival in the face of that affront. These so-called envelope stress responses are highly regulated, typically by alternate sigma factors referred to as extracytoplasmic function (ECF) sigmas and TCSs (Table 2), and there are often multiple response pathways in a given microorganism. In *E. coli*, for example, there are envelope stress response systems controlled by the sigma factor RpoE ($\sigma^E$)[123] and the TCSs CpxRA[124] and BaeSR,[125] each responsive to its own set of specific envelope 'stressors' (Table 2). Similarly, *B. subtilis* boasts four sigma factors (SigB, SigM, SigW and SigX) and at least two TCSs (BseRS and LiaRS) that respond to envelope stress, with unique and common stress 'triggers' amongst the six (Table 2). Given that many antimicrobials either target components of the cell envelope or must overcome the barrier provided by the cell envelope to gain access to targets within the cell, it is perhaps not surprising that changes manifested by envelope stress responses in the face of envelope stress can impact antimicrobial susceptibility.

### *TCSs: Gram-negative bacteria*

A variety of TCSs respond to envelope stress in Gram-negative bacteria, each reacting to different stressors and thus different physiological triggers that in many cases remain unknown. The

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**Table 2.** Cell envelope stress response regulators linked to antimicrobial resistance

| Regulator(s) | Activators[a] | Organism | Antimicrobial[b] | Reference(s) |
|---|---|---|---|---|
| Sigma factors | | | | |
| SigB ($\sigma^B$) | stationary phase, high salt, heat, ethanol, low temperature, acid pH, nitrosative stress, cell wall-active agents, nutrient starvation, energy stress | *B. subtilis* | rifampicin | 196 |
| | | *S. aureus* | β-lactams, CAPs, glycopeptides, pine oil cleaner | 197–202 |
| | | *L. monocytogenes* | biocides, tetracycline, gentamicin, β-lactams | 203–205 |
| SigM ($\sigma^M$) | cell wall/envelope-active agents, toxic peptides, high salt, ethanol | *B. subtilis* | moenomycin, ampicillin, bacitracin | 186–188 |
| SigW ($\sigma^W$) | cell envelope-active agents, alkaline shock | *B. subtilis* | fosfomycin, ampicillin, vancomycin | 188,189 |
| SigX ($\sigma^X$) | cell wall-active agents, tunicamycin, high temperature | *B. subtilis* | bacitracin, ampicillin, CAPs, nisin | 188,190,191 |
| RpoE ($\sigma^E$) | heat, ethanol, misfolded membrane proteins, abnormal LPS | *E. coli* | CAPs | 123 |
| | | *S. enterica* (Typhimurium) | CAPs | 178 |
| | | *C. glutamicum* | nalidixic acid, penicillin, vancomycin | 179 |
| | | *S. coelicolor* | vancomycin, bacitracin | 41 |
| AlgU (RpoE) | membrane-damaging solvents, detergents, antimicrobials | *P. aeruginosa* | chlorhexidine | 181 |
| Two-component systems | | | | |
| AmgRS | aminoglycoside-mediated membrane damage | *P. aeruginosa* | aminoglycosides | 138 |
| BaeSR | indole, spheroplasting, pilin overproduction, Na-tungstate, flavanoids | *E. coli* | novobiocin | 125,141–143 |
| BceRS | bacitracin | *B. subtilis* | bacitracin | 289 |
| | | *S. aureus* | bacitracin | 152 |
| | | *S. mutans* | bacitracin | 153 |
| BraRS | cell wall-damaging agents (bacitracin, nisin) | *S. aureus* | bacitracin, nisin | 154 |
| CpxRA | alkaline pH, high osmolarity, surface sensing, misfolded cell envelope proteins, overproduction of envelope proteins (e.g. NlpE) | *E. coli* | CAPs, β-lactams, aminoglycosides | 124,128–131,133 |
| | | *S. enterica* (Typhimurium) | CAPs, β-lactams | 129,132 |
| CroRS | cell wall-active agents (β-lactams, fosfomycin, glycopeptides, bacitracin, cycloserine) | *E. faecalis* | ceftriaxone | 176 |
| EnvZ/OmpR | osmotic pressure | *E. coli* | β-lactams | 133,134,136 |
| | | *S. enterica* (Typhimurium) | β-lactams | 132 |
| GraRS/Aps | CAPs | *S. aureus* | CAPs (including PXB), vancomycin | 16,166,173,174 |
| LiaFRS[c] | cell wall-active agents (e.g. lipid II cycle-interfering antibiotics, including vancomycin, bacitracin, nisin) | *E. faecalis* | daptomycin | 158 |
| | | *S. mutans* | bacitracin, vancomycin, chlorhexidine | 157 |
| NsaRS | cell envelope-active agents (fosfomycin, β-lactams, nisin, gramicidin, CCCP) | firmicutes (*Bacillus*, *Streptococcus*, *Staphylococcus, Listeria, Enterococcus*) | bacitracin | 170 |
| | | *S. aureus* | nisin | 171 |
| ParRS | CAPs | *P. aeruginosa* | CAPs (including PXB and colistin), aminoglycosides | 146,147 |
| PhoPQ | CAPs, low $Mg^{2+}$ | *S. enterica* | CAPs (including PXB) | 15 |
| | | *K. pneumoniae* | CAPs (including PXB) | 47,145 |
| | | *P. aeruginosa* | CAPs (including PXB) | 56 |
| | | *Y. pestis* | CAPs (including PXB) | 54 |

*Continued*



Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**Table 2.** *Continued*

| Regulator(s) | Activators[a] | Organism | Antimicrobial[b] | Reference(s) |
|---|---|---|---|---|
| RcsCDB | cell wall-active agents (β-lactams), CAPs (polymyxin B), low temperature, desiccation | *E. coli*<br>*S. enterica* (Typhimurium) | β-lactams<br>PXB | 156<br>151 |
| VsaRS[d] | cell wall/envelope-active agents (β-lactams, glycopeptides, bacitracin, cycloserine, CAPs) | *S. aureus* | vancomycin, teicoplanin, bacitracin, fosfomycin, β-lactams, CAPs | 159–168 |

[a]Stressors that activate sigma factors or TCSs whose activities have been linked to antimicrobial resistance.
[b]Resistance to these antimicrobials has been linked to the indicated stress response regulators.
[c]Originally described in *B. subtilis* as a two-component system, LiaRS, which responded to alkaline shock, organic solvents, detergents, CAPs and cell wall-active agents (vancomycin, bacitracin and nisin).[155,156]
[d]Homologue of LiaRS.

link to antimicrobial resistance comes from the observed impact of the stress and of TCS mutations on resistance.

### *CpxRA*

CpxRA generally responds to stresses that adversely impact cell envelope protein folding, and thus plays a role in envelope maintenance.[126] Triggering the *E. coli* CpxRA-dependent response promotes reduced susceptibility to β-lactams,[127] aminoglycosides,[127,128] novobiocin[127] and CAPs,[129] in part as a result of CpxRA-dependent up-regulation of multidrug efflux[127,130] and peptidoglycan amidase[129] genes. Mutations have also been identified in *cpxA* that are responsible for modest resistance to aminoglycosides[131] and β-lactams,[132] and cloned *cpxR* promotes modest resistance to aminoglycosides (kanamycin)[130] and β-lactams.[133] Interestingly, CpxRA also regulates expression of the major porin genes *ompF* and *ompC* in a manner reminiscent of EnvZ-OmpR-mediated osmoregulatory control of these genes[134] (see below)—activation of the CpxRA pathway increases expression of *ompC* at the expense of *ompF*.[135] OmpF is a major portal for entry of antimicrobials such as β-lactams (OmpC forms a smaller channel) and reduced *ompF* expression has been linked to β-lactam resistance.[136] Whether this explains CpxRA-promoted β-lactam resistance is, however, unclear. Paradoxically, CpxRA is activated by aminoglycosides and plays a role in aminoglycoside killing, being required for the generation of toxic ·OH that is formed upon membrane disruption by aminoglycoside-generated aberrant polypeptides and is responsible for killing of aminoglycoside-treated cells.[43] As such, *cpxRA* mutants show transiently reduced susceptibility to aminoglycosides.[43] Interestingly, however, ROS generation in aminoglycoside-exposed cells has also been linked to antimicrobial resistance development as a result of ROS-promoted mutation.[137]

### *AmgRS*

The *amgRS* locus in *P. aeruginosa* encodes a homologue of the *E. coli* OmpR-EnvZ TCS and was first identified in a screen of transposon-insertion mutants susceptible to aminoglycosides.[138] Unlike OmpR-EnvZ, however, AmgRS regulates a number of membrane transporter and protease genes more reminiscent of the *E. coli* CpxRA envelope stress response and does so in response to aminoglycoside exposure.[138] It has been suggested that AmgRS responds to envelope stress mediated by aberrant polypeptides that are proposed to accumulate upon aminoglycoside treatment,[43] with the AmgRS-controlled stress response functioning to protect cells from aberrant peptide-mediated membrane damage.[138,139] In support of this, a number of AmgRS-regulated protease and other genes have been shown to contribute to aminoglycoside resistance, their loss in mutants comprising resistance.[140] The observation that an *amgRS* mutant is more sensitive to alkaline and salt stress, and more susceptible to β-lactams, suggests that, like *E. coli* CpxRA, it may be a general envelope stress response regulator in *P. aeruginosa*.[140]

### *BaeSR*

The BaeSR envelope stress-triggered TCS in *E. coli*[125] is induced by possible membrane stressors sodium tungstate and plant flavanoids[141] and promotes resistance to these[141] as well as bile salts and novobiocin,[142,143] dependent upon the MdtABCD multidrug efflux system whose expression is positively regulated by BaeSR.[1,125,141–143]

### *PhoPQ*

Membrane-disrupting CAPs, including PXB and proteins of innate immunity, also trigger stress responses whose outcomes are a strengthening of membranes and, in the case of Gram-negative organisms, modification of the LPS to prevent CAP binding,[9] LPS being the initial site of CAP interaction with cells.[144] In *S. enterica*[15] and *K. pneumoniae*[145] this is mediated by the PhoPQ TCS that also responds to growth-limiting $Mg^{2+}$ and involves the same set of LPS modification genes (see above). In *P. aeruginosa*, CAP-promoted LPS modification is mediated by the ParRS TCS and not by PhoPQ.[146,147] In all cases, however, subinhibitory CAP exposure ultimately promotes LPS modification and resistance to CAPs, with these TCSs thus mediating inducible CAP resistance.[145,146] ParRS also mediates CAP induction of the *mexXY* multidrug efflux operon that is linked to aminoglycoside resistance in *P. aeruginosa*.[146]



Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

### Rcs phosphorelay system

Phosphorelay systems, more complex versions of TCSs that include additional components beyond the sensor kinase and response regulator,[148] have also been linked to envelope stress and antimicrobial resistance. The RcsCDB/F phosphorelay system that is widely distributed in the Enterobacteriaceae and was originally implicated in the regulation of capsular synthesis in *E. coli* is one such system, being activated by envelope stress (high osmolarity, desiccation, low temperature, high $Zn^{2+}$),[149] PXB[150] and peptidoglycan-disrupting β-lactams (e.g. cefsulodin and methicillin).[150] Interestingly, *rcsB* deletion mutants are β-lactam susceptible, suggesting that this system may control a global protective response to cell wall stress.[150] Consistent with this, mutational activation of the Rcs relay enhances β-lactam resistance independent of capsule synthesis.[150] The Rcs relay has also been linked to PXB resistance in *Salmonella* Typhimurium.[151]

## TCSs: Gram-positive bacteria

A number of TCSs responding to cell envelope stressors have been reported in Gram-positive bacteria, many of which have been linked to antimicrobial resistance. In most instances, however, this link comes from observations of enhanced antimicrobial susceptibility of mutants defective in these systems and not through any demonstration that they mediate envelope stress-dependent enhancement of resistance. Exceptions to this include the BceRS TCS found in *S. aureus*[152] and *Streptococcus mutans*[153] and the BraRS TCS also of *S. aureus*,[154] which respond to the envelope-stressing antimicrobials bacitracin[152,153] and bacitracin and nisin,[154] respectively. In both cases, however, the stress-responsive TCSs activate genes that promote resistance to these agents specifically, with BraRS activating genes encoding an efflux transporter responsible for detoxifying bacitracin and nisin.[154] Thus these TCSs may not be envelope stress responsive per se.

