The agricultural antibiotic carbadox induces phage-mediated gene transfer in *Salmonella*

**Bradley L. Bearson**¹*, **Heather K. Allen**²†, **Brian W. Brunelle**², **In Soo Lee**¹³, **Sherwood R. Casjens**⁴ and **Thaddeus B. Stanton**⁵

¹ Agroecosystems Management Research Unit, National Laboratory for Agriculture and the Environment, ARS, USDA, Ames, IA, USA  
² Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, ARS, USDA, Ames, IA, USA  
³ Department of Biological Sciences and Biotechnology, Hannam University, Daejeon, South Korea  
⁴ Division of Microbiology and Immunology, Department of Pathology, University of Utah, Salt Lake City, UT, USA

*Correspondence:*  
Bradley L. Bearson, Agroecosystems Management Research Unit, National Laboratory for Agriculture and the Environment, ARS, USDA, 2110 University Drive, NSRIC-2103, Ames, IA 50011, USA  
e-mail: brad.bearson@ars.usda.gov

†These authors have contributed equally to this work.

**INTRODUCTION**

Antibiotics are used for disease therapeutic or preventative effects in humans and animals, as well as for enhanced feed conversion efficiency in livestock. Antibiotics can also cause undesirable effects in microbial populations, including selection for antibiotic resistance, enhanced pathogen invasion, and stimulation of horizontal gene transfer. Carbadox is a veterinary antibiotic used in the US during the starter phase of swine production for improved feed efficiency and control of swine dysentery and bacterial swine enteritis. Carbadox has been shown *in vitro* to induce phage-encoded Shiga toxin in Shiga toxin-producing *Escherichia coli* (STEC) and a phage-like element transferring antibiotic resistance genes in *Brachyspira hyodysenteriae*, but the effect of carbadox on prophages in other bacteria is unknown. This study examined carbadox exposure on prophage induction and genetic transfer in *Salmonella enterica* serovar Typhimurium, a human foodborne pathogen that frequently colonizes swine without causing disease. *S. Typhimurium* LT2 exposed to carbadox induced prophage production, resulting in bacterial cell lysis and release of virions that were visible by electron microscopy. Carbadox induction of phage-mediated gene transfer was confirmed by monitoring the transduction of a sodCIII::neo cassette in the Fels-1 prophage from LT2 to a recipient *Salmonella* strain. Furthermore, carbadox frequently induced 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. Our research indicates that exposure of *Salmonella* to carbadox induces prophages that can transfer virulence and antibiotic resistance genes to susceptible bacterial hosts. Carbadox-induced, phage-mediated gene transfer could serve as a contributing factor in bacterial evolution during animal production, with prophages being a reservoir for bacterial fitness genes in the environment.

**Keywords:** *Salmonella*, bacteriophage, antibiotic, carbadox, prophage, gene transfer, transduction
et al., 2004). Sub-inhibitory antibiotics promote resistance gene transfer among gut bacteria via transposons (Shoemaker et al., 2001; Song et al., 2009), plasmids (Feld et al., 2008), and phage-like gene transfer agents (GTAs) (Stanton et al., 2008). The agricultural antibiotic carbadox is frequently used in the US during the starter phase of swine production for performance enhancement and control of enteric diseases. Carbadox is an antibacterial agent used exclusively in animals. For growth promotion and disease prophylaxis, swine feed contains 10–25 g/ton [11–28 mg/kg or parts-per-million (ppm)] and 50 g/ton [55 mg/kg (ppm)], respectively. Carbadox, a quinoxaline-di-N-oxide, is mutagenic, causing base pair substitutions and frameshift mutations in DNA (Beutin et al., 1981). A range of carbadox concentrations from 0.5 to 8 μg/ml (ppm) has been shown to induce prophages in Shiga toxin-producing Escherichia coli (STEC) (Kohler et al., 2000) and GTAs in Brachyspira hyodysenteriae (Stanton et al., 2008). However, it is unknown what effect carbadox would have on prophages encoded by other bacterial species, including those native to S. Typhimurium strains.

Salmonella strains have multiple prophage genomes integrated into their chromosomes. For example, the genome of S. Typhimurium strain LT2 contains four functional prophages: Gifsy-1 and -2 and Fels-1 and -2 (McClelland et al., 2001; Casjens, 2011). Investigation of prophages in S. Typhimurium indicates that many of these prophages can be induced to produce infectious virions by various environmental signals including DNA damage, antibiotics such as mitomycin C, and hydrogen peroxide (Schicklmaier et al., 1998; Figueroa-Bossi and Bossi, 1999; Schmieger and Schicklmaier, 1999; Frye et al., 2005; Garcia-Russell et al., 2009). Furthermore, prophages often encode virulence genes that enhance the pathogenesis of the bacterial strain into which the prophage is integrated (Groman, 1955; O’Brien et al., 1984; Cheetham and Katz, 1995; Waldor and Mekalanos, 1996; Figueroa-Bossi and Bossi, 1999; Mirold et al., 1999; Figueroa-Bossi et al., 2001; Ho et al., 2002; Casjens and Hendrix, 2005).

Since Salmonella strains usually contain multiple functional prophages and frequently colonize the swine intestinal tract, the goal of the current study was to evaluate prophage induction and genetic transfer in S. Typhimurium following carbadox exposure. Our research demonstrates that carbadox induced prophage production, thereby generating infectious virions capable of transferring virulence and antibiotic resistance genes via a prophage genome or generalized transduction.

