

Cryptic Prophages as Targets For Drug Development

Xiaoxue Wang^{1*} & Thomas K. Wood^{2,3*}

¹Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Marine
Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou
510301, PR China

²Department of Chemical Engineering and ³Department of Biochemistry and Molecular Biology,
Pennsylvania State University, University Park, Pennsylvania 16802-4400

*Correspondence should be addressed to xxwang@scsio.ac.cn or tuw14@psu.edu

Running title: Cryptic prophage drug targets

Keywords: Cryptic prophage, antibiotic resistance, antibiotic tolerance, horizontal gene transfer,
toxin-antitoxin system

ABSTRACT

Bacterial chromosomes may contain up to 20% phage DNA that encodes diverse proteins ranging from those for photosynthesis to those for autoimmunity; hence, phages contribute greatly to the metabolic potential of pathogens. Active prophages carrying genes encoding virulence factors and antibiotic resistance can be excised from the host chromosome to form active phages and are transmissible among different bacterial hosts upon SOS responses. Cryptic prophages are artifacts of mutagenesis in which lysogenic phage are captured in the bacterial chromosome: they may excise but they do not form active phage particles or lyse their captors. Hence, cryptic prophages are relatively permanent reservoirs of genes, many of which benefit pathogens, in ways we are just beginning to discern. Here we explore the role of active prophage- and cryptic prophage-derived proteins in terms of (i) virulence, (ii) antibiotic resistance, and (iii) antibiotic tolerance; antibiotic tolerance occurs as a result of the non-heritable phenotype of dormancy which is a result of activation of toxins of toxin/antitoxin loci that are frequently encoded in cryptic prophages. Therefore, cryptic prophages are promising targets for drug development.

INTRODUCTION

Bacteriophages and bacteria are the most abundant life forms on Earth. They also interact frequently, and each phage infection has the potential to introduce new genetic material into the bacterial host, thereby driving the evolution of bacteria. The introduction of novel genes by phages into the bacterial host can confer beneficial phenotypes that enable the exploitation of competitive environments (Canchaya et al 2003, Lawrence and Ochman 1998, Penadés et al 2015). For example, marine bacteriophage encode photosynthesis genes which may provide relief from intense sunlight in oceans for the phage and host (Mann et al 2003), as well as encode adaptive bacterial immune systems to provide immunity from competing phage known as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems (Bellas et al 2015).

Among the beneficial genes, phages provide DNA for virulence, resistance, and tolerance to antibiotics as three major factors for pathogens during infection. Resistance involves genetic mutations that allow for growth in the presence of antibiotics whereas tolerance involves metabolic dormancy from the activation of toxins of toxin/antitoxin (TA) systems that allows pathogens to sleep through a course of antibiotic treatment (Wood 2016).

Treating pathogenic bacteria that evade antibiotics has become a global issue. This review will focus on the virulence, resistance, and tolerance genes carried by prophages, the antibiotics or other chemicals that can trigger the spread of these genes by prophage excision/integration, and also some preventative strategies to treat pathogens by targeting prophages. We also emphasize ways to avoid the adverse effects of triggering virulence dissemination by prophages.

Prophages are reservoirs of virulence genes

Bacteriophages provide one of the most efficient vehicles for moving DNA sequences (their own and the host's DNA by mistake), via transduction, between bacterial cells. Horizontal transfer of genetic information by phages is much more prevalent than previously thought, and the environment plays a crucial role in the phage-mediated transfer of virulence genes (Penadés et al 2015). The relatively high numbers of phage (10^{30} phage and approximate ratio of 10 phage to each bacterium) leads to frequent lytic and lysogenic phage infections (10^{25} infections/sec) (Chibani-Chennoufi et al 2004). Unlike lytic phages, temperate phages are integrated into the bacterial genome and maintain a long-term lysogenic

45 relationship with their hosts (**Figure 1**). Lysogeny has a unique role within the bacterium-phage arms race in that it favors the development of a symbiotic relationship by providing an ecological window for the evolution of mutually-beneficial functions (Feiner et al 2015). Hence, these frequent phage infections provide ample opportunity to affect virulence.

There are major differences both between and within different bacterial species in their ability to
 50 cause infection. Opportunistic and pathogenic bacterial species include *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Listeria* spp., *Salmonella enterica*, *Enterococcus faecalis*, *Streptococcus* spp., and *Staphylococcus* spp. A major driving force in the emergence and evolution of pathogenic isolates is the horizontal transfer and acquisition of virulence factors. Several mobile genetic elements (insertion sequences, plasmids, bacteriophages, and pathogenicity islands) have been implicated in the horizontal
 55 transfer of virulence genes; one of the most significant groups is the bacteriophages (reviewed in (Hastings et al 2004)).

Lysogenic conversion by prophages encoding toxins and other virulence determinants is the most
 ostensible contribution to bacterial pathogenesis (Brussow et al 2004). Many diseases are caused by
 toxins that are encoded by phages such as diphtheria, cholera, dysentery, botulism, food poisoning,
 60 scalded skin syndrome, necrotizing pneumonia or scarlet fever (Hacker and Kaper 2000). Among these, exotoxin production such as scarlatinal toxin, cholera toxin and Shiga toxin is the among the best documented virulence factors (Wagner and Waldor 2002). Bacteriophages can also alter host physiology to increase virulence at different stages of infection, including bacterial adhesion, colonization, invasion, resistance to host immune defenses, and transmissibility among human host (as reviewed by (Wagner and
 65 Waldor 2002)).

The contribution of phages to pathogenicity was first discovered in streptococci in 1927 when it was
 shown that nontoxic streptococci acquired the ability to produce scarlatinal toxin through the phages
 released by the toxic streptococci (Frobisher and Brown 1927). In the 1950's, toxin-encoding
 bacteriophages of *Corynebacterium diphtheria* further linked phages to bacterial pathogenicity (Barksdale
 70 and Arden 1974, Freeman 1951, Groman 1953).

The cholera toxin of *Vibrio cholerae* illustrates well a case of how multiple phages contribute to
 bacterial pathogenicity (Brüssow and Hendrix 2002, Davis et al 2000, Karaolis et al 1998, Karaolis et al

1999, Waldor and Mekalanos 1996). Shiga toxin (Stx) is present in *Shigella dysenteriae* type 1 and shiga toxin producing *E. coli* (STEC). Two major classes of Stx are found in STEC, Stx1 and Stx2. The *stx* genes in *E. coli* strains are the central contributors to the virulence of enterohemorrhagic *E. coli* (EHEC), and EHEC infection can cause bloody diarrhea and can lead to hemolytic anemia, thrombocytopenia, renal failure, and even to death (Kaper et al 2004). Stx in *E. coli* O157:H7 is encoded as a late gene product by temperate bacteriophage integrated into the chromosome. Phage late genes, including *stx*, are silent in the lysogenic state, and Shiga toxin (Stx) production depends on the activation of the Stx prophage. Stress signals, including some induced by antibiotics, trigger the phage to enter the lytic cycle, and phage replication and Stx production occur concurrently (Kaper et al 2004).

Prophage also play a role in the virulence of *Pseudomonas aeruginosa*. *P. aeruginosa* is an important opportunistic pathogen with a broad host range (plants, invertebrates, and vertebrates) (Palleroni 1984), and *P. aeruginosa* is the most common cause of chronic lung infections in cystic fibrosis (CF) patients (Lyczak et al 2002). The Pf4 prophage is essential for several stages of the *P. aeruginosa* biofilm life cycle, and it significantly contributes to its virulence *in vivo* (Mai-Prochnow et al 2004, Rice et al 2009).