### LiaRS

LiaRS is a cell envelope stress responsive TCS first described in *B. subtilis*, where it is activated by exposure to alkaline shock, organic solvents, detergents and lipid II (peptidoglycan intermediate) cycle inhibitors (vancomycin, bacitracin, nisin) and CAPs.[155,156] This system, which functions as a three-component LiaFRS system in *S. mutans* and is similarly cell envelope stress inducible in this organism, has been linked to bacitracin, vancomycin and chlorhexidine resistance—LiaFRS$^{-}$ mutants are susceptible to these agents.[157] Moreover, a mutation in LiaF has been shown to contribute to daptomycin resistance in *E. faecalis*.[158] The LiaRS homologue in *S. aureus*, VraSR, is also induced by cell wall-active antibiotics (β-lactams, glycopeptides, bacitracin, cycloserine)[159,160] and CAPs,[161] and controls many cell wall genes.[159] Its inactivation increases β-lactam,[159,160,162] glycopeptide (vancomycin and teicoplanin),[160,162] bacitracin[160] and fosfomycin[160] susceptibility in methicillin-resistant *S. aureus* (MRSA) strains and CAPs, bacitracin, teicoplanin and β-lactam susceptibility in a type strain of this organism.[161] Up-regulation of the VraSR-dependent cell wall stress stimulon is also linked to intermediate vancomycin resistance in clinical isolates of *S. aureus* [vancomycin intermediate *S. aureus* (VISA)].[163] Mutations in *vraRS* are commonly seen in *S. aureus* showing intermediate resistance to teicoplanin[164,165] and are responsible for intermediate vancomycin resistance in some VISA isolates,[166–168] presumably as a result of activation of the VraRS stimulon. Finally, a transcriptomic study of *Listeria monocytogenes* highlighted the roles of several TCSs, including LiaRS, in this organism's cell envelope stress response to the β-lactam cefuroxime, with many TCS-regulated genes implicated in cell envelope functions or resistance to cefuroxime and other cell envelope stressors.[169]

### NsaRS

NsaRS is a TCS in the firmicute group of Gram-positive bacteria (e.g. *Bacillus*, *Staphylococcus*, *Streptococcus*, *Listeria*, *Enterococcus*) that modulates cell envelope stability by sensing disruptions in this structure.[170] In *S. aureus*, for example, it is activated by cell envelope-damaging antimicrobials such as fosfomycin, ampicillin, nisin, gramicidin, carbonyl cyanide m-chlorophenylhydrazone (CCCP) and penicillin, and regulates a variety of genes, including those associated with cell wall synthesis.[170] While an *nsaS* mutant showed a 200-fold reduced ability to develop resistance to bacitracin, suggesting this TCS might contribute to antimicrobial resistance, the drug susceptibility of this mutant was unaltered relative to wild-type.[170] However, *nsaS* mutations were found in nisin-resistant *S. aureus*, suggesting that NsaRS-regulated genes are linked to resistance to this agent.[171]

### GraRS

GraRS (also known as Aps) is yet another envelope stress-responsive TCS in *S. aureus*, one that responds to CAPs, including PXB,[172–174] and that has been linked to vancomycin[166,173] and CAP[173,174] resistance in this organism. Thus *graR* knockout mutants are hypersusceptible to vancomycin and PXB, and more susceptible to some anti-staphylococcal CAPs.[166,173,174] Similarly, overexpression of *graRS* is seen in clinical VISA strains, with inactivation of this locus increasing susceptibility to vancomycin and PXB.[16] GraRS-promoted resistance appears to result from an increased net positive surface charge as a result of its induction of genes associated with production of lysyl-phosphatidyl glycine (L-PG) (*mprF*)[16,173] and addition of D-Ala to teichoic acids (*dltABCD*),[16] the increased L-PG content of membranes and masking of anionic teichoic acids with D-Ala serving to reduce the binding of cationic agents such as vancomycin and PXB.[16,174] Increased L-PG incorporation as a result of a gain-of-function mutation in *mprF* has also been linked in part to daptomycin resistance in *S. aureus*.[175]

### CroRS

The *croRS* genes encode a second envelope stress-responsive TCS in *E. faecalis*, one that is inducible by β-lactams and other peptidoglycan inhibitors (fosfomycin, cycloserine, moenomycin, ramoplanin, vancomycin and bacitracin).[176] A *croRS* mutant exhibited a 4000-fold increase in susceptibility to ceftriaxone, a β-lactam for which resistance in *E. faecalis* is linked to production of penicillin-binding protein (PBP) 5.[176] Interestingly, the *pbp5* gene was expressed in the mutant, and a plasmid bearing the *pbp5* gene failed to restore the mutant's ceftriaxone

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

resistance,[176] indicating that some as yet unknown CroRS-controlled gene mediates PBP5-dependent ceftriaxone resistance in this organism.

### *Sigma factors: Gram-negative bacteria*

The major cell envelope stress response sigma factor in *E. coli* and other Gram-negative bacteria, $\sigma^E$, has been linked in a limited way to antimicrobial resistance. In *E. coli*, $\sigma^E$ has been shown to negatively control the PhoPQ TCS that promotes resistance to CAPs as a result of LPS modification, providing a link between envelope stress and LPS modification/CAP resistance in this organism.[177] $\sigma^E$ also plays a role in resistance to CAPs in *Salmonella* Typhimurium,[178] to vancomycin and bacitracin in *S. coelicolor*[41] and to nalidixic acid, penicillin and vancomycin in *Corynebacterium glutamicum*.[179] Perhaps the clearest link between envelope stress, $\sigma^E$ and antimicrobial resistance involves the *mexCD-oprJ* multidrug efflux operon of *P. aeruginosa*. Originally shown to be induced by a variety of membrane-active biocides (chlorhexidine and benzalkonium chloride) and dyes (ethidium bromide and rhodamine),[180] *mexCD-oprJ* was more recently shown to be inducible by a variety of membrane-damaging agents (MDAs), including detergents, organic solvents, biocides and CAPs.[181] Consistent with envelope stress being a signal for MexCD-OprJ recruitment, this induction was mediated by the *algU*-encoded envelope stress response sigma factor,[181] a functionally equivalent homologue of *E. coli* RpoE[182] that was first described as a regulator of alginate biosynthesis in *P. aeruginosa*.[183,184] While MexCD-OprJ would appear to be a component of an envelope stress response in this organism, its role in this is unclear. The exometabolome of an *nfxB* mutant hyperexpressing MexCD-OprJ does, however, show elevated levels of long-chain fatty acids, which have been proposed as possible MexCD-OprJ substrates.[185] Perhaps this efflux system plays a role in fatty acid export as part of a system for exchanging these components of membrane lipids as the cell responds to envelope stress and restructures its membranes accordingly. How AlgU responds to MDAs in promoting *mexCD-oprJ* expression is as yet unknown.

### *Sigma factors: Gram-positive bacteria*

No fewer than four envelope stress response sigma factors have been described in the Gram-positive bacterium *B. subtilis* (SigB, SigM, SigW and SigX), with homologues often present in other Gram-positive organisms. These have been linked in a limited way to resistance to β-lactams and other cell-wall active agents. *B. subtilis* SigM is activated by cell envelope stress elicited by antibiotics and by heat, acid, ethanol and superoxide stress,[186] and *sigM* mutants are more susceptible to bacitracin,[187,188] ampicillin[188] and moenomycin.[188] *B. subtilis* SigW responds to a variety of cell envelope stresses, including that promoted by cell wall-active antimicrobials,[189] and this sigma factor has been linked to fosfomycin, vancomycin and ampicillin resistance —*sigW* deletion mutants show increased susceptibility to these agents.[188] *B. subtilis sigX* is induced by inhibitors of peptidoglycan biosynthesis and tunicamycin[190] and its deletion in lab-constructed mutants renders *B. subtilis* more susceptible to CAPs,[63,188,191] ampicillin[188] and bacitracin.[188] The SigB general stress response sigma factor,[192] which responds to various stresses, including heat, salt, acid, nitrosative, cell wall, nutritional and energy stress,[193,194] has been described in *B. subtilis*, *Bacillus cereus*, *L. monocytogenes* and *S. aureus*.[195] In *B. subtilis* this sigma factor is linked to rifampicin resistance—a *sigB* mutant is more susceptible to this antimicrobial.[196] In *S. aureus*, SigB expression or activation is linked to CAP,[197] glycopeptide[198,199] and β-lactam resistance,[200] including methicillin,[198] with methicillin and glycopeptide resistance dependent upon the SigB-regulated *spoVG* TCS genes.[198] Inactivation of *sigB* also increases the oxacillin susceptibility of MRSA[201] and reverses the increased oxacillin and vancomycin resistance of *in vitro*-selected pine oil cleaner-resistant *S. aureus* mutants,[202] highlighting its contribution to acquired resistance in this organism. Finally, SigB is linked to disinfectant,[203] tetracycline,[204] gentamicin[204] and β-lactam[205] resistance in *L. monocytogenes*, with *sigB* mutants showing increased susceptibly to these agents.

## Heat stress

Aminoglycoside induction of heat shock genes has been reported in *E. coli*,[206] *Acinetobacter baumannii*[207] and *P. aeruginosa*.[208] In the case of *P. aeruginosa*, this is mediated by the alternate Lon protease AsrA, whose expression is induced in response to tobramycin or heat shock, dependent upon the RpoH heat shock sigma factor.[208] Intriguingly, *asrA* overexpression in an engineered strain had a modest positive impact on aminoglycoside resistance,[208] raising the possibility that the heat shock response might protect to some extent against aminoglycoside challenge. In agreement with this, pre-treatment of *A. baumannii* cells for 30 min at 45°C rendered the organism better able to withstand a subsequent streptomycin challenge as compared with cells pre-treated at 37°C.[207] One explanation for the link between aminoglycosides and heat shock is that components of the heat shock response charged with eliminating misfolded proteins might target the aberrant mistranslated polypeptides that are produced by aminoglycoside-disrupted ribosomes and that insert into and disrupt bacterial membranes.[139] Aminoglycoside-mediated membrane damage has been reported[139] and is likely a key step in the lethal activity of these agents,[43,138] and thus elimination of aberrant polypeptides that may be responsible would certainly reduce their toxicity to cells. Heat shock has also been linked to β-lactam resistance, in the form of the ClpL heat shock chaperone that is found mainly in Gram-positive bacteria. In *Streptococcus pneumoniae* its expression is associated with increased penicillin resistance—an overproducing mutant shows a 4-fold increase in resistance while a $ClpL^-$ mutant shows a 4-fold decrease, both relative to wild-type.[209] Interestingly, *clpL* is induced upon exposure of *S. pneumoniae* to penicillin (or a heat shock), where it positively influences both expression of the *pbp2x* cell wall synthesis gene and stability of its PBP2x product[209] and in so doing enhances penicillin resistance. Similarly, treatment of *S. aureus* with cell wall-active antimicrobials that inhibit peptidoglycan biosynthesis was shown to induce a number of heat shock genes (*dnaK*, *groES*, *clpB* and *clpL*),[210,211] although there was no indication that this impacted antimicrobial susceptibility. Heat has also been linked to antimicrobial resistance in *Cronobacter sakazaki*, an emerging food-borne pathogen,[212] and induction of a heat shock response

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

leads to macrolide resistance in *Lactococcus lactis*,[213] although no mechanisms were proposed.

## General stress response

The *rpoS* gene, which encodes the general stress response sigma factor that responds to nutrient starvation, hyperosmolarity, pH downshift, non-optimal high and low temperature in *E. coli*[214] and heat shock, hyperosmolarity and prolonged peroxide exposure in *P. aeruginosa*[215] has been linked to antibiotic resistance in both *E. coli* and *P. aeruginosa*. In *E. coli*, for example, constitutive expression of *rpoS* partially suppressed the drug hypersusceptibility of a mutant *E. coli* lacking the *acrAB* multidrug efflux genes.[216] Similarly its overexpression was shown to partially restore ofloxacin tolerance in an ofloxacin-susceptible LasIR$^-$ *P. aeruginosa* defective in acylhomoserine lactone production and quorum-sensing-dependent gene expression.[217] Conversely, an *rpoS* mutant strain of *P. aeruginosa* showed enhanced susceptibility to carbapenems, though only in the stationary phase.[218] Interestingly, RpoS was also shown to be required for a heat shock-promoted increase in carbapenem resistance in *P. aeruginosa*,[218] a clear example of a stress response sigma factor mediating stress-promoted antimicrobial resistance. Still, the RpoS targets responsible for resistance in *P. aeruginosa* or *E. coli* are unknown. Recently RpoS has been linked to persister formation in *E. coli* and *P. aeruginosa*—*rpoS* null mutants showed increased formation of *E. coli* persisters resistant to ampicillin and ciprofloxacin and *P. aeruginosa* persisters resistant to ciprofloxacin,[219] providing yet another way in which this sigma factor can contribute to antimicrobial resistance.