**METHODS**

**BACTERIAL STRAINS, MEDIA, AND CHEMICALS**

Bacterial strains (Table 1) were grown in LB (Lennox Laboratory Supplies, Dublin, Ireland) or E minimal medium containing 0.4% glucose (Vogel and Bonner, 1956). A 5 mg/ml carbadox (Sigma-Aldrich, St. Louis, MO, USA) stock solution was made in 0.1 N NaOH and used at a final concentration of 2.5 μg/ml unless noted otherwise. Other antibiotics were used at the following concentrations: ampicillin (100 μg/ml), kanamycin (50 μg/ml), tetracycline (20 μg/ml), chloramphenicol (30 μg/ml), and carbenicillin (50 μg/ml).

**S. TYPHIMURIUM GENE AND PROPHAGE KNOCKOUTS BY RECOMBINEEERING**

Oligonucleotide primers for PCR amplification and construction of gene and prophage knockouts are listed in Table 2. S. Typhimurium gene and prophage knockouts were constructed by recombineering (recombination-mediated genetic engineering) as previously described (Bearson and Bearson, 2008; Bearson et al., 2008). Briefly, the 5′ end of a gene knockout primer (bold, Table 2) has homology to 32–44 bp of the target gene whereas the 3′ end contains universal sequences (underlined) to amplify an antibiotic resistance gene and truncate potential translation of the target gene. A gene knockout primer set was used to PCR amplify either the neo or the cat gene. Gel electrophoresis was performed on the amplification product of a knockout fragment and the respective DNA fragment was gel extracted using a Freeze’n Squeeze column (Bio-Rad, Hercules, CA). Each knockout fragment was transformed (Sambrook and Russell, 2006) into an arabinose-induced S. Typhimurium strain containing the pKD46 plasmid (Datso and Wanner, 2000). Transformants containing the knockout were selected on LB agar medium containing kanamycin. If necessary, the gene knockout with the neo marker was moved to another strain background by transduction using a P22 phage with a high transduction frequency. Flp mediated deletion of the neo or cat gene was performed by transferring the pCP20 plasmid into the knockout strain by either transduction or transformation followed by a procedure to screen for loss of resistance to kanamycin (Cherepanov and Wackernagel, 1995).

**DETERMINATION OF PHAGE TITERS FOLLOWING CARBADOX INDUCTION OF BACTERIAL STRAINS LYSOGENIZED WITH THE MODEL PHAGES P22, λ, HK97, AND SF6**

Bacterial strains lysogenized with P22 (UB-1790), λ (UB-1703), HK97 (UB-1704), and SF6 (UB-1496) were grown in LB broth at 37°C with shaking. At a density of 1 × 10^8 bacterial cells/ml, carbadox was added to a final concentration of 0.5 μg/ml (ppm). At the indicated times after carbadox addition, aliquots of the cultures were removed, shaken with several drops of chloroform to complete lysis, and titered on the permissive host. Strains used to titer the phage lysates were DB7004 (P22), 594 (λ, and HK97), and UB-1458 (SF6).

**CARBADOX INDUCTION OF WILD-TYPE S. TYPHIMURIUM**

S. Typhimurium strains were grown in LB 0.4% glucose at 37°C with shaking at 180 rpm. Carbadox was added to cultures at OD_{560} = 0.2 at a final concentration of 2.5 μg/ml (ppm). Incubation of cultures was continued to monitor for bacterial cellular lysis.

**PHAGE TRANSDUCTION USING CARBADOX-INDUCED S. TYPHIMURIUM LYSATES**

Supernatants from non-induced and carbadox-induced bacterial cultures were harvested by adding 100 μl of chloroform, vortexing gently, and allowing the culture to incubate for ~15 min with shaking. The cultures were centrifuged at 1000× g for 20 min and the supernatant was transferred to a fresh tube containing 200 μl of chloroform for storage. Bacterial lysates were plated to LB to ensure that viable bacterial cells were not present. An
Table 1 | Bacterial strain list.