Salmonella enterica serovar Typhimurium harbors two functional prophages, *Gifsy-1* and *Gifsy-2* that contain virulence genes (Figuerola-Bossi and Bossi 1999). Prophage *Gifsy-2* carries the *sodC* gene for a periplasmic [Cu, Zn]-superoxide dismutase involved with the defense against killing by macrophages (Farrant et al 1997). The removal of both prophages leads to a significant attenuation of virulence, and the curing bacteria for the *Gifsy-2* prophage significantly reduces the ability of *S. enterica* serovar Typhimurium to establish a systemic infection in mice (Figuerola-Bossi and Bossi 1999).

In *Staphylococcus aureus*, expression of the phage encoded *sea*, *seg2*, *sek2* and *sak* toxins is greatly increased following prophage induction (Sumby and Waldor 2003). In *S. aureus*, *tst*, the gene that encodes toxic shock syndrome toxin, is carried by a 15 kb-long pathogenicity island (Hochhut and Waldor 1999). Prophage and prophage-like elements are also the major sources of variation between the genomes from *Streptococcus pyogenes* strains involved in two distinct pathologies, wound infections and rheumatic fever. The prophages encode several secreted proteins involved in the human–bacterium interaction, including the scarlet fever toxin.

The mammalian intestine is home to a dense community of bacteria and its associated bacteriophage,

which influence virulence. The Gram-positive bacterium *Enterococcus faecalis* is a natural inhabitant of the mammalian gastrointestinal tract and is commonly found in soil, sewage, water, and food, frequently through fecal contamination (Matos RC 2013). *E. faecalis* is an opportunistic pathogen that is a major cause of urinary tract infections, bacteremia and infective endocarditis, and *E. faecalis* V583 harbors a composite phage derived from two distinct chromosomally-encoded prophage elements. One prophage encodes the structural genes necessary for phage particle production and the other prophage is required for phage infection of susceptible host bacteria. *E. faecalis* V583 uses phage particles to establish and maintain dominance of its intestinal niche in the presence of closely-related competing strains. Recent studies of the human fecal virome show that temperate rather than lytic phages are long-term contributors to the microbial host phenotype through provision of adaptive genes (Reyes et al 2010).

Prophages are reservoirs of antibiotic resistance genes

Many microorganisms produce secondary metabolites with antimicrobial activities and release them into their natural habitats. These antibiotic-producing microorganisms are resistant to the antibiotics they produce, but for the non-resistant bacteria, they need to develop resistance mechanisms to ensure survival in these environments (Muniesa et al 2013). The presence of antibiotics in the environment may exert long-term selective pressure for the emergence and horizontal transmission of resistance mechanisms in the non-producing microorganisms. In recent years, the explosive spread of antibiotic-resistance determinants among pathogenic, commensal, and environmental bacteria has reached a global dimension. Prophages not only encode toxin genes for human pathogens but also carry genes that enhance the fitness of the bacterial cell in ecological niches (Hendrix 2003). For examples, prophage contain genes that provide protection from attack by other phages, such as restriction-modification systems (Vasu and Nagaraja 2013) and CRISPR/Cas systems (Deveau et al 2010). Growing evidence shows that phages also carry or transfer genes that participate in other cellular process such as inactivating antibiotics (Muniesa et al 2013). Erythromycin resistance methylases (Erm) confer resistance to three classes of clinically-important antibiotics (the macrolides, the lincosamides, and the streptogramins B), and are widespread in *Staphylococcus* and other bacterial species (Seppälä et al 1998). For example, in *Staphylococcus xylosus* isolated from bovine mastitis milk, a novel macrolide-lincosamide-streptogramin B resistance gene is located on a 53-kb prophage that is site-specifically integrated into the *S. xylosus*

chromosome (Wipf et al 2014). In *Staphylococcus fleurettii*, this gene is located in a genomic island
 130 which is site-specifically integrated into the housekeeping gene *guaA*, and exhibits the ability to
 circularize (Wipf et al 2015). Transfer of erythromycin resistance via prophages of clinically isolated
Streptococcus pyogenes was suggested to be responsible for the emergence of streptococci with multiple
 resistances in the clinical environment (Hyder and Streifled 1978). P1 bacteriophages lysogenize bacteria
 as independent plasmid-like elements, and a recent report shows that P1-like bacteriophage carrying
 135 SHV-2 extended-spectrum β -lactamase is present in clinical strains of *E. coli* (Billard-Pomares et al 2014).
 Acquired resistance to β -lactam antibiotics is conferred principally by β -lactamases and penicillin-binding
 proteins (Livermore and Woodford 2006). Two β -lactamase genes and one gene encoding a
 penicillin-binding protein have been detected in the bacteriophage DNA fraction of sewage, river water,
 and fecal waste from farmed animals, suggesting that bacteriophages can be environmental vectors for the
 140 horizontal transfer of antibiotic resistance genes (Colomer-Lluch and Muniesa 2011). Quinolone
 antibiotic resistance genes (*qnrA* and *qnrS*) have also been found in phage DNA isolated from urban
 wastewater and animal wastewater, suggesting that spreading genetic information via bacteriophages has
 gained importance in the resistance dissemination in environments (Colomer-Lluch et al 2014).

Prophages may also become trapped in the host genome due to mutation (Canchaya et al 2003); these
 145 inactive prophage elements are referred to as cryptic prophages (**Figure 1**), and they also play a role in
 antibiotic resistance and tolerance. For example, the *E. coli* K-12 genome has gained 1,600 kb of novel
 DNA (18%) since its divergence from *Salmonella* sp. 100 million years ago (Lawrence and Ochman
 1998), and contains one active Lambda prophage and nine cryptic prophages (Blattner et al 1997). At
 least cryptic prophage *rac* is a phage fossil that is present in other *E. coli* strains having been acquired
 150 over 4.5 million years ago, which appears more ancient than the Lambda prophage (Perna et al 2001).
 These cryptic prophage are not inactive DNA remnants generated in the course of host evolution but are
 important for host fitness in terms of both antibiotic and stress resistance (Wang et al 2010). For example,
 by deleting all the cryptic prophage genes in *E. coli* (166 kb), it has been shown that that cryptic
 prophages contribute significantly to resistance to sub-lethal concentrations of quinolone and β -lactam
 155 antibiotics and that the prophages are beneficial for withstanding osmotic, oxidative, and acid stresses
 (Wang et al 2010).

Specifically, the nine cryptic prophages in *E. coli* K-12 contain 165 putative genes, and 50 of them are related to antibiotic resistance either by transcriptome studies or in whole genome screening tests (Kohanski et al 2007, Peter et al 2004, Walker et al 2004). As shown in **Table 1**, 17 cryptic prophage genes affect antibiotic resistance by survival tests or metabolic activity assays using deletion strains or using plasmids to express these prophage genes. In particular, the products of *kilR* in *rac* prophage and *dicB* in *Qin* prophage are responsible for inhibiting cell division and are important for resistance to nalidixic acid (a quinolone) and azlocillin (a β -lactam) (Wang et al 2010). YdaC encoded by *rac* was identified in the screen for antibiotic resistance using pooled plasmids from the ASKA library that showed increased resistance to erythromycin (Soo et al 2011), and we have confirmed this phenotype in survival assays using two different constructs to express *ydaC* (unpublished data). Rac-like prophage is transmissible to other *E. coli* strains (Asadulghani et al 2009), thus enabling it to spread these resistance genes.