ClpXP is a protease that is broadly distributed in bacteria, where it is charged with turning over damaged proteins as part of the cell's protein quality control systems.[220] The *clpP* gene encoding the proteolytic component of ClpXP is induced by heat shock and other stresses in Gram-negative[221] and Gram-positive[222,223] bacteria and is important for stress tolerance—*clpP* mutants are susceptible to heat and other stresses.[222–225] Recently *clpP* mutations in *S. aureus* have also been shown to confer reduced susceptibility to vancomycin in several VISA isolates,[226] while *clpP* null mutants of *P. aeruginosa* showed modest (2-fold) increases in susceptibility to ciprofloxacin,[227] highlighting a link, hitherto unexplained, to antimicrobial resistance.

## Stress-induced mutagenesis

In the face of 'growth-limiting' stress owing to nutrient starvation, hypoxia, low pH, increased osmotic pressure, extreme temperature shifts or antimicrobial exposure, bacteria activate a stress-induced increase in the mutation rate (reviewed in Galhardo *et al.*,[17] Cirz and Romesberg[20] and Foster[228]). Also called adaptive mutagenesis, stress-induced mutagenesis arises from a stress-dependent introduction of double-stranded breaks in chromosomal DNA[229] and subsequent activation of error-prone repair under the control of the SOS response that typically follows DNA damage,[230] the RpoS-mediated general stress response[231] and the sigma E ($\sigma^E$)-dependent envelope stress response.[232] Presumably the increased mutation rate enhances the likelihood that mutations permitting adaptation to the stressor will arise. Since the stress-induced mutations are random, however, and unrelated to the initial stress that triggered mutagenesis, non-selected mutations, including those that impact antimicrobial resistance, also arise.[20] While studied predominantly in *E. coli*,[17,20,228] evidence for stress-induced mutagenesis is also seen in *B. subtilis*,[233] *Pseudomonas putida*,[234] *Caulobacter crescentus*[235] and *M. tuberculosis*,[236] and FQ-induced mutation typical of stress-induced mutagenesis has been demonstrated in *S. aureus*,[237,238] *S. pneumoniae*,[239] *Streptococcus uberis*,[240] *P. aeruginosa*,[241] *Salmonella* Typhimurium,[242] *Mycobacterium fortuitum*[243] and *Vibrio cholerae*.[244]

As with FQs,[20,206,237,245,246] β-lactams,[6,244,247,248] trimethoprim,[244] chloramphenicol,[244] tetracycline,[244] aminoglycosides[244] and bile[249] have also been shown to induce the SOS response and error-prone repair in certain bacteria and thus increase the mutation rate.[244,248,249] FQ (e.g. ciprofloxacin) promotion of ciprofloxacin resistance development as a result of the induction of the SOS response and error-prone mutagenic pathway is well documented[20] and there are reports of these agents promoting SOS response pathway-dependent development of β-lactam resistance as well.[246] Non-antibiotic stresses impacting mutational events have also been seen, including UV light exposure promoting SOS response-dependent development of ciprofloxacin resistance in *S. aureus*[237] and osmotic stress promoting chromosomal DNA rearrangements in *E. coli*.[250] Strikingly, nutrient starvation induction of the SOS and RpoS responses has also been linked to antibiotic resistance development—Lac$^-$ *E. coli* engineered to carry the *ampD ampC* determinants of β-lactam resistance yielded AmpC β-lactamase-expressing *ampD* mutants when starved of lactose.[251] This is significant because mutations in *ampD* are common in AmpC-overproducing β-lactam-resistant bacteria.[252]

## SOS response

The SOS response is activated by a number of 'stresses' (DNA damage, exposure to certain antimicrobials, pH extremes, oxidative stress, nutrient starvation[253]) and thus has the potential to promote antimicrobial resistance under a variety of stress conditions. Moreover, its contribution to resistance extends beyond its roles in stress-induced mutagenesis and chromosomal rearrangements. β-lactam stimulation of the SOS response in *E. coli*, for example, facilitates survival of a lethal β-lactam exposure as a result of an SOS response-promoted transient halt in cell division.[6] Because β-lactams only act on growing cells, the halt in cell division is protective. Expression of *qnrB*, one of several plasmid-borne quinolone-resistance (*qnr*) genes widespread in the Enterobacteriaceae, is ciprofloxacin inducible, dependent on the drug's induction of the SOS response.[254,255] Activation of the SOS response has recently been shown to be important for ciprofloxacin and rifampicin resistance development in *E. coli*, with suppression of this response blocking emergence of resistance to these agents *in vitro*[256] and enhancing FQ killing of drug-resistant, biofilm and persister cells of *E. coli*.[257]

The SOS response also contributes to resistance development by promoting the horizontal dissemination of drug resistance genes—activation of the SOS response in *V. cholerae* by ciprofloxacin as well as other stresses induces transfer of an antimicrobial resistance gene-carrying integrative conjugative

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

element (ICE) called STX.[258] The expression, and thus the activity of integron-encoded recombinases dubbed integrases, is also enhanced under circumstances that activate the SOS response.[259] Integrons are mobile genetic elements that typically carry multiple antibiotic resistance genes or cassettes, with integrases catalysing recombination events that result in the capture of new resistance genes/cassettes or the reshuffling of integron-resident resistance genes to promote their expression.[260] As such, the SOS response can promote acquisition and expression of resistance genes by integron elements, thereby promoting resistance development. Antibiotics known to stimulate the SOS response are capable of activating integrase expression and activity,[259] an interesting observation given that many of today's common integrons carry genes for resistance to these agents.[253] Binding sites for the LexA regulator of the SOS response are prevalent in the integrase gene promoters of a wide variety of integrons (and superintegrons, large chromosomally located integrons carrying many genes, including antibiotic resistance genes[260]) found in a wide range of bacterial species,[261] suggesting that SOS control of integrase-mediated integron capture and expression of antibiotic resistance genes is common in the bacterial world.

## Persisters

The ability of a small subpopulation of an apparently susceptible and genetically homogeneous population of bacteria to survive antibiotic exposure has been known for some time.[5,262] These so-called persisters are slow-growing or dormant organisms, slow growth and dormancy effectively protecting them from the lethal impact of antibiotics, which preferentially act on rapidly growing bacteria.[5] Persisters are an example of the phenotypic heterogeneity that exists naturally within genetically homogeneous microbial populations[262] and are suggested to arise either stochastically (randomly) and continuously during population growth (so-called type II or stochastic) or are formed in response to an external (i.e. environmental) trigger (type I or determinative).[5,262] Their presence ultimately increases the fitness of a bacterial population by ensuring that a proportion of its members are able to survive insults (e.g. antimicrobial exposure) that would otherwise eradicate a phenotypically homogeneous population. For example, the antimicrobial resistance of biofilms has been attributed to persisters,[23,263] as has the recalcitrance of chronic infections (e.g. *P. aeruginosa* CF lung infections, *M. tuberculosis* lung infections) to antimicrobial therapy.[264,265]

A possible link between persisters and stress was first made by Korch *et al.*,[266] who showed that persistence in *E. coli* was correlated with production of the ppGpp alarmone. These researchers showed that increasing ppGpp production with the cloned *relA* gene increased the frequency of persister formation and that compromising ppGpp production in a high-persistence mutant (one that yielded persisters at a high frequency) reduced or eliminated this high-persistence phenotype.[266] Still, there was no evidence that stress or stress-mediated ppGpp production enhanced persister formation. Indeed, there is some controversy over whether persister formation can, in fact, be inducible, although there is a report of ciprofloxacin treatment promoting the formation of ciprofloxacin-resistant persisters in *E. coli*, dependent, interestingly enough, on the SOS response.[21] Recent data suggest that toxin–antitoxin (TA) gene pairs/modules play an important role in persister formation.[267] Apparently the toxin components of these modules target key constituents of macromolecular synthesis and, in shutting them down, compromise growth (but not viability).[5] Thus stochastic or stress-mediated increases in toxin levels would promote dormancy, and thus formation of antibiotic-resistant persisters. Consistent with this model, expression of the *relE* gene encoding a known inhibitor of translation was shown to promote persister development in *E. coli*.[268] Moreover, TA genes are known to be expressed in drug-resistant persisters[21] and the aforementioned SOS response-mediated ciprofloxacin-inducible persister formation seen in *E. coli* is dependent on the SOS response-inducible TA locus *tisAB*/*istR*—knockout of this locus dramatically reduced the levels of ciprofloxacin-resistant persisters.[269] Of note, too, ciprofloxacin induces expression of the *tisB* toxin gene, and TisB-expressing cells are multidrug tolerant.[269] Additional support for stress being a trigger for persister formation comes from the demonstration that *E. coli* experiencing increased endogenous stress as a result of mutational inactivation of stress response genes or increased exogenous stress as a result of exposure to peroxide or acid pH produced markedly elevated levels of ampicillin-resistant persisters compared with wild-type or untreated cells.[219]

## Small-colony variants (SCVs)

SCVs are a slow-growing auxotrophic subpopulation of bacteria that have been described in a number of bacteria,[4,270] including *S. aureus*, where they have been linked to antimicrobial resistance.[4,270] *S. aureus* SCV auxotrophies typically impact the electron transport chain and/or the TCA cycle, ultimately compromising ATP production, and thus growth, and generation of an electrochemical gradient across the cytoplasmic membrane.[4,270] The slow growth of *S. aureus* SCVs reduces susceptibility to β-lactam antibiotics that typically act on growing cells while the reduced electrochemical gradient reduces susceptibility to aminoglycosides, which depend on it for uptake into cells. Selectable *in vitro*[271,272] and *in vivo*[271,273] with aminoglycosides, it is unclear whether *S. aureus* SCVs arise from natural (e.g. antibiotic) selection of auxotrophic mutants or in response to the growth inhibitory stress imposed by antibiotics. In a recent article, however, aminoglycosides were shown to markedly enhance SCV recovery *in vitro* and in an animal model of *S. aureus* mastitis, dependent on the SigB stress response sigma factor.[271] In addition, *in vitro* aminoglycoside-selected as well as clinical SCVs showed evidence of SigB activation,[271] again consistent with SigB (and thus environmental stress) playing a role in SCV formation. The *S. aureus* growth inhibitory molecule 4-hydroxy-2-heptylquinoline-N-oxide (HQNO), a *P. aeruginosa* exoproduct, also promotes SigB-dependent SCV formation by *S. aureus*,[274] further highlighting SCV formation and the attendant antimicrobial resistance as a stress response. Still, details of the mechanisms responsible for SCV formation remain largely unknown.

## Stress-induced biofilm formation

In nature and in the infected host[275] bacteria commonly exist in biofilms that, among other benefits, provide resistance to antimicrobials.[276] Environmental factors that promote bacterial biofilm



Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

formation will thus contribute positively to antimicrobial resistance. A variety of stresses have been linked to biofilm formation, with biofilm formation itself possibly being a stress response.[18] The observation, for example, that inactivating mutations in the *rpoS* gene encoding the general stress response sigma factor hampers biofilm formation by *E. coli*[18,277] supports biofilm formation being a stress response. Nutrient limitation (i.e. nutrient stress) in the form of Fe limitation has also been shown to promote biofilm formation in *S. aureus*[278] and *Legionella pneumophila*,[279] while $Mg^{2+}$ limitation has a positive impact on biofilm formation in *P. aeruginosa*.[280] The latter arises from a low $Mg^{2+}$-dependent repression of the *retS* 'biofilm repressor' gene, which is ultimately mediated by PhoPQ.[280] Fe limitation also increases transcription and translation of known biofilm determinants as well as biofilm quantity in the periodontopathogen *Aggregatibacter actinomycetemcomitans*.[281] Still, limiting Fe can also negatively impact biofilm formation in some organisms (e.g. *P. aeruginosa*[282]), and high metal levels have been shown to have a positive impact on biofilm formation by a number of organisms.[283] Ribosome disruption by ribosome-targeting agents (ribosome stress) also leads to strong biofilm induction in *E. coli* as a result of a SpoT-dependent decrease in ppGpp and an increase in the biofilm-promoting second messenger c-di-GMP [bis-(3′-5′)-cyclic di-GMP].[284] In this way the ppGpp alarmone links ribosome status to genes necessary for initiation of biofilm formation. ppGpp is also linked to biofilm formation in *E. faecalis*, as mutants unable to synthesize this alarmone exhibit a diminished ability to sustain biofilm formation.[285] Oxidative stress has also been implicated in biofilm formation by *E. coli*. An antitoxin, MqsA, that represses RpoS, leading to a reduction in c-di-GMP levels and thus a reduction in biofilm formation, is degraded (by the Lon protease) in response to oxidative stress.[286] Although not specifically studied, the resultant oxidative stress-promoted increase in *rpoS* expression[286] would be expected to enhance biofilm formation. RpoS has also been implicated in low-temperature-promoted biofilm development in *E. coli*.[287]

## Concluding remarks

Despite the link between stress and known antimicrobial resistance determinants, in many instances the contribution of the resistance genes to the stress response itself is unknown or unconfirmed. While several of the RND family multidrug exporters of *P. aeruginosa* are stress inducible, their roles in the corresponding stress responses and the identities of the inducing signals/effector molecules remain uncertain. Beyond fostering an understanding of the biology of these exporters, knowledge of the environmental circumstances and/or effector molecules responsible for recruiting multidrug efflux systems has relevance in terms of the potential generation of these effectors *in vivo* (in hospital or at a site of infection), where their induction of these resistance determinants may compromise antimicrobial chemotherapy. Knowing where and when a particular resistance mechanism might be recruited *in vivo* could inform an appropriate choice of therapeutic options. Interestingly, stressor induction of these efflux systems does not appear to enhance resistance,[3,109,187] their documented contributions to resistance being limited to circumstances of mutational overexpression.[288] This is true, as well, of other stress response-linked resistance genes, where mutational activation of stress response pathways has been linked to antimicrobial resistance (e.g. mutations in *soxRS* and several of the cell envelope stress response TCSs; see above) while, for the most part, stress activation of these pathways has not. Nonetheless, given that resistance determinants such as efflux are components of stress responses, the relevant stresses will provide a selective pressure for efflux-expressing antimicrobial-resistant mutants even in the absence of antimicrobials. Indeed, *in vitro* exposure of *P. aeruginosa* selects for MexXY-expressing pan-aminoglycoside-resistant mutants,[3] and growth in a mouse model of pneumonia selects for mutants expressing various RND pumps.[2] Still, given their link to antimicrobial resistance, mutations in stress response pathways may well be selected by the antimicrobials themselves, independent of the cognate stress.