| Strain no. | Strain background | Genotype | Phenotype | Source |
|------------|-------------------|----------|-----------|--------|
| LT2 (BSX 1) | S. enterica LT2 | Wild-type | | John Foster |
| UB-0020 | S. enterica LT2 | leuA414, Fels2−, r−, supR | | Miriam Susskind |
| UB-1731 (BSX 97) | S. enterica LT2 | Δ(Fels-2 Gifsy-1 Gifsy-2) P22 UC-911 prophage | KnrR | Kelly Hughes (Bunny et al., 2002) |
| DB7004 | S. enterica LT2 | leuA414, supE | | Padilla-Meier et al., 2012 |
| ATTC 14028s | S. enterica | Wild-type | | Winston et al., 1979 |
| UK1 | S. enterica UK1 | Wild-type | StrR | John Foster |
| SL1344 | S. enterica SL1344 | Wild-type | StrR | John Foster |
| x4232 (BSX 8) | S. enterica x4232 | Wild-type | NalR | Tom Stabel |
| NCTC13348 | S. enterica DT104 | Wild-type | ApR, CamR, TetR, StrR, SuR, SpR | Public Health England |
| DT104-530 | S. enterica DT104 | Wild-type | ApR, CamR, TetR, StrR | This study |
| DT104-745 | S. enterica DT104 | Wild-type | ApR, CamR, TetR, StrR, KnR | This study |
| DT104b-5414 | S. enterica DT104 | Wild-type | ApR, CamR, TetR, StrR, KnR | This study |
| DT120-150 | S. enterica DT120 | Wild-type | ApR, CamR, TetR, StrR | This study |
| DT120-305 | S. enterica DT120 | Wild-type | ApR, CamR, TetR, StrR | This study |
| DT120-613 | S. enterica DT120 | Wild-type | ApR, CamR, TetR, StrR | This study |
| DT120-7055 | S. enterica DT120 | Wild-type | ApR, CamR, TetR, StrR | This study |
| DT193-1434 | S. enterica DT193 | Wild-type | ApR, CamR, TetR, StrR, KnR, NalR | This study |
| DT208-2348 | S. enterica DT208 | Wild-type | ApR, CamR, TetR, NalR | This study |
| U302-4715 | S. enterica U302 | Wild-type | ApR, CamR, TetR, | This study |
| BSX 7 | S. enterica TT22971 (LT2) | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 | ApR, 30°C | John Roth via Max Wu |
| BBS 119 | S. enterica LT2 | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 | ApR, 30°C | BSX 7 cured of pKD46 |
| BBS 120 | S. enterica LT2 | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 | ApR, 30°C | BSX 119 pCP20 |
| BBS 231 | S. enterica LT2 | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 | KnR | BSX 7/oBBI 197/198 |
| BBS 233 | S. enterica x4232 | hisDCBHA::neo | NalR, KnR | BSX 8 × HT BBS231 |
| BBS 243 | S. enterica x4232 | ΔhisDCBHA | NalR | BBS 233 × HT BBS 120 |
| BBS 561 | S. enterica LT2 | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 sodCII::neo | KnR | BSX 7/oBBI 300/301 neo |
| BBS 565 | S. enterica LT2 | sodCII::neo | KnR | BSX 1 × HT BBS 561 |
| BBS 649 | S. enterica DT104-745 | ΔfloR Δtet Δpse-1 | KnR | BSX 97 × HT BBS 561 |
| BBS 651 | S. enterica DT104-745 | ΔfloR Δtet Δpse-1 pKD46 | KnR, ApR, 30°C | BBS 649/pKD46 |
| BBS 998 | S. enterica UB-1731 | sodCII::neo | KnR | BSX 97 × HT BBS 561 |
| BBS 1004 | S. enterica LT2 | metA22 metE551 trpD2 ilv-452 leu pto (leaky) hasdLT6 hasdSA29 hasdB strA120 P22 UC-911 prophage | KnR | BSX 7/oBBI 302/439 neo |
| BBS 1008 | S. enterica LT2 | Δ(Fels-2 Gifsy-1 Gifsy-2) Fels-1::neo | KnR | BSX 97 × HT BBS 1004 |
| BBS 1010 | S. enterica DT104-745 | ΔfloR Δtet Δpse-1 hisDCBHA::cat | KnR, CamR | BBS 651/oBBI 197/198 cat |
| BBS 1012 | S. enterica DT104-745 | ΔfloR Δtet Δpse-1 ΔhisDCBHA | KnR | BBS 1010/pCP20 |
| UB-1703 | E. coli K-12 | λ prophage | | Roger Hendrix (Hendrix and Duda, 1992) |
| UB-1704 | E. coli K-12 594 | HK97 prophage | | Roger Hendrix |
| E. coli K-12 594 | 594 | StrR | | Weigle, 1966 |
| UB-1458 | S. flexneri PE577 | Wild-type | | Renato Morona (Casjens et al., 2004) |
| UB-1496 | S. flexneri PE577 | S6 prophage | | Renato Morona |

*Known antibiotic resistance phenotypes. Ap, Ampicillin; Cam, Chloramphenicol; Tet, Tetracycline; Str, Streptomycin; Su, Sulfamethoxazole; Sp, Spectinomycin; Kn, Kanamycin; Nal, Nalidixic acid.
Table 2 | Primers used in this study.

| Gene/phage target | Primer | Sequence (5′–3′)                  | References |
|-------------------|--------|----------------------------------|------------|
| hisD-A            | oBBI 197 | ctgatggcgctgcggcttatcaggcctacgtaatgc | This study |
|                   | oBBI 198 | cgttttgccagcattggatggcctccttaacg   |            |
| sodCIII           | oBBI 300 | gtttaacctgttgaatgcagttggcctcagcaggtattttttaaagctatagagcagtgacgtagtcgc | This study |
|                   | oBBI 301 | gttgaacagtgcctcagcaggtattttttaaagctatagagcagtgacgtagtcgc |            |
| Fels-1            | oBBI 302 | cattcattaaaaggaagagattgaactgtgaaataatccgatacctaaataagttgacgtagtcgc | This study |
|                   | oBBI 439 | cattactaactaactactataatgtgcctcagcaggtattttttaaagctatagagcagtgacgtagtcgc |            |
| P22-like          | ST104Gp1F | gacgccccgcacagtgcagcagta             | This study |
|                   | ST104Gp1R | acccggcaccgttaatctg                  |            |

Bold text indicates homology to the target gene/phage.
Underlined text indicates sequence for neo or cat amplification.

Overnight culture of the transduction recipient was grown in LB or LB glucose at 37°C with shaking. The transduction was performed with equal volumes of both the S. Typhimurium recipient strain and either the non-induced or the carbadox-induced culture supernatant. The transduction was incubated at 37°C for ~1–3 h before plating on the appropriate selective medium. To determine the transduction frequency for transfer of the histidine operon, the transduction was plated onto E glucose minimal medium. Transduction frequency for antibiotic resistance gene transfer (sodCIII:: neo in Fels-1, plasmid-encoded kanamycin, and chromosomally encoded tetracycline) was determined by plating the transduction to LB containing either kanamycin or tetracycline.

PHAGE PURIFICATION FOR ELECTRON MICROSCOPY

Overnight cultures were diluted 1:100 in 400 ml E glucose minimal medium and incubated at 37°C with shaking. At OD<sub>600</sub> = 0.5, carbadox was added to a final concentration of 2.5 μg/ml, and incubation continued until lysis was achieved. Phages were purified and visualized by electron microscopy as described previously (Humphrey et al., 1997) with the following modifications. Purified phages were negatively stained by mixing phage samples with an equal volume of phosphotungstic acid (2%, pH 7.0). Samples were deposited on Formvar-coated 200-mesh carbon-reinforced copper grids (Electron Microscopy Sciences, Hatfield, PA) and viewed with a FEI Tecnai G2 BioTWIN electron microscope (80 kV; Hillsboro, OR).