In Gram-negative bacteria, antimicrobial agents must traverse both the outer membrane and plasma membrane to gain entry into the cell. One of many effective cellular resistance strategies involves the extrusion of the antimicrobial from the cell by transporters, which may be anchored in the inner membrane or reside in the trans-membrane space and which are also encoded by cryptic prophage. For example, the transporter ethidium multidrug resistance protein E (EmrE) in *E. coli* is a proton-dependent secondary transporter from cryptic prophage DLP12 (Yerushalmi et al 1995). EmrE confers resistance to positively charged hydrophobic antibiotics such as tetracycline by actively expelling the drug (Viveiros et al 2005). Outer membrane protease OmpT from prophage DLP12 has been shown to increase resistance to streptomycin and chlortetracycline (Li et al 2008). The impact of OmpT on *E. coli* resistance to urinary cationic peptides was investigated by testing an *ompT* knockout strain, and OmpT may help the host persist longer in the urinary tract by enabling it to resist the antimicrobial activity of urinary cationic peptides (Hui et al 2010).

In other species, many resistance genes are located on genomic islands, and there are several common features shared by cryptic prophage and genomic islands. Both of them harbor phage integrase or excisionase that directly regulate the integration or excision of these mobile elements. Another common feature is the presence of two perfect or near-perfect repeats at the borders of these mobile

185 genetic elements, and they are used as site-specific recombination events during excision. P4 or P4-like integrase genes are normally adjacent to the tRNAs or tRNA modification genes, which serve as the phage attachments (Williams 2002). Mobile genetic elements that carry P4-like integrases are termed cryptic prophages in *E. coli* (e.g. CP4-6, CP4-44, CP4-57) but often are referred to as genomic islands or pathogenicity islands in other species such as *Salmonella* and *Shigella*. For example, the genomic island that carries resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline in *Salmonella enterica* Typhimurium phage type DT104 is flanked by a near perfect 18-bp repeat and inserted in the *trmE* gene encoding a tRNA modification enzyme (Cabedo et al 1999). The resistance locus pathogenicity island in *Shigella* spp. mediates resistance to streptomycin, ampicillin, chloramphenicol, and tetracycline, and it can be excised from the chromosome via site-specific recombination mediated by the P4-related integrase (Turner et al 2004). We have shown in *E. coli* that excisionase AlpA in CP4-57 can lead to a complete removal of CP4-57 prophage (Wang et al 2009) and that excisionase XisR in *rac* and a modified host protein H-NS can lead to a complete removal of *rac* prophage (Hong et al 2010, Liu et al 2015). Therefore, the global dissemination of multiple antibiotic resistances harbored by mobile genetic islands in pathogenic bacteria seems to be closely related to the site-specific recombination events.

Prophages are reservoirs of antibiotic tolerance genes

In many cases, TA loci are closely linked to mobile genetic elements. For example, *V. cholera* has 13 TA pairs and all of them are clustered in the megaintegron on ChrII, the smaller of its two chromosomes (Budde et al 2007, Gerdes et al 2005). Also, type I, type II and type IV TA loci have been identified in the nine cryptic prophages of *E. coli* (**Table 1**), indicating TAs are overrepresented in *E. coli* prophages (~8%). The presence of these toxins are important in that activation of all toxins to date leads to a dramatic increase in persister cells (Chowdhury et al 2016a, Wood 2016). So these cryptic prophages not only provide the means for antibiotic resistance, but they also provide the means to make the cells dormant and more persistent.

210 The type I toxin/antitoxin pair RalR/RalA in *E. coli* *rac* cryptic prophage increases resistance to broad-spectrum fosfomycin (Guo et al 2014), and the underlying mechanism remains to be determined. RelE toxin of Type II TA RelB/RelE in *E. coli* prophage Qin is a sequence-specific endoribonuclease

which blocks translation by cleavage of mRNAs (Christensen et al 2001, Neubauer et al 2009). Critically, RelE leads to high persister cell formation in the presence of high concentrations of ciprofloxacin, ampicillin, and tobramycin (Keren et al 2004, Koga et al 2011). Also, the toxin of the type II TA system RnlA/RnlB of the *E. coli* cryptic prophage CP4-57 causes inhibition of cell growth and rapid degradation of cellular mRNAs. The toxin of first recognized type IV TA pair, CbtA/CbeA in *E. coli* cryptic prophage CP4-44, not only inhibits cell growth, but alters cell shape by inhibiting the polymerization of cytoskeletal proteins FtsZ and MreB via direct protein-protein interaction (Masuda et al 2012). Moreover, this TA pair has related to resistance to norfloxacin, novobiocin, and spectinomycin (Tan et al 2011) (Kohanski et al 2007, Masuda et al 2012). The only other two homologous TA loci of CbtA/CbeA also reside in prophages, YkfL/YafW on *E. coli* cryptic prophage CP4-6 and YpjF/YfjZ on *E. coli* cryptic prophage CP4-57 (Brown and Shaw 2003). Interestingly, YkfL/YafW is related to the resistance to bacteriocin colicin E3 (Walker et al 2004), and YpjF/YfjZ is related to resistance to novobiocin (Peter et al 2004). One of the most striking features of these P4-like cryptic prophages in *E. coli* is that they are pervasively mosaic, with different segments seem to have distinct evolutionary histories (Brussow et al 2004). The presence of three homologous TA loci in three P4-like prophages (CP4-6, CP4-44, and CP4-57) suggests that horizontal genetic exchange plays a dominant role in shaping these genome architectures.

Antibiotics trigger prophage excision

UV irradiation and mitomycin C (MMC) are classical agents that can efficiently induce prophage excision in lysogenic bacteria (Otsuji et al 1959). The SOS response is induced by UV radiation or MMC, and it can also be activated by antibiotics that inhibit DNA replication or inhibit DNA gyrase activities to produce single-strand DNA (ssDNA). During the course of repair of DNA damage, ssDNA is produced (e.g., DNA crosslinks produced as a result of MMC). Trimethoprim (dihydrofolate reductase inhibitor) is an example of an SOS-inducing antibiotic that inhibits DNA replication (Lewin and Amyes 1991). Fluoroquinolones, broad-spectrum antibiotics that inhibit bacterial DNA gyrase and topoisomerase activity, also lead to SOS responses by generating DNA double-strand breaks (Drlica and Zhao 1997).

The genes coding for Shiga toxins are silent in lysogenic bacteria, and prophage induction is necessary for their efficient expression and toxin production. Both toxins are usually encoded in the genomes of bacteriophages (Stx phages), and they can lysogenize *E. coli* strains, thereby allowing a

mechanism for toxin dissemination via transfer of bacteriophages (Huang et al 1987, O'Brien et al 1984). Shiga toxin-producing EHEC (O157 Sakai) possesses 18 prophages that encode numerous genes related to EHEC virulence, including those for Shiga toxins and two other potent cytotoxins (Hayashi et al 2001). Nine out of the 18 prophages can be excised to form a circle by MMC-mediated induction, and three of them are transferable to the non-pathogenic commensal *E. coli* strain K-12 and stably maintained in the new host (Asadulghani et al 2009). The induction of Shiga toxin-converting prophages in EHEC also occurs in the presence of norfloxacin and under oxidative stress (Łoś et al 2010). Hence, these cryptic prophages have a high potential for disseminating virulence-related genes and other genetic traits to other bacteria under stress conditions, and stress activates pathogenicity.