The fact that antimicrobials themselves are growth-compromising agents that activate bacterial stress responses also has important implications for antimicrobial resistance development, given the link between stress and resistance. While there are clear examples of stress-regulated genes/processes implicated in clinically relevant antimicrobial resistance (e.g. RND efflux systems and LPS modification in *P. aeruginosa*,[288] cell envelope stress-responsive TCS targets in VISA isolates,[16,163,166–168] possibly biofilms[276] and persisters[264,265] in several bacteria and SCVs in *S. aureus*[271,273]), in many cases the link between stress response genes/mutations and resistance in clinical strains is lacking and/or the impact on resistance is modest and unlikely to afford clinical resistance. Still, antimicrobial resistance in clinical strains is often multifactorial,[288] and even modest stress-related antimicrobial resistance, 'recruited' by stress induction or mutation, may well contribute to resistance development *in vivo*. Stress response pathways may thus be suitable targets for therapeutic intervention. In support of this, inactivation of the AmgRS regulators of an envelope stress response in *P. aeruginosa* increases susceptibility to aminoglycosides,[144] loss of the AlgU envelope stress response sigma factor reverses the resistance of a mutant *P. aeruginosa* hyperexpressing MexCD-OprJ,[187] and inactivation of the SOS response compromises FQ-promoted persister formation by *E. coli*[21] and renders cells more susceptible to FQs.[287] In addition, inactivation of the stringent response in *P. aeruginosa* and *E. coli* enhances susceptibility to several antimicrobials as well as survivability of *P. aeruginosa* in infected and antimicrobial-treated animals.[14] Ultimately, too, since antimicrobial lethality is typically dependent upon hydroxyl radical production/oxidative stress, which can be countered by bacterial antioxidant responses, targeting such responses may be generally useful in promoting antimicrobial efficacy. Still, a better understanding of the link between stress and antimicrobial resistance, including the identification of the stress-induced effectors that promote resistance and/or recruit resistance determinants and the genes involved, is needed in order to fully appreciate the importance of stress responses as resistance determinants, their value as therapeutic targets and how best to target them.

## Funding

Work in the author's laboratory on antimicrobial resistance is supported by the Canadian Institutes of Health Research and by Cystic Fibrosis Canada.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

## Transparency declarations

None to declare.

## References


**1** Asseray N, Jacqueline C, Le Mabecque V *et al*. Activity of glycopeptides against *Staphylococcus aureus* infection in a rabbit endocarditis model: MICs do not predict in vivo efficacy. *Antimicrob Agents Chemother* 2005; **49**: 857–9.

**2** Join-Lambert OF, Michea-Hamzehpour M, Kohler T *et al*. Differential selection of multidrug efflux mutants by trovafloxacin and ciprofloxacin in an experimental model of *Pseudomonas aeruginosa* acute pneumonia in rats. *Antimicrob Agents Chemother* 2001; **45**: 571–6.

**3** Fraud S, Poole K. Oxidative stress induction of the *mexXY* multidrug efflux genes and promotion of aminoglycoside resistance development in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2010; **55**: 1068–74.

**4** Proctor RA, von Eiff C, Kahl BC *et al*. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* 2006; **4**: 295–305.

**5** Lewis K. Persister cells. *Annu Rev Microbiol* 2010; **64**: 357–72.

**6** Miller C, Thomsen LE, Gaggero C *et al*. SOS response induction by β-lactams and bacterial defense against antibiotic lethality. *Science* 2004; **305**: 1629–31.

**7** Eng RH, Padberg FT, Smith SM *et al*. Bactericidal effects of antibiotics on slowly growing and nongrowing bacteria. *Antimicrob Agents Chemother* 1991; **35**: 1824–8.

**8** Poole K. Bacteria multidrug efflux pumps serve other functions. *Microbes* 2008; **3**: 179–85.

**9** Gunn JS. Bacterial modification of LPS and resistance to antimicrobial peptides. *J Endotoxin Res* 2001; **7**: 57–62.

**10** Wu J, Long Q, Xie J. (p)ppGpp and drug resistance. *J Cell Physiol* 2010; **224**: 300–4.

**11** Ishiguro EE, Ramey WD. Inhibition of in vitro peptidoglycan biosynthesis in *Escherichia coli* by guanosine 5′-diphosphate 3′-diphosphate. *Can J Microbiol* 1980; **26**: 1514–8.

**12** Han TH, Lee JH, Cho MH *et al*. Environmental factors affecting indole production in *Escherichia coli*. *Res Microbiol* 2011; **162**: 108–16.

**13** Tkachenko AG, Akhova AV, Shumkov MS *et al*. Polyamines reduce oxidative stress in *Escherichia coli* cells exposed to bactericidal antibiotics. *Res Microbiol* 2012; **163**: 83–91.

**14** Nguyen D, Joshi-Datar A, Lepine F *et al*. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* 2011; **334**: 982–6.

**15** Kato A, Groisman EA. The PhoQ/PhoP regulatory network of *Salmonella enterica*. *Adv Exp Med Biol* 2008; **631**: 7–21.

**16** Meehl M, Herbert S, Gotz F *et al*. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007; **51**: 2679–89.

**17** Galhardo RS, Hastings PJ, Rosenberg SM. Mutation as a stress response and the regulation of evolvability. *Crit Rev Biochem Mol Biol* 2007; **42**: 399–435.

**18** Landini P. Cross-talk mechanisms in biofilm formation and responses to environmental and physiological stress in *Escherichia coli*. *Res Microbiol* 2009; **160**: 259–66.

**19** Morita Y, Sobel ML, Poole K. Antibiotic inducibility of the MexXY multidrug efflux system of *Pseudomonas aeruginosa*: involvement of the antibiotic-inducible PA5471 gene product. *J Bacteriol* 2006; **188**: 1847–55.

**20** Cirz RT, Romesberg FE. Controlling mutation: intervening in evolution as a therapeutic strategy. *Crit Rev Biochem Mol Biol* 2007; **42**: 341–54.

**21** Dorr T, Lewis K, Vulic M. SOS response induces persistence to fluoroquinolones in *Escherichia coli*. *PLoS Genet* 2009; **5**: e1000760.

**22** Borriello G, Werner E, Roe F *et al*. Oxygen limitation contributes to antibiotic tolerance of *Pseudomonas aeruginosa* in biofilms. *Antimicrob Agents Chemother* 2004; **48**: 2659–64.

**23** Kim J, Hahn JS, Franklin MJ *et al*. Tolerance of dormant and active cells in *Pseudomonas aeruginosa* PA01 biofilm to antimicrobial agents. *J Antimicrob Chemother* 2009; **63**: 129–35.

**24** Borriello G, Richards L, Ehrlich GD *et al*. Arginine or nitrate enhances antibiotic susceptibility of *Pseudomonas aeruginosa* in biofilms. *Antimicrob Agents Chemother* 2006; **50**: 382–4.

**25** Wayne LG, Hayes LG. An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect Immun* 1996; **64**: 2062–9.

**26** Mitchison DA, Coates AR. Predictive in vitro models of the sterilizing activity of anti-tuberculosis drugs. *Curr Pharm Des* 2004; **10**: 3285–95.

**27** Baek SH, Li AH, Sassetti CM. Metabolic regulation of mycobacterial growth and antibiotic sensitivity. *PLoS Biol* 2011; **9**: e1001065.

**28** Chatterji D, Ojha AK. Revisiting the stringent response, ppGpp and starvation signaling. *Curr Opin Microbiol* 2001; **4**: 160–5.

**29** Potrykus K, Cashel M. (p)ppGpp: still magical? *Annu Rev Microbiol* 2008; **62**: 35–51.

**30** Sharma UK, Chatterji D. Transcriptional switching in *Escherichia coli* during stress and starvation by modulation of sigma activity. *FEMS Microbiol Rev* 2010; **34**: 646–57.

**31** Kusser W, Ishiguro EE. Involvement of the *relA* gene in the autolysis of *Escherichia coli* induced by inhibitors of peptidoglycan biosynthesis. *J Bacteriol* 1985; **164**: 861–5.

**32** Kusser W, Ishiguro EE. Suppression of mutations conferring penicillin tolerance by interference with the stringent control mechanism of *Escherichia coli*. *J Bacteriol* 1987; **169**: 4396–8.

**33** Rodionov DG, Ishiguro EE. Direct correlation between overproduction of guanosine 3′,5′-bispyrophosphate (ppGpp) and penicillin tolerance in *Escherichia coli*. *J Bacteriol* 1995; **177**: 4224–9.

**34** Vinella D, D'Ari R, Jaffe A *et al*. Penicillin binding protein 2 is dispensable in *Escherichia coli* when ppGpp synthesis is induced. *EMBO J* 1992; **11**: 1493–501.

**35** Vinella D, Albrecht C, Cashel M *et al*. Iron limitation induces SpoT-dependent accumulation of ppGpp in *Escherichia coli*. *Mol Microbiol* 2005; **56**: 958–70.

**36** Heath RJ, Jackowski S, Rock CO. Guanosine tetraphosphate inhibition of fatty acid and phospholipid synthesis in *Escherichia coli* is relieved by overexpression of glycerol-3-phosphate acyltransferase (*plsB*). *J Biol Chem* 1994; **269**: 26584–90.

**37** Greenway DL, England RR. The intrinsic resistance of Escherichia coli to various antimicrobial agents requires ppGpp and $\sigma^s$. *Lett Appl Microbiol* 1999; **29**: 323–6.

**38** Pomares MF, Vincent PA, Farias RN *et al*. Protective action of ppGpp in microcin J25-sensitive strains. *J Bacteriol* 2008; **190**: 4328–34.

**39** Viducic D, Ono T, Murakami K *et al*. Functional analysis of *spoT*, *relA* and *dksA* genes on quinolone tolerance in *Pseudomonas aeruginosa* under nongrowing condition. *Microbiol Immunol* 2006; **50**: 349–57.

**40** Abranches J, Martinez AR, Kajfasz JK *et al*. The molecular alarmone (p)ppGpp mediates stress responses, vancomycin tolerance, and virulence in *Enterococcus faecalis*. *J Bacteriol* 2009; **191**: 2248–56.


Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**41** Hesketh A, Hill C, Mokhtar J *et al*. Genome-wide dynamics of a bacterial response to antibiotics that target the cell envelope. *BMC Genomics* 2011; **12**: 226.

**42** Dwyer DJ, Kohanski MA, Collins JJ. Role of reactive oxygen species in antibiotic action and resistance. *Curr Opin Microbiol* 2009; **12**: 482–9.

**43** Kohanski MA, Dwyer DJ, Wierzbowski J *et al*. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* 2008; **135**: 679–90.

**44** Diggle SP, Matthijs S, Wright VJ *et al*. The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. *Chem Biol* 2007; **14**: 87–96.

**45** Haussler S, Becker T. The pseudomonas quinolone signal (PQS) balances life and death in *Pseudomonas aeruginosa* populations. *PLoS Pathog* 2008; **4**: e1000166.

**46** Fung DK, Chan EW, Chin ML *et al*. Delineation of a bacterial starvation stress response network which can mediate antibiotic tolerance development. *Antimicrob Agents Chemother* 2010; **54**: 1082–93.