RESULTS AND DISCUSSION

CARBADOX INDUCES S. TYPHIMURIUM AND OTHER Enterobacteriaceae PROPHAGES TO CAUSE PHAGE REPLICATION AND CELL LYSIS

To test whether well-characterized prophages in S. enterica and other Enterobacteriaceae species are induced by carbadox, we monitored the infectious phages produced by carbadox-treated cultures of bacterial strains that were lysogenized by the “model system” prophages P22 (S. enterica), λ and HK97 (E. coli), and Sf6 (Shigella flexneri). In each case, at least partial clearing of the culture indicated a substantial fraction of the cells in the culture had lysed by about 2 h. All four prophages gave an approximately three-log increase in free phage in the culture (Figure 1), with about 10–200 progeny phage produced per bacterium that were initially present at the time of carbadox addition. Several concentrations of carbadox were tested, and 0.5 μg/ml (ppm) is shown as the minimum that gave good induction of P22. Carbadox induces the P22 Salmonella prophage as well as E. coli and S. flexneri prophages under these conditions, and this is not surprising given its apparent mechanistic similarities to the action of mitomycin C. Carbadox is a DNA damaging agent, and DNA damage induced by mitomycin C is known to induce the bacterial SOS pathway, which induces prophages. It is likely capable of induction of prophages from many if not all Enterobacteriaceae bacterial species, as well as more distinctly related bacterial phyla.
To examine prophage induction by carbadox in wild-type S. Typhimurium isolates containing their natural prophages, we initially examined S. Typhimurium LT2, a strain that is widely used in the study of Salmonella genetics in the laboratory (Lilleengen, 1948). Cultures of wild-type LT2 in early log phase growth were exposed to carbadox. The bacterial density of the culture abruptly decreased at ~2 h following exposure to 2.5 µg/ml (ppm) of carbadox (Figure 2). Mitomycin C exposure is known to result in the induction of prophage Fels-1 from strain LT2 (Garcia-Russell et al., 2009). The fact that carbadox induces wild-type phage λ (above), which is only known to be induced by the SOS function of activated RecA protein (Little, 1993), strongly supports this mechanism for carbadox-mediated induction. Thus, the decrease in LT2 cell density is almost certainly due to bacterial cell lysis resulting from prophage induction.

LT2 is known to carry four prophages. Amplification of the integrase gene from DNA extracted from phage heads indicated that at least one of these, the Fels-1 prophage, was induced following carbadox exposure (Stanton and Humphrey, unpublished data). The BBS 1008 strain is an LT2 derivative from which all four prophages have been deleted. Following exposure of BBS 1008 to 2.5 µg/ml of carbadox, the bacterial culture density did not decrease (Figure 2), indicating that these prophages are responsible for the bacterial cell lysis phenotype induced by carbadox exposure.

Examination of the purified phages by electron microscopy demonstrated the presence of mostly empty phage heads in the carbadox-induced culture of LT2 (data not shown). Treatment of S. Typhimurium LT2 and other strains with mitomycin C is known to induce Fels-1 plaque-forming phages with poor efficiency (Figueroa-Bossi and Bossi, 1999), so it is perhaps not surprising that whole phage particles were not seen. Nonetheless, phage heads were either greatly reduced or were not observed in the absence of carbadox for the LT2 strain and in the presence of carbadox for BBS 1008. These results confirm prophage induction in a natural wild-type S. Typhimurium isolate following carbadox exposure.

### CARBADOX EXPOSURE OF S. TYPHIMURIUM LT2 PROMOTES PHAGE TRANSDUCTION INTO A SUSCEPTIBLE BACTERIAL HOST

To monitor phage transduction frequency, a neo gene was inserted by recombining into the putative virulence factor sodCIII on the Fels-1 prophage of strain LT2 to create strain S. Typhimurium BBS 565. The carbadox-induced phage lysate from BBS 565 (LT2 sodCIII::neo) efficiently transduced the kanamycin-sensitive strain BBS 243, as demonstrated by $8 \times 10^3$ kanamycin-resistant transductants per ml of lysate. In the absence of carbadox induction, BBS 243 remained susceptible to kanamycin following transduction with the control supernatant from BBS 565. This indicates that carbadox-induced prophages can carry genetic material from a donor into a recipient bacterial strain.

### CARBADOX-INDUCTION OF MULTIDRUG-RESISTANT S. TYPHIMURIUM DT104 RESULTS IN GENERALIZED TRANSDUCTION OF CHROMOSOMAL AND PLASMID DNA

Generalized transduction involves the packaging of random host DNA (genomic or plasmid) into a bacteriophage particle and the transfer of that DNA to a recipient strain. Bacteriophage P22 is a generalized transducing phage that is commonly used for genetic experiments with Salmonella (Zinder and Lederberg, 1952; Kropinski et al., 2007). Although S. Typhimurium LT2 contains multiple prophages, it does not contain a P22-like prophage that performs generalized transduction. Some multidrug-resistant S. Typhimurium isolates like phage type DT104 harbor a P22-like prophage. This prophage has been described as PDT17, ST104, and prophage 1 (Schmieder and Schicklmaier, 1999; Tanaka et al., 2004; Cooke et al., 2008). To determine whether carbadox exposure could induce generalized transduction, S. Typhimurium DT104 NCTC13348 was exposed to carbadox. The culture lysed, and the phage-containing supernatant was harvested. The BBS 243 strain, a histidine auxotroph lacking the genes hisDCBAH, could be successfully transduced to his<sup>+</sup> with this lysate. Generalized transduction was observed by growth on minimal medium lacking histidine, demonstrating the transfer of the his operon from the his<sup>+</sup> donor (DT104) to the his<sup>−</sup> recipient (BBS 243) (Table 3). The results show that carbadox exposure of multidrug-resistant S. Typhimurium DT104 can stimulate generalized transduction and therefore chromosomal gene transfer.