Increased virulence caused by increased Stx production has been related to stx prophage induction both *in vitro* (Mühldorfer et al 1996) and *in vivo* (Zhang et al 2000). In particular, clinically-used antibiotics known to trigger the SOS response, including ciprofloxacin, have been shown to enhance Stx production (Mühldorfer et al 1996). The SOS response induced by MMC or fluoroquinolones causes enhanced intra-intestinal transfer of Stx2 prophages *in vivo* (Zhang et al 2000). Prophages of *E. faecalis* V583 excise from the bacterial chromosome in the presence of a fluoroquinolone, and are able to produce active phage progeny (Matos RC 2013). The *S. typhimurium* functional prophages, *Gifsy-1* and *Gifsy-2*, can be induced by exposing bacteria to hydrogen peroxide (Figueroa-Bossi and Bossi 1999). Recent studies have demonstrated that oxidative stress conditions may occur during colonization of the human intestine by enteric bacteria (Kumar et al., 2007). Moreover, earlier studies on a clinical isolate of EHEC suggested that hydrogen peroxide produced by human neutrophils, may increase the production of Stx2 (Wagner et al 2001).

Carbadox is a quinoxaline-di-N-oxide, and exposure of *Salmonella* sp. to carbadox induces prophages that can transfer virulence and antibiotic resistance genes to susceptible bacterial hosts (Stanton et al 2008). Carbadox frequently induces generalized transducing phages in multidrug-resistant phage type DT104 and DT120 isolates, resulting in the transfer of chromosomal and plasmid DNA that included antibiotic resistance genes (Brunelle B W 2014). Metagenomics approaches were used to evaluate the effect of two antibiotics in feed (carbadox and ASP250 [chlortetracycline, sulfamethazine, and penicillin]) on swine intestinal phage metagenomes (viromes), and the abundance of phage integrase-encoding genes

was significantly increased in the viromes of medicated swine over that in the viromes of non-medicated swine (Allen et al 2011). Prophage-like VSH-1 was detected in *Brachyspira hyodysenteriae* cultures treated with mitomycin C, carbadox, metronidazole, and hydrogen peroxide. Carbadox- and metronidazole-induced VSH-1 particles transmitted tylosin and chloramphenicol resistance determinants between *B. hyodysenteriae* strains (Stanton et al 2008).

As previously described for SOS induction by MMC, fluoroquinolone antibiotics, and trimethoprim (Goerke et al 2006), β -lactams are also capable of triggering prophage induction in *S. aureus* lysogens. β -lactam-mediated phage induction also resulted in replication and high-frequency transfer of the staphylococcal pathogenicity islands, showing that such antibiotics may have the unintended consequence of promoting the spread of bacterial virulence factors (Maiques et al 2006). β -lactam antibiotics are extracellular stimuli of the SOS response in *S. aureus* as well as in *E. coli* and demonstrate another case for horizontal dissemination of virulence factors.

Integrating conjugative elements (ICE) can also carry antibiotic resistance genes and recruit SOS responses to mobilize themselves from one bacterial genome to another by cell-to-cell contact (Hastings et al 2004). Therapeutic agents such as ciprofloxacin and MMC promote the spread of antibiotic resistance genes carried on ICE in *V. cholerae* among a variety of Gram-negative species including *E. coli* (Beaber et al 2004, Hochhut and Waldor 1999).

Drugs and phage

Since MMC and other antibiotics trigger SOS responses that may contribute the augmentation of toxin production by inducing Stx prophage induction, the treatment of infections using antibiotics that result in DNA damage and phage induction may lead to unexpected adverse consequences. Stimulation of gene transfer following bacterial exposure to fluoroquinolones should be considered an adverse effect, and clinical decisions regarding antibiotic selection for infectious disease therapy should include this potential risk. Antibiotics that inhibit protein synthesis, such as chloramphenicol, tetracycline and streptomycin, do not induce SOS responses, and neither do agents that act upon the outer membrane (Hastings et al 2004). The use of fosfomycin which is an inhibitor of cell-wall synthesis did not cause the intrainestinal transfer of Stx2 prophage transfer in mice (Zhang et al 2000), and effectively reduced the risks of hemolytic-uremic syndrome (Takeda 1998). Thus, efforts should be made in developing new

compounds with antimicrobial activities targeting more specific cellular functions/components rather than DNA replication. For example, lassomycin is a newly identified antibiotic that exhibits potent bactericidal activity against both growing and dormant mycobacteria. It binds to a highly acidic region of the ClpC1 ATPase complex and markedly stimulates its ATPase activity without stimulating ClpP1P2-catalyzed protein breakdown, which is essential for viability of mycobacteria (Gavrish et al 2014). Another newly identified antibiotic, teixobactin, from uncultured bacteria, inhibits bacterial cell wall synthesis by binding to a highly conserved motif of lipid II (precursor of peptidoglycan) and lipid III (precursor of cell wall teichoic acid) (Ling et al 2015).

In contrast to active prophages that are triggered to excise by the DNA repair (SOS) response, cryptic prophages usually stay as stable residents on the host chromosome under adverse growing conditions including during the SOS response. For example, for the nine prophages in *E. coli* K-12, ϕ 14 was the only inducible prophage upon MMC treatment (Wang et al 2010). Among the eight cryptic prophages that do not excise with MMC, two prophages were induced to excise during *E. coli* biofilm formation, thus providing benefits for the population by creating a subpopulation of prophage-excised cells with different biofilm-related phenotypes (Liu et al 2015, Wang et al 2009). Furthermore, ϕ 4 prophage excision has been linked to both cell death and lysis for *P. aeruginosa* cells in biofilms as filamentous-like prophage excision increases diversity in dispersing cells as well as impacts biofilm architecture and virulence (Rice et al 2009, Webb et al 2003). It is well established that biofilm provides increased tolerance towards antibiotic treatment (Costerton et al 1995), thus prophage can also indirectly contribute to antibiotic tolerance by promoting biofilm formation.

Moreover, cryptic prophages carrying TA systems can be activated during stress (Yamaguchi and Inouye 2011). During oxidative stress and starvation, proteases such as Lon and ClpXP degrade unstable antitoxins and releases free toxins (Christensen et al 2004, Maisonneuve et al 2013, Wang et al 2011, Wang and Wood 2011). It has been suggested that activating toxins by deactivating antitoxins of TA systems would be beneficial in terms fighting pathogens (Chan et al 2015); however, this approach is short-sighted in that this approach will indubitably lead to an increase in the numbers of pathogen persister cells (Shapiro 2013) since proteins that reduce growth such as toxins increase persistence (Chowdhury et al 2016a). Perhaps the best approach then, to target pathogens that utilize TA systems like

325 those encoded by phages to form persister cells, is to utilize a combination of drugs with one used to kill growing cells and another one to kill persister cells. Examples of this approach of targeting both growing and dormant cells include combining rifampicin with the acyldepsipeptide ADEP4 (Conlon et al 2013) and by combining cefoperazone and doxycycline with daptomycin (Feng et al 2015). Other possible approaches include using either mitomycin C (Kwan et al 2015) (for infections where it does not lead to
 330 extracellular toxin production like Shiga toxins) or cisplatin (Chowdhury et al 2016b) to kill simultaneously both actively growing pathogens as well as their persister cells; both compounds kill active and persister cells by crosslinking their DNA, both have been shown to be broadly effective against pathogens such as *P. aeruginosa*, EHEC, *S. aureus*, and *Borrelia burgdorferi* (Chowdhury et al 2016b, Kwan et al 2015, Sharma et al 2015), and both are approved by the Food and Drug Administration for
 335 human use.