**47** Cheng HY, Chen YF, Peng HL. Molecular characterization of the PhoPQ-PmrD-PmrAB mediated pathway regulating polymyxin B resistance in *Klebsiella pneumoniae* CG43. *J Biomed Sci* 2010; **17**: 60.

**48** Macfarlane EL, Kwasnicka A, Ochs MM *et al*. PhoP-PhoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and polymyxin B resistance. *Mol Microbiol* 1999; **34**: 305–16.

**49** Groisman EA, Kayser J, Soncini FC. Regulation of polymyxin resistance and adaptation to low-$Mg^{2+}$ environments. *J Bacteriol* 1997; **179**: 7040–5.

**50** Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* 2003; **67**: 593–656.

**51** Richards SM, Strandberg KL, Gunn JS. *Salmonella*-regulated lipopolysaccharide modifications. *Subcell Biochem* 2010; **53**: 101–22.

**52** Shi Y, Cromie MJ, Hsu FF *et al*. PhoP-regulated *Salmonella resistance* to the antimicrobial peptides magainin 2 and polymyxin B. *Mol Microbiol* 2004; **53**: 229–41.

**53** Gunn JS. The *Salmonella* PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends Microbiol* 2008; **16**: 284–90.

**54** Zhou D, Han Y, Qin L *et al*. Transcriptome analysis of the $Mg^{2+}$-responsive PhoP regulator in *Yersinia pestis*. *FEMS Microbiol Lett* 2005; **250**: 85–95.

**55** Macfarlane EL, Kwasnicka A, Hancock RE. Role of *Pseudomonas aeruginosa* PhoP-PhoQ in resistance to antimicrobial cationic peptides and aminoglycosides. *Microbiology* 2000; **146**: 2543–54.

**56** McPhee JB, Bains M, Winsor G *et al*. Contribution of the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems to $Mg^{2+}$-induced gene regulation in *Pseudomonas aeruginosa*. *J Bacteriol* 2006; **188**: 3995–4006.

**57** Barrow K, Kwon DH. Alterations in two-component regulatory systems of *phoPQ* and *pmrAB* are associated with polymyxin B resistance in clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009; **53**: 5150–4.

**58** Miller AK, Brannon MK, Stevens L *et al*. PhoQ mutations promote lipid A modification and polymyxin resistance of *Pseudomonas aeruginosa* found in colistin-treated cystic fibrosis patients. *Antimicrob Agents Chemother* 2011; **55**: 5761–9.

**59** Abraham N, Kwon DH. A single amino acid substitution in PmrB is associated with polymyxin B resistance in clinical isolate of *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 2009; **298**: 249–54.

**60** Moskowitz SM, Brannon MK, Dasgupta N *et al*. PmrB mutations promote polymyxin resistance of *Pseudomonas aeruginosa* isolated from colistin-treated cystic fibrosis patients. *Antimicrob Agents Chemother* 2012; **56**: 1019–30.

**61** Tamae C, Liu A, Kim K *et al*. Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *J Bacteriol* 2008; **190**: 5981–8.

**62** Dorrer E, Teuber M. Induction of polymyxin resistance in *Pseudomonas fluorescens* by phosphate limitation. *Arch Microbiol* 1977; **114**: 87–9.

**63** Lewenza S, Falsafi R, Bains M *et al*. The *olsA* gene mediates the synthesis of an ornithine lipid in *Pseudomonas aeruginosa* during growth under phosphate-limiting conditions, but is not involved in antimicrobial peptide susceptibility. *FEMS Microbiol Lett* 2011; **320**: 95–102.

**64** Kwon DH, Lu CD. Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in *Pseudomonas aeruginosa* PAO1. *Antimicrob Agents Chemother* 2006; **50**: 1615–22.

**65** Johnson L, Mulcahy H, Kanevets U *et al*. Surface-localized spermidine protects the *Pseudomonas aeruginosa* outer membrane from antibiotic treatment and oxidative stress. *J Bacteriol* 2012; **194**: 813–26.

**66** Storz G, Imlay JA. Oxidative stress. *Curr Opin Microbiol* 1999; **2**: 188–94.

**67** Kolodkin-Gal I, Sat B, Keshet A *et al*. The communication factor EDF and the toxin-antitoxin module *mazEF* determine the mode of action of antibiotics. *PLoS Biol* 2008; **6**: e319.

**68** Touati D. Sensing and protecting against superoxide stress in *Escherichia coli*—how many ways are there to trigger SoxRS response? *Redox Rep* 2000; **5**: 287–93.

**69** Gu M, Imlay JA. The SoxRS response of *Escherichia coli* is directly activated by redox-cycling drugs rather than by superoxide. *Mol Microbiol* 2011; **79**: 1136–50.

**70** Miller PF, Sulavik MC. Overlaps and parallels in the regulation of intrinsic multiple-antibiotic resistance in *Escherichia coli*. *Mol Microbiol* 1996; **21**: 441–8.

**71** Lu C, Bentley WE, Rao G. Comparisons of oxidative stress response genes in aerobic *Escherichia coli* fermentations. *Biotechnol Bioeng* 2003; **83**: 864–70.

**72** Hartog E, Ben Shalom L, Shachar D *et al*. Regulation of *marA*, *soxS*, *rob*, *acrAB* and *micF* in *Salmonella enterica* serovar Typhimurium. *Microbiol Immunol* 2008; **52**: 565–74.

**73** Nikaido E, Shirosaka I, Yamaguchi A *et al*. Regulation of the AcrAB multidrug efflux pump in *Salmonella enterica* serovar Typhimurium in response to indole and paraquat. *Microbiology* 2011; **157**: 648–55.

**74** Koutsolioutsou A, Pena-Llopis S, Demple B. Constitutive *soxR* mutations contribute to multiple-antibiotic resistance in clinical *Escherichia coli* isolates. *Antimicrob Agents Chemother* 2005; **49**: 2746–52.

**75** Fabrega A, Martin RG, Rosner JL *et al*. Constitutive SoxS expression in a fluoroquinolone-resistant strain with a truncated SoxR protein and identification of a new member of the *marA-soxS-rob* regulon, *mdtG*. *Antimicrob Agents Chemother* 2010; **54**: 1218–25.

**76** Koutsolioutsou A, Martins EA, White DG *et al*. A *soxRS*-constitutive mutation contributing to antibiotic resistance in a clinical isolate of *Salmonella enterica* (serovar typhimurium). *Antimicrob Agents Chemother* 2001; **45**: 38–43.

**77** Kehrenberg C, Cloeckaert A, Klein G *et al*. Decreased fluoroquinolone susceptibility in mutants of *Salmonella* serovars other than Typhimurium: detection of novel mutations involved in modulated expression of *ramA* and *soxS*. *J Antimicrob Chemother* 2009; **64**: 1175–80.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**78** O'Regan E, Quinn T, Pages JM *et al*. Multiple regulatory pathways associated with high-level ciprofloxacin and multidrug resistance in *Salmonella enterica* serovar enteritidis: involvement of RamA and other global regulators. *Antimicrob Agents Chemother* 2009; **53**: 1080–7.

**79** Bialek-Davenet S, Marcon E, Leflon-Guibout V *et al*. In vitro selection of *ramR* and *soxR* mutants overexpressing efflux systems by fluoroquinolones as well as cefoxitin in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2011; **55**: 2795–802.

**80** Chou JH, Greenberg JT, Demple B. Posttranscriptional repression of *Escherichia coli* OmpF protein in response to redox stress: positive control of the *micF* antisense RNA by the *soxRS* locus. *J Bacteriol* 1993; **175**: 1026–31.

**81** Miller PF, Gambino LF, Sulavik MC *et al*. Genetic relationship between *soxRS* and *mar* loci in promoting multiple antibiotic resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 1994; **38**: 1773–9.

**82** Lee JH, Lee KL, Yeo WS *et al*. SoxRS-mediated lipopolysaccharide modification enhances resistance against multiple drugs in *Escherichia coli*. *J Bacteriol* 2009; **191**: 4441–50.

**83** Chen H, Hu J, Chen PR *et al*. The *Pseudomonas aeruginosa* multidrug efflux regulator MexR uses an oxidation-sensing mechanism. *Proc Natl Acad Sci USA* 2008; **105**: 13586–91.

**84** Chen PR, Bae T, Williams WA *et al*. An oxidation-sensing mechanism is used by the global regulator MgrA in *Staphylococcus aureus*. *Nat Chem Biol* 2006; **2**: 591–5.

**85** Chen PR, Nishida S, Poor CB *et al*. A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in *Staphylococcus aureus*. *Mol Microbiol* 2009; **71**: 198–211.

**86** Chang W, Small DA, Toghrol F *et al*. Microarray analysis of *Pseudomonas aeruginosa* reveals induction of pyocin genes in response to hydrogen peroxide. *BMC Genomics* 2005; **6**: 115.

**87** Chang W, Small DA, Toghrol F *et al*. Microarray analysis of toxicogenomic effects of peracetic acid on *Pseudomonas aeruginosa*. *Environ Sci Technol* 2005; **39**: 5893–9.

**88** Kaatz GW, Thyagarajan RV, Seo SM. Effect of promoter region mutations and *mgrA* overexpression on transcription of *norA*, which encodes a *Staphylococcus aureus* multidrug efflux transporter. *Antimicrob Agents Chemother* 2005; **49**: 161–9.

**89** Truong-Bolduc QC, Dunman PM, Strahilevitz J *et al*. MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. *J Bacteriol* 2005; **187**: 2395–405.

**90** Truong-Bolduc QC, Bolduc GR, Okumura R *et al*. Implication of the NorB efflux pump in the adaptation of *Staphylococcus aureus* to growth at acid pH and in resistance to moxifloxacin. *Antimicrob Agents Chemother* 2011; **55**: 3214–9.

**91** Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 479–87.

**92** Ciofu O, Riis B, Pressler T *et al*. Occurrence of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis patients is associated with the oxidative stress caused by chronic lung inflammation. *Antimicrob Agents Chemother* 2005; **49**: 2276–82.

**93** Jeannot K, Sobel ML, El Garch F *et al*. Induction of the MexXY efflux pump in *Pseudomonas aeruginosa* is dependent on drug-ribosome interaction. *J Bacteriol* 2005; **187**: 5341–6.

**94** Caughlan RE, Sriram S, Daigle DM *et al*. Fmt bypass in *Pseudomonas aeruginosa* causes induction of MexXY efflux pump expression. *Antimicrob Agents Chemother* 2009; **53**: 5015–21.

**95** Ling J, Soll D. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proc Natl Acad Sci USA* 2010; **107**: 4028–33.

**96** Dukan S, Farewell A, Ballesteros M *et al*. Protein oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci USA* 2000; **97**: 5746–9.

**97** Palyada K, Sun YQ, Flint A *et al*. Characterization of the oxidative stress stimulon and PerR regulon of *Campylobacter jejuni*. *BMC Genomics* 2009; **10**: 481.

**98** Muller JF, Stevens AM, Craig J *et al*. Transcriptome analysis reveals that multidrug efflux genes are upregulated to protect *Pseudomonas aeruginosa* from pentachlorophenol stress. *Appl Environ Microbiol* 2007; **73**: 4550–8.

**99** Ghosh S, Cremers CM, Jakob U *et al*. Chlorinated phenols control the expression of the multi-drug resistance efflux pump MexAB-OprM in *Pseudomonas aeruginosa* by activating NalC. *Mol Microbiol* 2011; **79**: 1547–56.

**100** Starr LM, Fruci M, Poole K. Pentachlorophenol induction of the *Pseudomonas aeruginosa mexAB-oprM* efflux operon: involvement of repressors NalC and MexR and the antirepressor ArmR. *PLoS ONE* 2012; **7**: e32684.

**101** McLamore ES, Zhang W, Porterfield DM *et al*. Membrane-aerated biofilm proton and oxygen flux during chemical toxin exposure. *Environ Sci Technol* 2010; **44**: 7050–7.

**102** Köhler T, Michea-Hamzehpour M, Henze U *et al*. Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. *Mol Microbiol* 1997; **23**: 345–54.

**103** Fetar H, Gilmour C, Klinoski R *et al*. *mexEF-oprN* multidrug efflux operon of *Pseudomonas aeruginosa*: regulation by the MexT activator in response to nitrosative stress and chloramphenicol. *Antimicrob Agents Chemother* 2011; **55**: 508–14.

**104** Köhler T, Epp SF, Curty LK *et al*. Characterization of MexT, the regulator of the MexE-MexF-OprN multidrug efflux system of *Pseudomonas aeruginosa*. *J Bacteriol* 1999; **181**: 6300–5.

**105** Ochs MM, McCusker MP, Bains M *et al*. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. *Antimicrob Agents Chemother* 1999; **43**: 1085–90.

**106** Maseda H, Saito K, Nakajima A *et al*. Variation of the *mexT* gene, a regulator of the MexEF-OprN efflux pump expression in wild-type strains of *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 2000; **192**: 107–12.