The Salmonella genomic island-1 (SGI-1) is ~43 kb and typically contains chromosomally-encoded resistance genes for multiple antibiotics including ampicillin, chloramphenicol, and tetracycline within an integron (Boyd et al., 2001; Mulvey et al., 2006). We attempted to transduce SGI-1 but were unsuccessful. Due to the size of SGI-1, transduction of this entire island by P22 into another Salmonella strain that does not already contain a segment of SGI-1 is inefficient [P22 packages about 43.4 Kbp of DNA (Kropinski et al., 2007)]. To overcome this experimental limitation, we utilized a DT104 derivative (BBS 1012) as our recipient strain. The BBS 1012 strain is a histidine auxotroph that has an internal deletion within SGI-1, resulting in sensitivity to ampicillin, chloramphenicol, and tetracycline due to the loss of multiple antibiotic resistance genes. In addition, BBS 1012 contains a natural plasmid that confers kanamycin resistance. Transduction of BBS 1012 with the carbadox-induced phage lysate from wild-type S. Typhimurium

---

**TABLE 3**

| Strain | Transduction Efficiency |
|--------|-------------------------|
| S. Typhimurium BBS 243 | 8 × 10³ kanamycin-resistant transductants per ml of lysate |
| S. Typhimurium DT104 NCTC13348 | 0 |
| S. Typhimurium DT104 NCTC13348 + carbadox | 8 × 10³ kanamycin-resistant transductants per ml of lysate |

---

**FIGURE 2** Carbadox exposure of wild-type S. Typhimurium LT2 results in bacterial cell lysis. S. Typhimurium strains (LT2 and BBS 1008) were grown in LB glucose medium at 37°C with shaking. At OD<sub>600</sub> = 0.2 (arrow), carbadox (2.5 µg/ml) was added to cultures indicated by the closed symbols. The open symbols indicate control cultures without carbadox.

---

www.frontiersin.org
DT104-530 (kanamycin sensitive) resulted in the transfer of tetracycline resistance following selection on tetracycline-containing LB agar medium. Transduction into the BBS 1012 strain was confirmed by growth on medium containing kanamycin and the absence of growth on minimal medium without histidine. Furthermore, the initial selection of BBS 1012 on LB medium containing tetracycline resulted in 100% co-transduction of resistance to both chloramphenicol and carbenicillin. The floR and pse-1 genes encode resistance to chloramphenicol and carbenicillin, respectively, and these genes are located adjacent to tetG on SGI-1. These results indicate that exposing multidrug-resistant S. Typhimurium DT104 to carbadox can promote the transfer of numerous genes co-located within SGI-1 that encode resistance to multiple classes of antibiotics. Thus generalized transduction could participate in bacterial strain evolution by providing an assortment of antibiotic resistance genes for recombination within this important genomic region.

Although the SGI-1 integron in S. Typhimurium DT104 encodes multiple antibiotic resistance genes, some strains contain additional antibiotic resistance genes encoded on plasmids. Transduction of BBS 243 (kanamycin sensitive) with the carbadox-induced phage lysate from S. Typhimurium DT104 (745) resulted in bacterial growth on LB medium containing kanamycin, demonstrating the transfer of the plasmid encoding kanamycin resistance. Thus, carbadox exposure promoted generalized transduction of this natural plasmid as well as chromosomally-encoded antibiotic resistance genes in multidrug-resistant S. Typhimurium DT104.

### CARBADOX-INDUCED GENE TRANSFER IS A GENERAL PHENOMENON THAT OCCURS IN MULTIDRUG-RESISTANT S. TYPHIMURIUM STRAINS DT120 AND DT104

Since the prevalence of multidrug-resistant S. Typhimurium strains has increased over the last few decades, we wanted to determine whether carbadox-induced gene transfer is unique to S. Typhimurium DT104 or is a general property of multidrug-resistant S. Typhimurium strains. Phage lysates were harvested from several S. Typhimurium phage types following carbadox exposure and used to transduce the recipient strain BBS 243 (ΔhisDCBHA) with selection on E glucose minimal medium; several different phage types were investigated as these, by definition (i.e., DT), should have varying prophage content. Carbadox-induced phage lysates from several S. Typhimurium DT104 and DT120 isolates resulted in growth on minimal medium, indicating the transfer of the his operon to BBS 243 (Table 3). The results suggest that generalized transduction following carbadox induction is a common phenomenon for multidrug-resistant S. Typhimurium DT104 and DT120.

We PCR amplified the P22 gene l (encoding gp1/portal protein) from several of the DT104 and DT120 isolates that we have shown are capable of generalized transduction, suggesting that these multidrug-resistant isolates contain a P22-like prophage. To confirm that a phage capable of generalized transduction is required for carbadox-induced gene transfer, the P22-like prophage (prophage 1) was deleted from DT104 using recombineering. Gene transfer into BBS 243 was eliminated following transduction with a carbadox-induced phage lysate from the DT104 P22-like prophage knockout strain, indicating that the P22-like prophage is responsible for the generalized transduction from S. Typhimurium DT104. In support of this, the S. Typhimurium strains LT2, UK1, SL1344, and χ4232 (frequently investigated strains in the literature) are incapable of generalized transduction and do not contain a P22-like prophage. In contrast, genome scanning has identified integrated P22-like prophages in the genome sequences of isolates of S. enterica serovars Arizona, Cholerae, Dublin, Hadar, Heidelberg, Houtenae, Johannesburg, Mississippi, Montevideo, Newport, Paratyphi, Rubislaw, Schwarzengrund, Tennessee, Typhimurium, Uganda, Wandsworth, and Welteverden; this suggests that P22-like prophages are common among Salmonella serovars and the potential for strain evolution due to generalized transduction is perhaps underappreciated. The capability of generalized transduction among Salmonella serovars is reinforced with the knowledge that P22 phage lysates are known to be stable for many years in the laboratory. Likewise, an ecological significance of phages in the environment is that DNA encapsulated within a phage head is protected from nucleases and therefore can survive outside of a bacterial host until encountering a recipient.