As an interesting use of TA systems from cryptic prophage as drugs, toxin RelE from cryptic prophage Qin causes apoptosis when it is produced in a human osteosarcoma cell line (Yamamoto et al 2002). Unfortunately, although toxins of TA systems have many of the same targets as antibiotics, they are active only intracellularly; i.e., they are not effective when added extracellularly but must be
 340 translocated to the cell interior. For example, the Hok toxin of the Hok/Sok TA system is not active with Gram positive or Gram negative bacteria unless it is electroporated into the cell (Pecota et al 2003).

Also, other non-TA components of prophage have potential as drugs. For example, recent progress has been made in HIV-1 therapy by directed evolution of a site specific recombinase that can recognize a 34-bp sequence flanking the majority of the integrated provirus HIV-1; this evolved recombinase can
 345 efficiently and precisely remove the integrated provirus from infected cells and is efficacious on clinical HIV-1 isolates *in vitro* and *in vivo* (Karpinski et al 2016). Thus, targeting the excisionase of prophage may be a promising approach for treating both viral and bacterial infections.

PERSPECTIVES

There is growing need to understand phage-host interactions and bacterial-host interactions in
 350 complex systems, such as among gut microbiota. Phages can regulate the microbiome using different strategies, such as killing competing bacteria to allow lysogenic bacteria to thrive in niches with limited nutrients, by encoding toxins or virulence factors that increase pathogenicity, by encoding genes that

increase antibiotic tolerance, and by functioning as vehicles for the horizontal transfer of genes among different species of bacteria. Clearly, the spread of antibiotic resistance among pathogenic bacteria has become a serious global issue for public health, and the role of phage in this process should not be neglected. Increasing evidence has shown that prophages of commensal and environmental bacteria are also reservoirs of antibiotic resistance and tolerance, and their roles in the dissemination of resistance and tolerance to pathogenic bacteria through horizontal gene transfer should be recognized. Prophages can also carry new families of virulence, resistance and tolerance genes. Prophages or prophage elements can be identified and annotated in the sequenced bacterial genomes through web servers such as PHAST (Zhou et al 2011), Prophage Finder (Bose and Barber 2006), IslandViewer (Dhillon et al 2015), and MobilomerFINDER (Ou et al 2007). In addition, the virome sequences that are present in publicly available databases (e.g., MG-RAST (Keegan KP et al 2016)) can also be mined for the presence of virulence, resistance and tolerance genes inside phages and prophages.

The human gut also contains large amounts of free viral particles, most of them bacteriophages probably released after spontaneous induction of prophages of lysogenic bacteria in the gut (Breitbart et al 2003). A recent study by Gordon's group shows that temperate phages are prominent in fecal microbiota, and an *in vivo* mice study demonstrated the prophage induction in a fecal community occurs upon exiting the host (Penadés et al 2015). Moreover, host-associated bacteria often encounter various host-related stresses such as nutritional deprivation, oxidants, temperature upshifts, and low pH which can also trigger prophage excision. Therefore, the human microbiome and environmental microbiome projects that have been initiated throughout the world (Dubilier et al 2015), should strive to identify prophages and functional genes embedded in the prophages, given the prominent role of phage in virulence, antibiotic resistance, and antibiotic tolerance.

ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (Grant No. 2013CB955701 to XW), by the National Natural Science Foundation of China (NFSC31270214, NFSC31290233 to XW), and the Army Research Office (W911NF-14-1-0279 to T.W.). We thank for Drs. Yunxue Guo and Pengxia Wang at the South China Sea Institute of Oceanology for their help with the

380 table and the figure for this manuscript. XW is the recipient of the 1000-Youth Elite Program (the Recruitment Program of Global Experts in China), and T.W. is the Biotechnology Endowed Professor at the Pennsylvania State University.

***Conflict of interest statement.* None declared.**

Accepted Manuscript

REFERENCES

- Allen HK, Looft T, Bayles DO, Humphrey S, Levine UY, Alt D *et al* (2011). Antibiotics in feed induce prophages in swine fecal microbiomes. *mBio* **2**: e00260-00211.
- Asadulghani M, Ogura Y, Ooka T, Itoh T, Sawaguchi A, Iguchi A *et al* (2009). The defective prophage pool of *Escherichia coli* O157: Prophage–prophage interactions potentiate horizontal transfer of virulence determinants. *PLoS Pathog* **5**: e1000408.
- Barksdale L, Arden SB (1974). Persisting bacteriophage infections, lysogeny, and phage conversions. *Annu Rev Microbiol* **28**: 265-299.
- Beaber JW, Hochhut B, Waldor MK (2004). SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427**: 72-74.
- Bellas C, Anesio A, Barker G (2015). Analysis of virus genomes from glacial environments reveals novel virus groups with unusual host interactions. *Front Microbiol* **6**: 656.
- Bhaskar V, Kumar M, Manicka S, Tripathi S, Venkatraman A, S K (2011). Identification of biochemical and putative biological role of a xenolog from *Escherichia coli* using structural analysis. *Proteins: Structure, Function, and Bioinformatics* **79**: 1132-1142.
- Billard-Pomares T, Fouteau S, Jacquet ME, Roche D, Barbe V, Castellanos M *et al* (2014). Characterization of a P1-Like bacteriophage carrying an SHV-2 extended-spectrum β -lactamase from an *Escherichia coli* strain. *Antimicrob Agents Chemother* **58**: 6550-6557.
- Blattner FR, Guy Plunkett I, Bloch CA, Perna NT, Burland V, Riley M *et al* (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.
- Brüssow H, Hendrix RW (2002). Phage genomics: Small is beautiful. *Cell* **108**: 13-16.
- Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P *et al* (2003). Metagenomic analyses of an uncultured viral community from human feces. *J Bacteriol* **185**: 6220-6223.
- Brown JM, Shaw KJ (2003). A novel family of *Escherichia coli* toxin-antitoxin gene pairs. *J. Bacteriol.* **185**: 6600-6608.
- Brunelle B W BBL, Bearson S M D. [J]. , 2014, 5. (2014). Chloramphenicol and tetracycline decrease motility and increase invasion and attachment gene expression in specific isolates of multidrug-resistant *Salmonella enterica* serovar Typhimurium. *Front Microbiol* **5**: 801.
- Brussow H, Canchaya C, Hardt WD (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol Mol Biol Rev* **68**: 560 - 602.
- Budde PP, Davis BM, Yuan J, Waldor MK (2007). Characterization of a *higBA* toxin-antitoxin locus in

Vibrio cholerae. *J Bacteriol* **189**: 491-500.

Cabedo H, Macián F, Villarroja M, Escudero JC, Martínez-Vicente M, Knecht E *et al* (1999). The *Escherichia coli trmE* (*mnme*) gene, involved in tRNA modification, codes for an evolutionarily conserved GTPase with unusual biochemical properties. *EMBO J* **18**: 7063-7076.

Canchaya C, Proux C, Fournous G, Bruttin A, Brüssow H (2003). Prophage genomics. *Microbiol. Mol. Biol. Rev.* **67**: 238-276.

Chan WT, Balsa D, Espinosa M (2015). One cannot rule them all: Are bacterial toxins-antitoxins druggable? *FEMS Microbiology Reviews* **10.1093/femsre/fuv002**.

Chibani-Chennoufi S, Bruttin A, Dillmann M-L, Brüssow H (2004). Phage-host interaction: an ecological perspective. *J. Bacteriol.* **186**: 3677-3686.