**107** Sobel ML, Poole K, Neshat S. Mutations in PA2491 (*mexS*) promote MexT-dependent *mexEF-oprN* expression and multidrug resistance in a clinical strain of *Pseudomonas aeruginosa*. *J Bacteriol* 2005; **187**: 1246–53.

**108** Tian ZX, Fargier E, Mac AM *et al*. Transcriptome profiling defines a novel regulon modulated by the LysR-type transcriptional regulator MexT in *Pseudomonas aeruginosa*. *Nucleic Acids Res* 2009; **37**: 7546–59.

**109** Blehert DS, Fox BG, Chambliss GH. Cloning and sequence analysis of two *Pseudomonas* flavoprotein xenobiotic reductases. *J Bacteriol* 1999; **181**: 6254–63.

**110** Pak JW, Knoke KL, Noguera DR *et al*. Transformation of 2,4,6-trinitrotoluene by purified xenobiotic reductase B from *Pseudomonas fluorescens* I-C. *Appl Environ Microbiol* 2000; **66**: 4742–50.

**111** Goswami M, Mangoli SH, Jawali N. Involvement of reactive oxygen species in the action of ciprofloxacin against *Escherichia coli*. *Antimicrob Agents Chemother* 2006; **50**: 949–54.

**112** Kuczynska-Wisnik D, Matuszewska E, Laskowska E. *Escherichia coli* heat-shock proteins IbpA and IbpB affect biofilm formation by influencing the level of extracellular indole. *Microbiology* 2010; **156**: 148–57.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**113** Kuczynska-Wisnik D, Matuszewska E, Furmanek-Blaszk B *et al*. Antibiotics promoting oxidative stress inhibit formation of *Escherichia coli* biofilm via indole signalling. *Res Microbiol* 2010; **161**: 847–53.

**114** Lee HH, Molla MN, Cantor CR *et al*. Bacterial charity work leads to population-wide resistance. *Nature* 2010; **467**: 82–5.

**115** Lee J, Attila C, Cirillo SL *et al*. Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microb Biotechnol* 2009; **2**: 75–90.

**116** Gusarov I, Nudler E. NO-mediated cytoprotection: instant adaptation to oxidative stress in bacteria. *Proc Natl Acad Sci USA* 2005; **102**: 13855–60.

**117** Gusarov I, Shatalin K, Starodubtseva M *et al*. Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. *Science* 2009; **325**: 1380–4.

**118** Shatalin K, Shatalina E, Mironov A *et al*. $H_2S$: a universal defense against antibiotics in bacteria. *Science* 2011; **334**: 986–90.

**119** Tkachenko AG, Fedotova MV. Dependence of protective functions of *Escherichia coli* polyamines on strength of stress caused by superoxide radicals. *Biochemistry (Mosc)* 2007; **72**: 109–16.

**120** Tkachenko A, Nesterova L, Pshenichnov M. The role of the natural polyamine putrescine in defense against oxidative stress in *Escherichia coli*. *Arch Microbiol* 2001; **176**: 155–7.

**121** Jung IL, Kim IG. Transcription of *ahpC*, *katG*, and *katE* genes in *Escherichia coli* is regulated by polyamines: polyamine-deficient mutant sensitive to $H_2O_2$-induced oxidative damage. *Biochem Biophys Res Commun* 2003; **301**: 915–22.

**122** Aiassa V, Barnes AI, Smania AM *et al*. Sublethal ciprofloxacin treatment leads to resistance via antioxidant systems in *Proteus mirabilis*. *FEMS Microbiol Lett* 2012; **327**: 25–32.

**123** Ades SE. Control of the alternative sigma factor $\sigma^E$ in *Escherichia coli*. *Curr Opin Microbiol* 2004; **7**: 157–62.

**124** Dorel C, Lejeune P, Rodrigue A. The Cpx system of *Escherichia coli*, a strategic signaling pathway for confronting adverse conditions and for settling biofilm communities? *Res Microbiol* 2006; **157**: 306–14.

**125** Raffa RG, Raivio TL. A third envelope stress signal transduction pathway in *Escherichia coli*. *Mol Microbiol* 2002; **45**: 1599–611.

**126** Ruiz N, Silhavy TJ. Sensing external stress: watchdogs of the *Escherichia coli* cell envelope. *Curr Opin Microbiol* 2005; **8**: 122–6.

**127** Nishino K, Yamasaki S, Hayashi-Nishino M *et al*. Effect of NlpE overproduction on multidrug resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 2010; **54**: 2239–43.

**128** Mileykovskaya E, Dowhan W. The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine. *J Bacteriol* 1997; **179**: 1029–34.

**129** Weatherspoon-Griffin N, Zhao G, Kong W *et al*. The CpxR/CpxA two-component system up-regulates two Tat-dependent peptidoglycan amidases to confer bacterial resistance to antimicrobial peptide. *J Biol Chem* 2011; **286**: 5529–39.

**130** Hirakawa H, Nishino K, Hirata T *et al*. Comprehensive studies of drug resistance mediated by overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. *J Bacteriol* 2003; **185**: 1851–6.

**131** Rainwater S, Silverman PM. The Cpx proteins of *Escherichia coli* K-12: evidence that *cpxA*, *ecfB*, *ssd*, and *eup* mutations all identify the same gene. *J Bacteriol* 1990; **172**: 2456–61.

**132** Sun S, Berg OG, Roth JR *et al*. Contribution of gene amplification to evolution of increased antibiotic resistance in *Salmonella typhimurium*. *Genetics* 2009; **182**: 1183–95.

**133** Hirakawa H, Nishino K, Yamada J *et al*. β-Lactam resistance modulated by the overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. *J Antimicrob Chemother* 2003; **52**: 576–82.

**134** Mizuno T, Mizushima S. Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. *Mol Microbiol* 1990; **4**: 1077–82.

**135** Batchelor E, Walthers D, Kenney LJ *et al*. The *Escherichia coli* CpxA-CpxR envelope stress response system regulates expression of the porins OmpF and OmpC. *J Bacteriol* 2005; **187**: 5723–31.

**136** Jaffe A, Chabbert YA, Derlot E. Selection and characterization of β-lactam-resistant *Escherichia coli* K-12 mutants. *Antimicrob Agents Chemother* 1983; **23**: 622–5.

**137** Kohanski MA, DePristo MA, Collins JJ. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. *Mol Cell* 2010; **37**: 311–20.

**138** Lee S, Hinz A, Bauerle E *et al*. Targeting a bacterial stress response to enhance antibiotic action. *Proc Natl Acad Sci USA* 2009; **106**: 14570–5.

**139** Davis BD, Chen LL, Tai PC. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc Natl Acad Sci USA* 1986; **83**: 6164–8.

**140** Hinz A, Lee S, Jacoby K *et al*. Membrane proteases and aminoglycoside antibiotic resistance. *J Bacteriol* 2011; **193**: 4790–7.

**141** Leblanc SK, Oates CW, Raivio TL. Characterization of the induction and cellular role of the BaeSR two-component envelope stress response of *Escherichia coli*. *J Bacteriol* 2011; **193**: 3367–75.

**142** Baranova N, Nikaido H. The BaeSR two-component regulatory system activates transcription of the *yegMNOB* (*mdtABCD*) transporter gene cluster in *Escherichia coli* and increases its resistance to novobiocin and deoxycholate. *J Bacteriol* 2002; **184**: 4168–76.

**143** Nagakubo S, Nishino K, Hirata T *et al*. The putative response regulator BaeR stimulates multidrug resistance of *Escherichia coli* via a novel multidrug exporter system, MdtABC. *J Bacteriol* 2002; **184**: 4161–7.

**144** Gutsmann T, Hagge SO, David A *et al*. Lipid-mediated resistance of Gram-negative bacteria against various pore-forming antimicrobial peptides. *J Endotoxin Res* 2005; **11**: 167–73.

**145** Llobet E, Campos MA, Gimenez P *et al*. Analysis of the networks controlling the antimicrobial-peptide-dependent induction of *Klebsiella pneumoniae* virulence factors. *Infect Immun* 2011; **79**: 3718–32.

**146** Fernandez L, Gooderham WJ, Bains M *et al*. Adaptive resistance to the “last hope” antibiotics polymyxin B and colistin in *Pseudomonas aeruginosa* is mediated by the novel two-component regulatory system ParR-ParS. *Antimicrob Agents Chemother* 2010; **54**: 3372–82.

**147** Muller C, Plesiat P, Jeannot K. A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β-lactams in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2010; **55**: 1211–21.

**148** Clarke DJ. The Rcs phosphorelay: more than just a two-component pathway. *Future Microbiol* 2010; **5**: 1173–84.

**149** Huang YH, Ferrieres L, Clarke DJ. The role of the Rcs phosphorelay in Enterobacteriaceae. *Res Microbiol* 2006; **157**: 206–12.

**150** Erickson KD, Detweiler CS. The Rcs phosphorelay system is specific to enteric pathogens/commensals and activates *ydeI*, a gene important for persistent *Salmonella* infection of mice. *Mol Microbiol* 2006; **62**: 883–94.

**151** Detweiler CS, Monack DM, Brodsky IE *et al*. *virK*, *somA* and *rcsC* are important for systemic *Salmonella enterica* serovar Typhimurium infection and cationic peptide resistance. *Mol Microbiol* 2003; **48**: 385–400.

**152** Kawada-Matsuo M, Yoshida Y, Nakamura N *et al*. Role of two-component systems in the resistance of *Staphylococcus aureus* to antibacterial agents. *Virulence* 2011; **2**: 427–30.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**153** Ou J, Tian XL, Versey J *et al*. The BceABRS four-component system regulates the bacitracin-induced cell envelope stress response in *Streptococcus mutans*. *Antimicrob Agents Chemother* 2010; **54**: 3895–906.

**154** Hiron A, Falord M, Valle J *et al*. Bacitracin and nisin resistance in *Staphylococcus aureus*: a novel pathway involving the BraS/BraR two-component system (SA2417/SA2418) and both the BraD/BraE and VraD/VraE ABC transporters. *Mol Microbiol* 2011; **81**: 602–22.

**155** Mascher T, Zimmer SL, Smith TA *et al*. Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. *Antimicrob Agents Chemother* 2004; **48**: 2888–96.

**156** Pietiainen M, Gardemeister M, Mecklin M *et al*. Cationic antimicrobial peptides elicit a complex stress response in *Bacillus subtilis* that involves ECF-type sigma factors and two-component signal transduction systems. *Microbiology* 2005; **151**: 1577–92.

**157** Suntharalingam P, Senadheera MD, Mair RW *et al*. The LiaFSR system regulates the cell envelope stress response in *Streptococcus mutans*. *J Bacteriol* 2009; **191**: 2973–84.

**158** Arias CA, Panesso D, McGrath DM *et al*. Genetic basis for *in vivo* daptomycin resistance in enterococci. *N Engl J Med* 2011; **365**: 892–900.

**159** Boyle-Vavra S, Yin S, Daum RS. The VraS/VraR two-component regulatory system required for oxacillin resistance in community-acquired methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 2006; **262**: 163–71.

**160** Kuroda M, Kuroda H, Oshima T *et al*. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. *Mol Microbiol* 2003; **49**: 807–21.

**161** Pietiainen M, Francois P, Hyyrylainen HL *et al*. Transcriptome analysis of the responses of *Staphylococcus aureus* to antimicrobial peptides and characterization of the roles of *vraDE* and *vraSR* in antimicrobial resistance. *BMC Genomics* 2009; **10**: 429.

**162** Gardete S, Wu SW, Gill S *et al*. Role of VraSR in antibiotic resistance and antibiotic-induced stress response in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 3424–34.

**163** McAleese F, Wu SW, Sieradzki K *et al*. Overexpression of genes of the cell wall stimulon in clinical isolates of *Staphylococcus aureus* exhibiting vancomycin-intermediate- *S. aureus*-type resistance to vancomycin. *J Bacteriol* 2006; **188**: 1120–33.

**164** Kato Y, Suzuki T, Ida T *et al*. Genetic changes associated with glycopeptide resistance in *Staphylococcus aureus*: predominance of amino acid substitutions in YvqF/VraSR. *J Antimicrob Chemother* 2010; **65**: 37–45.

**165** Kato Y, Suzuki T, Ida T *et al*. Microbiological and clinical study of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying VraS mutation: changes in susceptibility to glycopeptides and clinical significance. *Int J Antimicrob Agents* 2008; **31**: 64–70.

**166** Cui L, Neoh HM, Shoji M *et al*. Contribution of *vraSR* and *graSR* point mutations to vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; **53**: 1231–4.

**167** Katayama Y, Murakami-Kuroda H, Cui L *et al*. Selection of heterogeneous vancomycin-intermediate *Staphylococcus aureus* by imipenem. *Antimicrob Agents Chemother* 2009; **53**: 3190–6.