Swine environments, including swine manure, have been shown to contain abundant phage populations (McLaughlin et al., 2006; Wang et al., 2010). Bacteriophage populations present in manure could be derived principally from prophage induction of bacteria present in manure or a combination of induction from within the swine gastrointestinal tract and in manure. Prophage induction can be stimulated by various environmental signals and stresses including ultraviolet light, hydrogen peroxide, mitomycin C, and carbadox. Analysis of fecal phage metagenomes from medicated swine administered in-feed antibiotics [carbadox or ASP250 (chlortetracycline, sulfamethazine, and penicillin)] compared to non-medicated swine suggested that prophages were induced with antibiotic treatment (Allen et al., 2011). Similar work with mouse fecal phage metagenomes has shown that

### Table 3 | Average frequency of generalized transduction per 0.5 ml of lysate from numerous S. Typhimurium donor strains into BBS 243.

| Salmonella donor strain | Not induced | Carbadox induced ± s.e.m. |
|-------------------------|-------------|-------------------------|
| LT2                     | 0           | 0                       |
| UB-1731                 | 0           | 0                       |
| ATTC 14028s             | 0           | 0                       |
| UK1                     | 0           | 0                       |
| SL1344                  | 0           | 0                       |
| χ4232                   | 0           | 0                       |
| DT104 (NCTC13348)      | 0           | 6 ± 1.7                 |
| DT104-530               | 0           | 291 ± 100.8             |
| DT104b-5414             | <1          | 117 ± 52.8              |
| DT120-150               | 0           | 124 ± 19.3              |
| DT120-305               | <1          | 420 ± 94.8              |
| DT120-613               | <1          | 86 ± 16.7               |
| DT120-7055              | 0           | 0                       |
| DT193-1434              | 0           | 0                       |
| DT208-2348              | 0           | 0                       |
| US02-4715               | 0           | 0                       |

The P22-like prophage (prophage 1) was deleted from DT104 suggesting that the P22-like prophage is responsible for the generalized transduction following carbadox induction is a common phenomenon for multidrug-resistant S. Typhimurium DT104 and DT120. We PCR amplified the P22 gene l (encoding gp1/portal protein) from several of the DT104 and DT120 isolates that we have shown are capable of generalized transduction, suggesting that these multidrug-resistant isolates contain a P22-like prophage. To confirm that a phage capable of generalized transduction is required for carbadox-induced gene transfer, the P22-like prophage (prophage 1) was deleted from DT104 using recombineering. Gene transfer into BBS 243 was eliminated following transduction with a carbadox-induced phage lysate from the DT104 P22-like prophage knockout strain, indicating that the P22-like prophage is responsible for the generalized transduction from S. Typhimurium DT104. In support of this, the S. Typhimurium strains LT2, UK1, SL1344, and χ4232 (frequently investigated strains in the literature) are incapable of generalized transduction and do not contain a P22-like prophage. In contrast, genome scanning has identified integrated P22-like prophages in the genome sequences of isolates of S. enterica serovars Arizona, Cholerae, Dublin, Hadar, Heidelberg, Houtenae, Johannesburg, Mississippi, Montevideo, Newport, Paratyphi, Rubislaw, Schwarzengrund, Tennessee, Typhimurium, Uganda, Wandsworth, and Welteverden; this suggests that P22-like prophages are common among Salmonella serovars and the potential for strain evolution due to generalized transduction is perhaps underappreciated. The capability of generalized transduction among Salmonella serovars is reinforced with the knowledge that P22 phage lysates are known to be stable for many years in the laboratory. Likewise, an ecological significance of phages in the environment is that DNA encapsulated within a phage head is protected from nucleases and therefore can survive outside of a bacterial host until encountering a recipient.

Swine environments, including swine manure, have been shown to contain abundant phage populations (McLaughlin et al., 2006; Wang et al., 2010). Bacteriophage populations present in manure could be derived principally from prophage induction of bacteria present in manure or a combination of induction from within the swine gastrointestinal tract and in manure. Prophage induction can be stimulated by various environmental signals and stresses including ultraviolet light, hydrogen peroxide, mitomycin C, and carbadox. Analysis of fecal phage metagenomes from medicated swine administered in-feed antibiotics [carbadox or ASP250 (chlortetracycline, sulfamethazine, and penicillin)] compared to non-medicated swine suggested that prophages were induced with antibiotic treatment (Allen et al., 2011). Similar work with mouse fecal phage metagenomes has shown that...
antibiotic treatment caused an increase in the abundance of phage-encoded antibiotic resistance genes (Modi et al., 2013). This suggests that antibiotic-induced phase-mediated transduction may contribute to antibiotic resistance gene transfer during animal production. Relatively little information is available concerning the extent of carbadox-induced prophage from bacteria, as *Salmonella* is only the third bacterial genus for which this response has been described (Kohler et al., 2000; Stanton et al., 2008). Additional information is needed to understand the capacity for carbadox to induce prophages during swine production since there is a potential for dissemination into the environment following manure application onto agricultural soils.