Chowdhury N, Kwan BW, Wood TK (2016a). Persistence increases in the absence of the alarmone guanosine tetraphosphate by reducing cell growth. *Sci Rep* **6**: 20519.

Chowdhury N, Wood TL, Martínez-Vázquez M, García-Contreras R, Wood TK (2016b). DNA-crosslinker cisplatin eradicates bacterial persister cells. *Biotechnol Bioeng*/ DOI 10.1002/bit.25963.

Christensen SK, Mikkelsen M, Pedersen K, Gerdes K (2001). RelE, a global inhibitor of translation, is activated during nutritional stress. *Proc Natl Acad Sci USA* **98**: 14328-14333.

Christensen SK, Maenhaut-Michel G, Mine N, Gottesman S, Gerdes K, Van Melderen L (2004). Overproduction of the Lon protease triggers inhibition of translation in *Escherichia coli*: involvement of the *yefM-yoeB* toxin-antitoxin system. *Mol Microbiol* **51**: 1705-1717.

Colomer-Lluch M, Jofre J, Muniesa M (2014). Quinolone resistance genes (*qnrA* and *qnrS*) in bacteriophage particles from wastewater samples and the effect of inducing agents on packaged antibiotic resistance genes. *J Antimicrob Chemother* **69**: 1265-1274.

Colomer-Lluch M, Muniesa M (2011). Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS ONE* **6**: e17549.

Conlon BP, Nakayasu ES, Fleck LE, LaFleur MD, Isabella VM, Coleman K *et al* (2013). Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature* **503**: 365-370.

Costerton W, Lewandowski Z, Caldwell D, Korber D, Lappin-Scott H (1995). Microbial biofilms. *Annu Rev Microbiol* **49**: 711-745.

Davis BM, Lawson EH, Sandkvist M, Ali A, Sozhamannan S, Waldor MK (2000). Convergence of the secretory pathways for cholera toxin and the filamentous phage, CTX ϕ . *Science* **288**: 333-335.

- Deveau H, Garneau JE, Moineau S (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annu Rev Microbiol* **64**: 475-493.
- Drlica K, Zhao XL (1997). DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **61**: 377-394.
- Dubilier N, McFall-Ngai M, Zhao LP (2015). Microbiology: Create a global microbiome effort. *Nature* **526**: 631-634.
- Farrant JL, Sansone A, Canvin JR, Pallen MJ, Langford PR, Wallis TS *et al* (1997). Bacterial copper- and zinc-cofactored superoxide dismutase contributes to the pathogenesis of systemic salmonellosis. *Mol Microbiol* **25**: 785-796.
- Feiner R, Argov T, Rabinovich L, Sigal N, Borovok I, Herskovits AA (2015). A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nat Rev Micro* **13**: 641-650.
- Feng J, Auwaerter PG, Zhang Y (2015). Drug combinations against *Borrelia burgdorferi* persists *in vitro*: Eradication achieved by using daptomycin, cefoperazone and doxycycline. *PLoS ONE* **10**: e0117207.
- Figuerola-Bossi N, Bossi L (1999). Inducible prophages contribute to *Salmonella* virulence in mice. *Mol Microbiol* **33**: 167-176.
- Freeman VJ (1951). Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. *J Bacteriol* **61**: 675.
- Frobisher M, Brown J (1927). Transmissible toxicogenicity of streptococci. *Bull. Johns Hopkins Hosp.* **41**: 167-173.
- Gavriš E, Sit Clarissa S, Cao S, Kandror O, Spoering A, Peoples A *et al* (2014). Lassomycin, a ribosomally synthesized cyclic peptide, kills *Mycobacterium tuberculosis* by targeting the ATP-dependent protease ClpC1P1P2. *Chem Biol* **21**: 509-518.
- Gerdes K, Christensen S, Lobner-Olesen A (2005). Prokaryotic toxin-antitoxin stress response loci. *Nat Rev Microbiol* **3**: 371 - 382.
- Goerke C, Köller J, Wolz C (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. *Antimicrob Agents Chemothe* **50**: 171-177.
- Gotfredsen M, Gerdes K (1998). The *Escherichia coli relBE* genes belong to a new toxin-antitoxin gene family. *Mol Microbiol* **29**: 1065-1076.
- Groman NB (1953). Evidence for the induced nature of the change from nontoxigenicity to toxigenicity in

Corynebacterium diphtheriae as a result of exposure to specific bacteriophage. *J Bacteriol* **66**: 184.

Guo Y, Quiroga C, Chen Q, McAnulty MJ, Benedik MJ, Wood TK *et al* (2014). RalR (a DNase) and RalA (a small RNA) form a type I toxin–antitoxin system in *Escherichia coli*. *Nucl Acids Res* **42**: 6448-6462.

Hacker J, Kaper J (2000). Pathogenicity islands and the evolution of microbes *Annu Rev Microbiol* **54**: 641-679.

Hastings PJ, Rosenberg SM, Slack A (2004). Antibiotic-induced lateral transfer of antibiotic resistance. *Trends Microbiol* **12**: 401-404.

Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K *et al* (2001). Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res.* **8**: 11-22.

Hendrix RW (2003). Bacteriophage genomics. *Curr Opin Microbiol* **6**: 506-511.

Hochhut B, Waldor MK (1999). Site-specific integration of the conjugal *Vibrio cholerae* SXT element into *prfC*. *Mol Microbiol* **32**: 99-110.

Hong S, Wang X, Wood T (2010). Controlling biofilm formation, prophage excision and cell death by rewiring global regulator H-NS of *Escherichia coli*. *Microb Biotechnol* **3**: 344-356.

Huang A, Friesen J, Brunton JL (1987). Characterization of a bacteriophage that carries the genes for production of Shiga-like toxin 1 in *Escherichia coli*. *J Bacteriol* **169**: 4308-4312.

Hui C-Y, Guo Y, He Q-S, Peng L, Wu S-C, Cao H *et al* (2010). *Escherichia coli* outer membrane protease OmpT confers resistance to urinary cationic peptides. *Microbiol Immunol* **54**: 452-459.

Hyder S L, Streifeld MM (1978). Transfer of erythromycin resistance from clinically isolated lysogenic strains of *Streptococcus pyogenes* via their endogenous phage. *J Infect Dis* **138**: 281-286.

Kaper JB, Nataro JP, Mobley HL (2004). Pathogenic *Escherichia coli*. *Nat Rev Micro*: 123-140.

Karaolis DKR, Johnson JA, Bailey CC, Boedeker EC, Kaper JB, Reeves PR (1998). A *Vibrio cholerae* pathogenicity island associated with epidemic and pandemic strains. *Proc Natl Acad Sci USA* **95**: 3134-3139.

Karaolis DKR, Somara S, Maneval DR, Johnson JA, Kaper JB (1999). A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* **399**: 375-379.

Karpinski J, Hauber I, Chemnitz J, Schafer C, Paszkowski-Rogacz M, Chakraborty D *et al* (2016). Directed evolution of a recombinase that excises the provirus of most HIV-1 primary isolates with high

specificity. *Nat Biotech* **34**:401-409.

Keren I, Shah D, Spoering A, Kaldalu N, Lewis K (2004). Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J. Bacteriol.* **186**: 8172-8180.

Koga M, Otsuka Y, Lemire Sb, Yonesaki T (2011). *Escherichia coli* *rnlA* and *rnlB* compose a novel toxin-antitoxin system. *Genetics* **187**: 123-130.

Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ (2007). A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**: 797-810.

Kwan BW, Chowdhury N, Wood TK (2015). Combatting bacterial infections by killing persister cells with mitomycin C. *Environ Microbiol* **17** 4406-4414.