**168** Howden BP, Stinear TP, Allen DL *et al*. Genomic analysis reveals a point mutation in the two-component sensor gene *graS* that leads to intermediate vancomycin resistance in clinical *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008; **52**: 3755–62.

**169** Nielsen PK, Andersen AZ, Mols M *et al*. Genome-wide transcriptional profiling of the cell envelope stress response and the role of LisRK and CesRK in *Listeria monocytogenes*. *Microbiology* 2012; **158**: 963–74.

**170** Kolar SL, Nagarajan V, Oszmiana A *et al*. NsaRS is a cell-envelope-stress-sensing two-component system of *Staphylococcus aureus*. *Microbiology* 2011; **157**: 2206–19.

**171** Blake KL, Randall CP, O'Neill AJ. In vitro studies indicate a high resistance potential for the lantibiotic nisin in *Staphylococcus aureus* and define a genetic basis for nisin resistance. *Antimicrob Agents Chemother* 2011; **55**: 2362–8.

**172** Otto M. Bacterial sensing of antimicrobial peptides. *Contrib Microbiol* 2009; **16**: 136–49.

**173** Yang SJ, Bayer AS, Mishra NN *et al*. The *Staphylococcus aureus* two-component regulatory system, GraRS, senses and confers resistance to selected cationic antimicrobial peptides. *Infect Immun* 2012; **80**: 74–81.

**174** Li M, Cha DJ, Lai Y *et al*. The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*. *Mol Microbiol* 2007; **66**: 1136–47.

**175** Jones T, Yeaman MR, Sakoulas G *et al*. Failures in clinical treatment of *Staphylococcus aureus* infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. *Antimicrob Agents Chemother* 2008; **52**: 269–78.

**176** Comenge Y, Quintiliani R Jr, Li L *et al*. The CroRS two-component regulatory system is required for intrinsic β-lactam resistance in *Enterococcus faecalis*. *J Bacteriol* 2003; **185**: 7184–92.

**177** Coornaert A, Lu A, Mandin P *et al*. MicA sRNA links the PhoP regulon to cell envelope stress. *Mol Microbiol* 2010; **76**: 467–79.

**178** Humphreys S, Stevenson A, Bacon A *et al*. The alternative sigma factor, $\sigma^{E,}$ is critically important for the virulence of *Salmonella typhimurium*. *Infect Immun* 1999; **67**: 1560–8.

**179** Park SD, Youn JW, Kim YJ *et al*. *Corynebacterium glutamicum* $\sigma^E$ is involved in responses to cell surface stresses and its activity is controlled by the anti-sigma factor CseE. *Microbiology* 2008; **154**: 915–23.

**180** Morita Y, Murata T, Mima T *et al*. Induction of *mexCD-oprJ* operon for a multidrug efflux pump by disinfectants in wild-type *Pseudomonas aeruginosa* PAO1. *J Antimicrob Chemother* 2003; **5**: 991–4.

**181** Fraud S, Campigotto AJ, Chen Z *et al*. The MexCD-OprJ multidrug efflux system of *Pseudomonas aeruginosa*: involvement in chlorhexidine resistance and induction by membrane damaging agents dependent upon the AlgU stress-response sigma factor. *Antimicrob Agents Chemother* 2008; **52**: 4478–82.

**182** Yu H, Schurr MJ, Deretic V. Functional equivalence of *Escherichia coli* $\sigma^E$ and *Pseudomonas aeruginosa* AlgU: *E. coli rpoE* restores mucoidy and reduces sensitivity to reactive oxygen intermediates in *algU* mutants of *P. aeruginosa*. *J Bacteriol* 1995; **177**: 3259–68.

**183** Martin DW, Holloway BW, Deretic V. Characterization of a locus determining the mucoid status of *Pseudomonas aeruginosa*: AlgU shows sequence similarities with a *Bacillus* sigma factor. *J Bacteriol* 1993; **175**: 1153–64.

**184** Hershberger CD, Ye RW, Parsek MR *et al*. The *algT* (*algU*) gene of *Pseudomonas aeruginosa*, a key regulator involved in alginate biosynthesis, encodes an alternative sigma factor ($\sigma^E$). *Proc Natl Acad Sci USA* 1995; **92**: 7941–5.

**185** Stickland HG, Davenport PW, Lilley KS *et al*. Mutation of *nfxB* causes global changes in the physiology and metabolism of *Pseudomonas aeruginosa*. *J Proteome Res* 2010; **9**: 2957–67.

**186** Eiamphungporn W, Helmann JD. The *Bacillus subtilis* $\sigma^M$ regulon and its contribution to cell envelope stress responses. *Mol Microbiol* 2008; **67**: 830–48.

**187** Thackray PD, Moir A. SigM, an extracytoplasmic function sigma factor of *Bacillus subtilis*, is activated in response to cell wall antibiotics, ethanol, heat, acid, and superoxide stress. *J Bacteriol* 2003; **185**: 3491–8.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**188** Mascher T, Hachmann AB, Helmann JD. Regulatory overlap and functional redundancy among *Bacillus subtilis* extracytoplasmic function sigma factors. *J Bacteriol* 2007; **189**: 6919–27.

**189** Helmann JD. Deciphering a complex genetic regulatory network: the *Bacillus subtilis* $\sigma^W$ protein and intrinsic resistance to antimicrobial compounds. *Sci Prog* 2006; **89**: 243–66.

**190** Helmann JD. The extracytoplasmic function (ECF) sigma factors. *Adv Microb Physiol* 2002; **46**: 47–110.

**191** Cao M, Helmann JD. The *Bacillus subtilis* extracytoplasmic-function $\sigma^X$ factor regulates modification of the cell envelope and resistance to cationic antimicrobial peptides. *J Bacteriol* 2004; **186**: 1136–46.

**192** Boylan SA, Redfield AR, Brody MS *et al*. Stress-induced activation of the $\sigma^B$ transcription factor of *Bacillus subtilis*. *J Bacteriol* 1993; **175**: 7931–7.

**193** Hecker M, Pane-Farre J, Volker U. SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. *Annu Rev Microbiol* 2007; **61**: 215–36.

**194** Shin JH, Kim J, Kim SM *et al*. $\sigma^B$-dependent protein induction in *Listeria monocytogenes* during vancomycin stress. *FEMS Microbiol Lett* 2010; **308**: 94–100.

**195** van Schaik W, Abee T. The role of $\sigma^B$ in the stress response of Gram-positive bacteria—targets for food preservation and safety. *Curr Opin Biotechnol* 2005; **16**: 218–24.

**196** Bandow JE, Brotz H, Hecker M. *Bacillus subtilis* tolerance of moderate concentrations of rifampin involves the $\sigma^B$-dependent general and multiple stress response. *J Bacteriol* 2002; **184**: 459–67.

**197** Zhang H, Morikawa K, Ohta T *et al*. In vitro resistance to the CS$\alpha\beta$-type antimicrobial peptide ASABF-$\alpha$ is conferred by overexpression of sigma factor *sigB* in *Staphylococcus aureus*. *J Antimicrob Chemother* 2005; **55**: 686–91.

**198** Schulthess B, Meier S, Homerova D *et al*. Functional characterization of the $\sigma^B$-dependent *yabJ-spoVG* operon in *Staphylococcus aureus*: role in methicillin and glycopeptide resistance. *Antimicrob Agents Chemother* 2009; **53**: 1832–9.

**199** Bischoff M, Berger-Bachi B. Teicoplanin stress-selected mutations increasing $\sigma^B$ activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; **45**: 1714–20.

**200** Morikawa K, Maruyama A, Inose Y *et al*. Overexpression of sigma factor, $\sigma^B$, urges *Staphylococcus aureus* to thicken the cell wall and to resist β-lactams. *Biochem Biophys Res Commun* 2001; **288**: 385–9.

**201** Singh VK, Schmidt JL, Jayaswal RK *et al*. Impact of *sigB* mutation on *Staphylococcus aureus* oxacillin and vancomycin resistance varies with parental background and method of assessment. *Int J Antimicrob Agents* 2003; **21**: 256–61.

**202** Price CT, Singh VK, Jayaswal RK *et al*. Pine oil cleaner-resistant *Staphylococcus aureus*: reduced susceptibility to vancomycin and oxacillin and involvement of SigB. *Appl Environ Microbiol* 2002; **68**: 5417–21.

**203** van der Veen S, Abee T. Importance of SigB for *Listeria monocytogenes* static and continuous-flow biofilm formation and disinfectant resistance. *Appl Environ Microbiol* 2010; **76**: 7854–60.

**204** Zhou Q, Wang L, Yin X *et al*. SigB-dependent tolerance to protein synthesis-inhibiting antibiotics in *Listeria monocytogenes* EGDe. *Curr Microbiol* 2012; **64**: 234–41.

**205** Begley M, Hill C, Ross RP. Tolerance of *Listeria monocytogenes* to cell envelope-acting antimicrobial agents is dependent on SigB. *Appl Environ Microbiol* 2006; **72**: 2231–4.

**206** Shaw KJ, Miller N, Liu X *et al*. Comparison of the changes in global gene expression of *Escherichia coli* induced by four bactericidal agents. *J Mol Microbiol Biotechnol* 2003; **5**: 105–22.

**207** Cardoso K, Gandra RF, Wisniewski ES *et al*. DnaK and GroEL are induced in response to antibiotic and heat shock in *Acinetobacter baumannii*. *J Med Microbiol* 2010; **59**: 1061–8.

**208** Kindrachuk KN, Fernandez L, Bains M *et al*. Involvement of an ATP-dependent protease, PA0779/AsrA, in inducing heat shock in response to tobramycin in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2011; **55**: 1874–82.

**209** Tran TD, Kwon HY, Kim EH *et al*. Decrease in penicillin susceptibility due to heat shock protein ClpL in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2011; **55**: 2714–28.

**210** Pechous R, Ledala N, Wilkinson BJ *et al*. Regulation of the expression of cell wall stress stimulon member gene *msrA1* in methicillin-susceptible or -resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48**: 3057–63.

**211** Kuroda H, Kuroda M, Cui L *et al*. Subinhibitory concentrations of β-lactam induce haemolytic activity in *Staphylococcus aureus* through the SaeRS two-component system. *FEMS Microbiol Lett* 2007; **268**: 98–105.

**212** Al Nabulsi AA, Osaili TM, Elabedeen NA *et al*. Impact of environmental stress desiccation, acidity, alkalinity, heat or cold on antibiotic susceptibility of *Cronobacter sakazakii*. *Int J Food Microbiol* 2011; **146**: 137–43.

**213** Dorrian JM, Briggs DA, Ridley ML *et al*. Induction of a stress response in *Lactococcus lactis* is associated with a resistance to ribosomally active antibiotics. *FEBS J* 2011; **278**: 4015–24.

**214** Hengge-Aronis R. Signal transduction and regulatory mechanisms involved in control of the $\sigma^S$ (RpoS) subunit of RNA polymerase. *Microbiol Mol Biol Rev* 2002; **66**: 373–95.

**215** Suh SJ, Silo-Suh L, Woods DE *et al*. Effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. *J Bacteriol* 1999; **181**: 3890–7.

**216** Rami A, Toutain CM, Jacq A. An increased level of alternative sigma factor RpoS partially suppresses drug hypersensitivity associated with inactivation of the multidrug resistance pump AcrAB in *Escherichia coli*. *Res Microbiol* 2005; **156**: 356–60.

**217** Kayama S, Murakami K, Ono T *et al*. The role of *rpoS* gene and quorum-sensing system in ofloxacin tolerance in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 2009; **298**: 184–92.

**218** Murakami K, Ono T, Viducic D *et al*. Role for *rpoS* gene of *Pseudomonas aeruginosa* in antibiotic tolerance. *FEMS Microbiol Lett* 2005; **242**: 161–7.

**219** Hong SH, Wang X, O'Connor HF *et al*. Bacterial persistence increases as environmental fitness decreases. *Microb Biotechnol* 2012; doi:10.1111/j.1751-7915.2011.00327.x.

**220** Baker TA, Sauer RT. ClpXP, an ATP-powered unfolding and protein-degradation machine. *Biochim Biophys Acta* 2012; **1823**: 15–28.

**221** Kroh HE, Simon LD. The ClpP component of Clp protease is the $\sigma^{32}$-dependent heat shock protein F21.5. *J Bacteriol* 1990; **172**: 6026–34.

**222** Gerth U, Kruger E, Derre I *et al*. Stress induction of the *Bacillus subtilis clpP* gene encoding a homologue of the proteolytic component of the Clp protease and the involvement of ClpP and ClpX in stress tolerance. *Mol Microbiol* 1998; **28**: 787–802.