CONCLUSIONS

Prophages are a potential environmental reservoir for bacterial fitness genes and may drive the emergence of new epidemic clones (Brussow et al., 2004). Prophages integrated in the genomes of *Salmonella* strains can encode genes associated with virulence or antimicrobial resistance (Figueroa-Bossi and Bossi, 1999; Figueroa-Bossi et al., 2001; Moreno Switt et al., 2013). Therefore, the pathogenic potential of a particular *Salmonella* strain depends in part upon the prophage repertoire integrated into the bacterial genome, and acquisition of prophages could conceivably result in enhanced bacterial virulence or survival during host colonization. In this report, we demonstrate that exposure of several different *S. Typhimurium* isolates to the agricultural antibiotic carbadox resulted in the production of transducing particles capable of transferring the individual phage genome as well as chromosomal and plasmid DNA by generalized transduction.

AUTHOR CONTRIBUTIONS

Conceived and designed experiments: Bradley L. Bearson, Heather K. Allen, Sherwood R. Casjens, Thaddeus B. Stanton, and Brian W. Brunelle. Performed the experiments: Bradley L. Bearson, Heather K. Allen, Sherwood R. Casjens, In S. Lee, Brian W. Brunelle, and Thaddeus B. Stanton. Wrote and edited the manuscript: Bradley L. Bearson, Heather K. Allen, Sherwood R. Casjens, Brian W. Brunelle, and Thaddeus B. Stanton.

ACKNOWLEDGMENTS

We are indebted to Stephanie Jones, Kellie Winter, Samuel Humphrey, and Briony Atkinson for outstanding technical assistance, to Judi Stasko for excellence with electron microscopy, to Kassandra Wilson and Eddie Gilcrease for measurement of phage titers, and to Kelly Hughes, Roger Hendrix, and Renato Morona for the gift of bacterial strains. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

REFERENCES

Allen, H. K., Looft, T., Bayles, D. O., Humphrey, S., Levine, U. Y., Alt, D., et al. (2011). Antibiotics in feed induce prophages in swine fecal microbiomes. *MBio* 2, 1–9. doi: 10.1128/mBio.00260-11

Bearson, B. L., and Bearson, S. M. (2008). The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar Typhimurium. *MBio* 2, 271–278. doi:10.1128/mBio.00260-11

Bearson, B. L., Bearson, S. M., Utte, J. J., Dowd, S. E., Houghton, J. O., Lee, I., et al. (2008). Iron regulated genes of *Salmonella enterica* serovar Typhimurium in response to norepinephrine and the requirement of *fepGDC* for norepinephrine-enhanced growth. *Microbes Infect.* 10, 807–816. doi: 10.1016/j.micinf.2008.04.011

Beutin, L., Preller, E., and Kowalski, B. (1981). Mutagenicity of quindoxin, its metabolites, and two substituted quinoxaline-di-N-oxides. *Antimicrob. Agents Chemother.* 20, 336–343. doi: 10.1128/AAC.20.3.336

Boyd, D., Peters, G. A., Cloeckaert, A., Boumedine, K. S., Chaslas-Dandala, E., Imberechts, H., et al. (2001). Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT104 and serovar Agona. *J. Bacteriol.* 183, 5725–5732. doi: 10.1128/JB.183.19.5725-5732.2001

Brazas, M. D., and Hancock, R. E. (2005). Using microarray gene signatures to elucidate mechanisms of antibiotic action and resistance. *Drug Discov. Today* 10, 1245–1252. doi:10.1016/S1359-6446(05)03566-X

Brunelle, B. W., Bearson, S. M., and Bearson, B. L. (2013). Tetracycline accelerates the temporally-regulated invasion response in specific isolates of multidrug-resistant *Salmonella enterica* serovar Typhimurium. *BMC Microbiol.* 13:202. doi: 10.1186/1471-2180-13-202

Bearson, H., Canchaya, C., and Hardt, W. D. (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lyogenic conversion. *Microbiol. Mol. Biol. Rev.* 68, 560–602, table of contents. doi: 10.1123/MMBR.68.3.560-602.2004

Bunny, K., Liu, J., and Roth, J. (2002). Phenotypes of *lexA* mutations in *Salmonella enterica*: evidence for a lethal *lexA* null phenotype due to the Fels-2 phage. *J. Bacteriol.* 184, 6235–6249. doi:10.1128/JB.184.22.6235-6249.2002

Casjens, S. (2011). “A plethora of putative phages and prophages,” in *A Tribute to John Roth*, eds S. Maloy, K. Hughes, and J. Casadesus (Washington, DC: ASM Press), 291–306.

Casjens, S., and Hendrix, R. W. (2005). “Bacteriophages and the bacterial genome,” in *The Bacterial Chromosome*, ed N. P. Higgins (Washington, DC: ASM Press), 39–52.

Casjens, S., Winn-Stapley, D. A., Gilcrease, E. B., Morona, R., Kuhlewein, C., Chua, J. E., et al. (2004). The chromosome of *Shigella flexneri* bacteriophage Sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. *J. Mol. Biol.* 339, 379–394. doi: 10.1016/j.jmb.2004.03.068

Cheetham, B. F., and Katz, M. E. (1995). A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. *Mol. Microbiol.* 18, 201–208. doi:10.1111/j.1365-2958.1995.mmbi_180201.x

Cherepanov, P. P., and Wackernagel, W. (1995). Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* 158, 9–14. doi:10.1016/0378-1119(95)00193-A

Cooke, F. J., Brown, D. J., Fookes, M., Pickard, D., Ivens, A., Wain, J., et al. (2008). Characterization of the genomes of a diverse collection of *Salmonella enterica* serovar Typhimurium definitive phage type 104. *J. Bacteriol.* 190, 8155–8162. doi:10.1128/JB.00636-08

Datsenko, K. A., and Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6640–6645. doi:10.1073/pnas.120163297

Davies, J., Spiegelman, G. B., and Yim, G. (2006). The world of subinhibitory antibiotic concentrations. *Curr. Opin. Microbiol.* 9, 445–453. doi: 10.1016/j.mib.2006.08.006