Lawrence JG, Ochman H (1998). Molecular archaeology of the *Escherichia coli* genome. *Proc. Natl. Acad. Sci. USA* **95**: 9413-9417.

Lewin CS, Amyes SGB (1991). The role of the SOS response in bacteria exposed to zidovudine or trimethoprim. *J Med Microbiol* **34**: 329-332.

Li H, Wang B-C, Xu W-J, Lin X-M, Peng X-X (2008). Identification and network of outer membrane proteins regulating streptomycin resistance in *Escherichia coli*. *J Proteome Res* **7**: 4040-4049.

Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP *et al* (2015). A new antibiotic kills pathogens without detectable resistance. *Nature* **517**: 455-459.

Liu X, Li Y, Guo Y, Zeng Z, Li B, Wood TK *et al* (2015). Physiological function of *rac* prophage during biofilm formation and regulation of *rac* excision in *Escherichia coli* K-12. *Sci Rep* **5**: 16074.

Livermore DM, Woodford N (2006). The β -lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol* **14**: 413-420.

Łoś JM, Łoś M, Węgrzyn A, Węgrzyn G (2010). Hydrogen peroxide-mediated induction of the Shiga toxinconverting lambdoid prophage ST2-8624 in *Escherichia coli* O157:H7. *FEMBS Immunol Med Microbiol* **58**: 322-329.

Lyczak JB, Cannon CL, Pier GB (2002). Lung infections associated with cystic fibrosis. *Clinical Microbiol Rev* **15**: 194-222.

Mühldorfer I, Hacker J, Keusch GT, Acheson DW, Tschäpe H, Kane AV *et al* (1996). Regulation of the Shiga-like toxin II operon in *Escherichia coli*. *Infect Immun* **64**: 495-502.

Mai-Prochnow A, Evans F, Dalisay-Saludes D, Stelzer S, Egan S, James S *et al* (2004). Biofilm development and cell death in the marine bacterium *Pseudoalteromonas tunicata*. *Appl Environ*

Microbiol **70**: 3232-3238.

Maiques E, Úbeda C, Campoy S, Salvador N, Lasa Í, Novick RP *et al* (2006). β -Lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in *Staphylococcus aureus*. *J Bacteriol* **188**: 2726-2729.

Maisonneuve E, Shakespeare LJ, Jørgensen MG, Gerdes K (2011). Bacterial persistence by RNA endonucleases *Proc. Natl. Acad. Sci. USA* **108**: 13206-13211.

Maisonneuve E, Castro-Camargo M, Gerdes K (2013). (p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell* **154**: 1140-1150.

Mann NH, Cook A, Millard A, Bailey S, Clokie M (2003). Marine ecosystems: Bacterial photosynthesis genes in a virus. *Nature* **424**: 741-741.

Masuda H, Tan Q, Awano N, Wu K, Inouye M (2012). YeeU enhances the bundling of cytoskeletal polymers of MreB and FtsZ, antagonizing the CbtA (YeeV) toxicity in *Escherichia coli*. *Mol. Microbiol.* **84**: 979-989.

Matos RC LN, Rigottier-Gois L, Debarbieux L, Meylheuc T, Gonzalez-Zorn B, *et al.* (2013). *Enterococcus faecalis* prophage dynamics and contributions to pathogenic traits. *PLoS Genet* **9**(6): e1003539.

Morimyo M, Hongo E, Hamainaba H, Machida I (1992). Cloning and characterization of the Mvrc gene of *Escherichia coli* K-12 which confers resistance against methyl viologen toxicity. *Nucleic Acids Res* **20**: 3159-3165.

Muniesa M, Colomer-Lluch M, Jofre J (2013). Potential impact of environmental bacteriophages in spreading antibiotic resistance genes. *Future Microbiol* **8**: 739-751.

Neubauer C, Gao Y-G, Andersen KR, Dunham CM, Kelley AC, Hentschel J *et al* (2009). The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell* **139**: 1084-1095.

O'Brien A, Newland J, Miller S, Holmes R, Smith H, Formal S (1984). Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* **226**: 694-696.

Otsuji N, Sekiguchi M, Iijima T, Takagi Y (1959). Induction of phage formation in the lysogenic *Escherichia coli* K-12 by Mitomycin C. *Nature* **184**: 1079-1080.

Palleroni NJ (1984). Pseudomonadaceae. In: Kreig N.R HJG (ed). *Bergey's manual of systematic bacteriology*. Williams & Wilkins Co.: Baltimore, MD. pp 141-199.

- Pecota DC, Osapay G, Selsted ME, Wood TK (2003). Antimicrobial properties of the *Escherichia coli* R1 plasmid host killing peptide. *J Biotech* **100**: 1-12.
- Penadés JR, Chen J, Quiles-Puchalt N, Carpena N, Novick RP (2015). Bacteriophage-mediated spread of bacterial virulence genes. *Curr Opin Microbiol* **23**: 171-178.
- Perna NT, Plunkett G, Burland V, Mau B, Glasner JD, Rose DJ *et al* (2001). Genome sequence of enterohaemorrhagic *Escherichia coli* O157: H7. *Nature* **409**: 529-533.
- Peter B, Arsuaga J, Breier A, Khodursky A, Brown P, Cozzarelli N (2004). Genomic transcriptional response to loss of chromosomal supercoiling in *Escherichia coli*. *Genome Biol* **5**: R87.
- Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F *et al* (2010). Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* **466**: 334-338.
- Rice SA, Tan CH, Mikkelsen PJ, Kung V, Woo J, Tay M *et al* (2009). The biofilm life cycle and virulence of *Pseudomonas aeruginosa* are dependent on a filamentous prophage. *ISME J* **3**: 271-282.
- Sabina J, Dover N, Templeton LJ, Smulski DR, Soll D D, LaRossa RA (2003). Interfering with different steps of protein synthesis explored by transcriptional profiling of *Escherichia coli* K-12. *J. Bacteriol.* **185**: 6158-6170.
- Seppälä H, Skurnik M, Soini H, Roberts MC, Huovinen P (1998). A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. *Antimicrob Agents Chemother* **42**: 257-262.
- Shapiro S (2013). Speculative strategies for new antibacterials: all roads should not lead to Rome. *J Antibiot* **66**: 371-386.
- Sharma B, Brown AV, Matluck NE, Hu LT, Lewis K (2015). *Borrelia burgdorferi*, the causative agent of Lyme disease, forms drug-tolerant persister cells. *Antimicrob Agents Chemother* **59** (8): 4616-4624.
- Soo VW, Hanson-Manful P, Patrick WM (2011). Artificial gene amplification reveals an abundance of promiscuous resistance determinants in *Escherichia coli*. *Proc Natl Acad Sci U S A* **108**: 1484-1489.
- Stanton TB, Humphrey SB, Sharma VK, Zuerner RL (2008). Collateral effects of antibiotics: carbadox and metronidazole induce VSH-1 and facilitate gene transfer among *Brachyspira hyodysenteriae* strains. *Appl Environ Microbiol* **74**: 2950-2956.
- Sumby P, Waldor MK (2003). Transcription of the toxin genes present within the *Staphylococcal* phage ϕ Sa3ms is intimately linked with the phage's life cycle. *J Bacteriol* **185**: 6841-6851.
- Takeda T (1998). Early use of fosfomycin for Shiga toxin-producing *Escherichia coli* O157 infection reduces the risk of Hemolytic-Uremic syndrome. *Escherichia coli* O157:H7 and other shiga toxin producing *E.coli* strains.: 385-387.