**223** Chastanet A, Prudhomme M, Claverys JP *et al*. Regulation of *Streptococcus pneumoniae clp* genes and their role in competence development and stress survival. *J Bacteriol* 2001; **183**: 7295–307.

**224** Frees D, Qazi SN, Hill PJ *et al*. Alternative roles of ClpX and ClpP in *Staphylococcus aureus* stress tolerance and virulence. *Mol Microbiol* 2003; **48**: 1565–78.

**225** Thomsen LE, Olsen JE, Foster JW *et al*. ClpP is involved in the stress response and degradation of misfolded proteins in *Salmonella enterica* serovar Typhimurium. *Microbiology* 2002; **148**: 2727–33.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**226** Shoji M, Cui L, Iizuka R *et al*. *walK* and *clpP* mutations confer reduced vancomycin susceptibility in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2011; **55**: 3870–81.

**227** Fernandez L, Breidenstein EB, Song D *et al*. Role of intracellular proteases in the antibiotic resistance, motility, and biofilm formation of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2012; **56**: 1128–32.

**228** Foster PL. Stress-induced mutagenesis in bacteria. *Crit Rev Biochem Mol Biol* 2007; **42**: 373–97.

**229** Shee C, Gibson JL, Darrow MC *et al*. Impact of a stress-inducible switch to mutagenic repair of DNA breaks on mutation in *Escherichia coli*. *Proc Natl Acad Sci USA* 2011; **108**: 13659–64.

**230** Galhardo RS, Do R, Yamada M *et al*. DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in *Escherichia coli*. *Genetics* 2009; **182**: 55–68.

**231** Frisch RL, Su Y, Thornton PC *et al*. Separate DNA Pol II- and Pol IV-dependent pathways of stress-induced mutation during double-strand-break repair in *Escherichia coli* are controlled by RpoS. *J Bacteriol* 2010; **192**: 4694–700.

**232** Gibson JL, Lombardo MJ, Thornton PC *et al*. The $\sigma^E$ stress response is required for stress-induced mutation and amplification in *Escherichia coli*. *Mol Microbiol* 2010; **77**: 415–30.

**233** Sung HM, Yeamans G, Ross CA *et al*. Roles of YqjH and YqjW, homologs of the *Escherichia coli* UmuC/DinB or Y superfamily of DNA polymerases, in stationary-phase mutagenesis and UV-induced mutagenesis of *Bacillus subtilis*. *J Bacteriol* 2003; **185**: 2153–60.

**234** Tegova R, Tover A, Tarassova K *et al*. Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in *Pseudomonas putida*. *J Bacteriol* 2004; **186**: 2735–44.

**235** Galhardo RS, Rocha RP, Marques MV *et al*. An SOS-regulated operon involved in damage-inducible mutagenesis in *Caulobacter crescentus*. *Nucleic Acids Res* 2005; **33**: 2603–14.

**236** Boshoff HI, Reed MB, Barry CE III *et al*. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell* 2003; **113**: 183–93.

**237** Cirz RT, Jones MB, Gingles NA *et al*. Complete and SOS-mediated response of *Staphylococcus aureus* to the antibiotic ciprofloxacin. *J Bacteriol* 2007; **189**: 531–9.

**238** Mesak LR, Davies J. Phenotypic changes in ciprofloxacin-resistant *Staphylococcus aureus*. *Res Microbiol* 2009; **160**: 785–91.

**239** Henderson-Begg SK, Livermore DM, Hall LM. Effect of subinhibitory concentrations of antibiotics on mutation frequency in *Streptococcus pneumoniae*. *J Antimicrob Chemother* 2006; **57**: 849–54.

**240** Varhimo E, Savijoki K, Jefremoff H *et al*. Ciprofloxacin induces mutagenesis to antibiotic resistance independent of UmuC in *Streptococcus uberis*. *Environ Microbiol* 2008; **10**: 2179–83.

**241** Cirz RT, O'Neill BM, Hammond JA *et al*. Defining the *Pseudomonas aeruginosa* SOS response and its role in the global response to the antibiotic ciprofloxacin. *J Bacteriol* 2006; **188**: 7101–10.

**242** Power EG, Phillips I. Correlation between *umuC* induction and *Salmonella* mutagenicity assay for quinolone antimicrobial agents. *FEMS Microbiol Lett* 1993; **112**: 251–4.

**243** Gillespie SH, Basu S, Dickens AL *et al*. Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. *J Antimicrob Chemother* 2005; **56**: 344–8.

**244** Baharoglu Z, Mazel D. *Vibrio cholerae* triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. *Antimicrob Agents Chemother* 2011; **55**: 2438–41.

**245** Ysern P, Clerch B, Castano M *et al*. Induction of SOS genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. *Mutagenesis* 1990; **5**: 63–6.

**246** Tattevin P, Basuino L, Chambers HF. Subinhibitory fluoroquinolone exposure selects for reduced β-lactam susceptibility in methicillin-resistant *Staphylococcus aureus* and alterations in the SOS-mediated response. *Res Microbiol* 2009; **160**: 187–92.

**247** Blazquez J, Gomez-Gomez JM, Oliver A *et al*. PBP3 inhibition elicits adaptive responses in *Pseudomonas aeruginosa*. *Mol Microbiol* 2006; **62**: 84–99.

**248** Perez-Capilla T, Baquero MR, Gomez-Gomez JM *et al*. SOS-independent induction of *dinB* transcription by β-lactam-mediated inhibition of cell wall synthesis in *Escherichia coli*. *J Bacteriol* 2005; **187**: 1515–8.

**249** Prieto AI, Ramos-Morales F, Casadesus J. Repair of DNA damage induced by bile salts in *Salmonella enterica*. *Genetics* 2006; **174**: 575–84.

**250** Jolivet-Gougeon A, David-Jobert S, Tamanai-Shacoori Z *et al*. Osmotic stress-induced genetic rearrangements in *Escherichia coli* H10407 detected by randomly amplified polymorphic DNA analysis. *Appl Environ Microbiol* 2000; **66**: 5484–7.

**251** Petrosino JF, Galhardo RS, Morales LD *et al*. Stress-induced β-lactam antibiotic resistance mutation and sequences of stationary-phase mutations in the *Escherichia coli* chromosome. *J Bacteriol* 2009; **191**: 5881–9.

**252** Poole K. Resistance to β-lactam antibiotics. *Cell Mol Life Sci* 2004; **61**: 2200–23.

**253** Erill I, Campoy S, Barbe J. Aeons of distress: an evolutionary perspective on the bacterial SOS response. *FEMS Microbiol Rev* 2007; **31**: 637–56.

**254** Da Re S, Garnier F, Guerin E *et al*. The SOS response promotes *qnrB* quinolone-resistance determinant expression. *EMBO Rep* 2009; **10**: 929–33.

**255** Wang M, Jacoby GA, Mills DM *et al*. SOS regulation of *qnrB* expression. *Antimicrob Agents Chemother* 2009; **53**: 821–3.

**256** Cirz RT, Chin JK, Andes DR *et al*. Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biol* 2005; **3**: e176.

**257** Lu TK, Collins JJ. Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc Natl Acad Sci USA* 2009; **106**: 4629–34.

**258** Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 2004; **427**: 72–4.

**259** Guerin E, Cambray G, Sanchez-Alberola N *et al*. The SOS response controls integron recombination. *Science* 2009; **324**: 1034.

**260** Cambray G, Guerout AM, Mazel D. Integrons. *Annu Rev Genet* 2010; **44**: 141–66.

**261** Cambray G, Sanchez-Alberola N, Campoy S *et al*. Prevalence of SOS-mediated control of integron integrase expression as an adaptive trait of chromosomal and mobile integrons. *Mob DNA* 2011; **2**: 6.

**262** Gefen O, Balaban NQ. The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress. *FEMS Microbiol Rev* 2009; **33**: 704–17.

**263** Lewis K. Multidrug tolerance of biofilms and persister cells. *Curr Top Microbiol Immunol* 2008; **322**: 107–31.

**264** Mulcahy LR, Burns JL, Lory S *et al*. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. *J Bacteriol* 2010; **192**: 6191–9.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022

**265** Fauvart M, De Groote VN, Michiels J. Role of persister cells in chronic infections: clinical relevance and perspectives on anti-persister therapies. *J Med Microbiol* 2011; **60**: 699–709.

**266** Korch SB, Henderson TA, Hill TM. Characterization of the *hipA7* allele of *Escherichia coli* and evidence that high persistence is governed by (p)ppGpp synthesis. *Mol Microbiol* 2003; **50**: 1199–213.

**267** Wang X, Wood TK. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. *Appl Environ Microbiol* 2011; **77**: 5577–83.

**268** Keren I, Shah D, Spoering A *et al*. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J Bacteriol* 2004; **186**: 8172–80.

**269** Dorr T, Vulic M, Lewis K. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. *PLoS Biol* 2010; **8**: e1000317.

**270** von Eiff C. *Staphylococcus aureus* small colony variants: a challenge to microbiologists and clinicians. *Int J Antimicrob Agents* 2008; **31**: 507–10.

**271** Mitchell G, Brouillette E, Seguin DL *et al*. A role for sigma factor B in the emergence of *Staphylococcus aureus* small-colony variants and elevated biofilm production resulting from an exposure to aminoglycosides. *Microb Pathog* 2010; **48**: 18–27.

**272** Schaaff F, Bierbaum G, Baumert N *et al*. Mutations are involved in emergence of aminoglycoside-induced small colony variants of *Staphylococcus aureus*. *Int J Med Microbiol* 2003; **293**: 427–35.

**273** von Eiff C, Bettin D, Proctor RA *et al*. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. *Clin Infect Dis* 1997; **25**: 1250–1.

**274** Mitchell G, Seguin DL, Asselin AE *et al*. *Staphylococcus aureus* sigma B-dependent emergence of small-colony variants and biofilm production following exposure to *Pseudomonas aeruginosa* 4-hydroxy-2-heptylquinoline-N-oxide. *BMC Microbiol* 2010; **10**: 33.

**275** Lopez D, Vlamakis H, Kolter R. Biofilms. *Cold Spring Harb Perspect Biol* 2010; **2**: a000398.

**276** Hoiby N, Bjarnsholt T, Givskov M *et al*. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010; **35**: 322–32.

**277** Ito A, May T, Kawata K *et al*. Significance of *rpoS* during maturation of *Escherichia coli* biofilms. *Biotechnol Bioeng* 2008; **99**: 1462–71.

**278** Johnson M, Cockayne A, Morrissey JA. Iron-regulated biofilm formation in *Staphylococcus aureus* Newman requires *ica* and the secreted protein Emp. *Infect Immun* 2008; **76**: 1756–65.

**279** Hindre T, Bruggemann H, Buchrieser C *et al*. Transcriptional profiling of *Legionella pneumophila* biofilm cells and the influence of iron on biofilm formation. *Microbiology* 2008; **154**: 30–41.

**280** Mulcahy H, Lewenza S. Magnesium limitation is an environmental trigger of the *Pseudomonas aeruginosa* biofilm lifestyle. *PLoS ONE* 2011; **6**: e23307.

**281** Amarasinghe JJ, Scannapieco FA, Haase EM. Transcriptional and translational analysis of biofilm determinants of *Aggregatibacter actinomycetemcomitans* in response to environmental perturbation. *Infect Immun* 2009; **77**: 2896–907.

**282** Patriquin GM, Banin E, Gilmour C *et al*. Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. *J Bacteriol* 2008; **190**: 662–71.

**283** Harrison JJ, Ceri H, Turner RJ. Multimetal resistance and tolerance in microbial biofilms. *Nat Rev Microbiol* 2007; **5**: 928–38.

**284** Boehm A, Steiner S, Zaehringer F *et al*. Second messenger signalling governs *Escherichia coli* biofilm induction upon ribosomal stress. *Mol Microbiol* 2009; **72**: 1500–16.

**285** Chavez de Paz LE, Lemos JA, Wickstrom C *et al*. Role of (p)ppGpp in biofilm formation by *Enterococcus faecalis*. *Appl Environ Microbiol* 2012; **78**: 1627–30.

**286** Wang X, Kim Y, Hong SH *et al*. Antitoxin MqsA helps mediate the bacterial general stress response. *Nat Chem Biol* 2011; **7**: 359–66.

**287** White-Ziegler CA, Um S, Perez NM *et al*. Low temperature (23°C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. *Microbiology* 2008; **154**: 148–66.

**288** Poole K. *Pseudomonas aeruginosa*: resistance to the max. *Front Microbiol* 2011; **2**: 65.

**289** Ohki R, Giyanto, Tateno K *et al*. The BceRS two-component regulatory system induces expression of the bacitracin transporter, BceAB, in *Bacillus subtilis*. *Mol Microbiol* 2003; **49**: 1135–44.

Downloaded from https://academic.oup.com/jac/article/67/9/2069/878480 by guest on 20 August 2022