Tan Q, Awano N, Inouye M (2011). YeeV is an *Escherichia coli* toxin that inhibits cell division by targeting the cytoskeleton proteins, FtsZ and MreB. *Mol Microbiol* **79**: 109-118.

Turner SA, Luck SN, Sakellaris H, Rajakumar K, Adler B (2004). Role of attP in integrase-mediated integration of the *Shigella* resistance locus pathogenicity island of *Shigella flexneri*. *Antimicrob Agents Chemother* **48**: 1028-1031.

Vasu K, Nagaraja V (2013). Diverse functions of restriction-modification systems in addition to cellular defense. *Microbiol Mol Biol Rev* **77**: 53-72.

Viveiros M, Jesus A, Brito M, Leandro C, Martins M, Ordway D *et al* (2005). Inducement and reversal of tetracycline resistance in *Escherichia coli* K-12 and expression of proton gradient-dependent multidrug efflux pump genes. *Antimicrob Agents Chemother* **49**: 3578-3582.

Wagner PL, Acheson DWK, Waldor MK (2001). Human neutrophils and their products induce Shiga toxin production by Enterohemorrhagic *Escherichia coli*. *Infect Immun* **69**: 1934-1937.

Wagner PL, Waldor MK (2002). Bacteriophage control of bacterial virulence. *Infect Immun* **70**: 3985-3993.

Waldor MK, Mekalanos JJ (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272**: 1910 - 1914.

Walker D, Rolfe M, Thompson A, Moore GR, James R, Hinton JCD *et al* (2004). Transcriptional profiling of colicin-induced cell death of *Escherichia coli* MG1655 identifies potential mechanisms by which bacteriocins promote bacterial diversity. *J Bacteriol* **186**: 866-869.

Wang X, Kim Y, Wood TK (2009). Control and benefits of CP4-57 prophage excision in *Escherichia coli* biofilms. *ISME J*. **3**: 1164-1179.

Wang X, Kim Y, Ma Q, Hong SH, Pokusaeva K, Sturino JM *et al* (2010). Cryptic prophages help bacteria cope with adverse environments. *Nat Commun* **1**: 147.

Wang X, Kim Y, Hong SH, Ma Q, Brown BL, Pu M *et al* (2011). Antitoxin MqsA helps mediate the bacterial general stress response. *Nat. Chem. Biol.* **7**: 359-366.

Wang X, Wood TK (2011). Toxin/antitoxin systems influence biofilm and persister cell formation and the general stress response. *Appl. Environ. Microbiol.* **77**: 5577-5583.

Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B *et al* (2003). Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* **185**: 4585-4592.

Williams KP (2002). Integration sites for genetic elements in prokaryotic tRNA and tmRNA genes:

sublocation preference of integrase subfamilies. *Nucl Acids Res* **30**: 866-875.

Wipf JRK, Schwendener S, Perreten V (2014). The novel macrolide-lincosamide-streptogramin B resistance gene *erm*(44) is associated with a prophage in *Staphylococcus xylosus*. *Antimicrob Agents Chemother* **58**: 6133-6138.

Wipf JRK, Schwendener S, Nielsen JB, Westh H, Perreten V (2015). The New Macrolide-Lincosamide-Streptogramin B resistance gene *erm*(45) is located within a genomic island in *Staphylococcus fleurettii*. *Antimicrob Agents Chemother* **59**: 3578-3581.

Wood TK (2016). Combatting bacterial persister cells. *Biotechnol Bioeng* **113**: 476-483.

Yamaguchi Y, Inouye M (2011). Regulation of growth and death in *Escherichia coli* by toxin-antitoxin systems. *Nat Rev Micro* **9**: 779-790.

Yamamoto TAM, Gerdes K, Tunnacliffe A (2002). Bacterial toxin RelE induces apoptosis in human cells. *FEBS Lett* **519**: 191-194.

Yerushalmi H, Lebendiker M, Schuldiner S (1995). EmrE, an *Escherichia coli* 12-kDa multidrug transporter, exchanges toxic cations and H⁺ and is soluble in organic solvents. *J Biol Chem* **270**: 6856-6863.

Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DWK (2000). Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in Mice. *J Infect Dis* **181**: 664-670.

Table 1. Summary of genes that participate in antibiotic resistance or tolerance in the cryptic prophages of *E. coli* K-12.

Gene name	Prophage	Function	Antibiotics tested	References
<i>ydaC</i>	Rac	Putative double-strand break reduction protein	Erythromycin	(Soo et al 2011)
<i>ralR</i>	Rac	DNase, toxin of type I TA pair RalR/RalA	Fosfomycin	(Guo et al 2014)
<i>kilR</i>	Rac	Toxin, Ftsz inhibitor	Novobiocin; Bicyclomycin; Azlocillin	(Peter et al 2004, Sabina et al 2003)
<i>relE</i>	Qin	Toxin of TA pair RelE/RelB, Sequence-specific endoribonuclease	Cefotaxime; Ofloxacin; Tobramycin; Ciprofloxacin; Ampicillin	(Gottfredsen and Gerdes 1998, Keren et al 2004, Maisonneuve et al 2011)
<i>dicB</i>	Qin	Control of cell division	Azlocillin	(Wang et al 2010)
<i>hokD</i>	Qin	Small toxic membrane polypeptide	Kanamycin; Novobiocin	(Kohanski et al 2007, Peter et al 2004)
<i>emrE</i>	DLP12	Multidrug resistance pump	Methyl viologen; Tetracycline; Ethidium; Tetraphenylphosphonium	(Morimyo et al 1992, Yerushalmi et al 1995),
<i>ompT</i>	DLP12	Outer membrane protease	Streptomycin; Chlortetracycline	(Hui et al 2010, Li et al 2008)
<i>yfdO</i>	CPS-53	Uncharacterized protein	Lidocane; Nalidixic acid	(Soo et al 2011)
<i>rnlA</i>	CP4-57	Toxin of RnlA/RnlB TA pair	Gentamicin	(Koga et al 2011)
<i>yfiZ</i>	CP4-57	Antitoxin of putative TA pair Ypjf-Yfjz	Novobiocin	(Peter et al 2004)
<i>ypjF</i>	CP4-57	Toxin of putative TA pair Ypjf-Yfjz	Novobiocin	(Peter et al 2004)
<i>yeeU</i>	CP4-44	Antitoxin of TA pair YeeU/YeeV, cytoskeleton bundling-enhancing factor A	Norfloxacin; Novobiocin	(Masuda et al 2012, Sabina et al 2003)
<i>yeeV</i>	CP4-4	Toxin of TA pair YeeU/YeeV, cytoskeleton binding toxin	Norfloxacin; Spectinomycin	(Masuda et al 2012)
<i>ykfI</i>	CP4-6	Toxin of putative TA pair YkfI-YafW	Colicin E3	(Walker et al 2004)
<i>yafW</i>	CP4-6	Antitoxin of putative TA pair YkfI-YafW	Colicin E3; Kasugamycin	(Walker et al 2004)
<i>yagE</i>	CP4-6	2-keto-3-deoxy gluconate (KDG) aldolase	Novobiocin; Norfloxacin; Ampicillin; Streptomycin	(Bhaskar V et al 2011, Peter et al 2004)

Figure 1. Different types of phages and prophages. Genes in cryptic prophages are abbreviated as *int*: integrase, *xis*: excisionase, RM: Restriction-modification system, and TA: Toxin-antitoxin systems.