Feld, L., Schjorring, S., Hammer, K., Licht, T. R., Danielsen, M., Krogfelt, K., et al. (2008). Selective pressure affects transfer and establishment of a *Lactobacillus plantarum* resistance plasmid in the gastrointestinal environment. *J. Antimicrob. Chemother.* 61, 845–852. doi: 10.1093/jac/dkn033

Figueroa-Bossi, N., and Bossi, L. (1999). Inducible prophages contribute to *Salmonella* virulence in mice. *Mol. Microbiol.* 33, 167–176. doi: 10.1111/j.1365-2988.1999.01461.x

Figueroa-Bossi, N., Coissac, E., Netter, P., and Bossi, L. (1997). Unsuspected prophage-like elements in *Salmonella typhimurium*. *Mol. Microbiol.* 25, 161–173. doi: 10.1111/j.1365-2988.1997.441807.x

Figueroa-Bossi, N., Uzzau, S., Maloriol, D., and Bossi, L. (2001). Variable assortment of prophages provides a transferable repertoire of pathogenic *Salmonella typhimurium*. *MBio* 2, 271–278. doi:10.1128/mBio.00260-11
determinants in Salmonella. Mol. Microbiol. 39, 260–271. doi: 10.1046/j.1365-2958.2001.02234.x

Frye, J. G., Porwollik, S., Blackmer, F., Cheng, P., and McClelland, M. (2005). Host gene expression changes and DNA amplification during temperate phage induction. J. Bacteriol. 187, 1485–1492. doi: 10.1128/JB.187.4.1485-1492.2005

Garcia-Russell, N., Elrod, B., and Dominguez, K. (2009). Stress-induced prophage DNA replication in Salmonella enterica serovar Typhimurium. Infect. Genet. Evol. 9, 889–895. doi: 10.1016/j.meegid.2009.05.017

Groman, N. B. (1955). Evidence for the active role of bacteriophage in the conversion of nontoxicogenic Corynebacterium diphtheriae to toxin production. J. Bacteriol. 69, 9–15.

Hendrix, R. W., and Duda, R. L. (1992). Bacteriophage lambda PaPa: not the mother of all lambda phages. Science 258, 1145–1148. doi: 10.1126/science.1439823

Ho, T. D., Figueroa-Bossi, N., Wang, M., Uzzau, S., Bossi, L., and Slauch, J. M. (2002). Identification of GtGe, a novel virulence factor encoded on the Gifsy-2 bacteriophage of Salmonella enterica serovar Typhimurium. J. Bacteriol. 184, 5234–5239. doi: 10.1128/JB.184.19.5234-5239.2002

Humphrey, S. B., Stanton, T. B., Jensen, N. S., and Zuerner, R. L. (1997). Purification and characterization of VSH-1, a generalized transducing bacte-
riophage of Serpulina hyodysenteriae. J. Bacteriol. 179, 323–329.

Kohler, B., Karch, H., and Schmidt, H. (2000). Antibiotics that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-
2-converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. Microbiology 146, 1085–1090.

Kropinski, A. M., Sulakvelidze, A., Konczy, P., and Poppe, J. M. (2007). Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-2-converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. Microbiology 146, 1085–1090.

Lilleeneng, K. (1948). Typing Salmonella typhimurium by means of bacteriophage. Acta Pathol. Microbiol. Scand. Suppl. 77, 11–25.

Little, J. W. (1993). LexA cleavage and other self-processing reactions. J. Bacteriol. 175, 4943–4950.

McClendon, M., Sanderson, K. E., Spieth, J., Clifton, S. W., Latreille, P., Courtney, L., et al. (2001). Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature 413, 852–856. doi: 10.1038/35101614

McLaughlin, M. R., Balaa, M. F., Sims, J., and King, R. L. (1993). LexA cleavage and other self-processing reactions. J. Bacteriol. 175, 4943–4950.

Mirold, S., Rabsch, W., Rohde, M., Stender, S., Tschape, H., Russmann, H., et al. (2001). Complete genome sequence of Salmonella enterica serovar Typhimurium DT104. J. Bacteriol. 183, 219–226. doi: 10.1128/JB.183.1.219-226.2001

Salmonella enterica serovar Typhimurium DT104. J. Bacteriol. 183, 219–226. doi: 10.1128/JB.183.1.219-226.2001

Salmonella enterica serovar Typhimurium DT104. J. Bacteriol. 183, 219–226. doi: 10.1128/JB.183.1.219-226.2001

Schmieger, H., and Schicklmaier, P. (1999). Transduction of multiple drug resis-
tance of Salmonella enterica serovar typhimurium DT104. FEMS Microbiol. Lett. 170, 251–256. doi: 10.1111/j.1574-6968.1999.tb13381.x

Shoemaker, N. B., Vlamakis, H., Hayes, K., and Salyers, A. A. (2001). Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon. Appl. Environ. Microbiol. 67, 561–568. doi: 10.1128/AEM.67.3.561-568.2001

Seng, B., Wang, G. R., Shoemaker, N. B., and Salyers, A. A. (2009). An unex-
pected effect of tetracycline concentration: growth phase-associated excision of the Bacteroides mobilizable transposon NBUT1. J. Bacteriol. 191, 1078–1082. doi: 10.1128/JB.00637-08

Stanton, T. B., Humphrey, S. B., Sharma, V. K., and Zuerner, R. L. (2008). Collateral effects of antibiotics: carbadox and metronidazole induce VSH-1 and facil-
itate gene transfer among Brachyspira hyodysenteriae strains. Appl. Environ. Microbiol. 74, 2950–2956. doi: 10.1128/AEM.00189-08

Tanaka, K., Nishimori, K., Makino, S., Nishimori, T., Kanno, T., Ishihara, R., et al. (2004). Molecular characterization of a prophage of Salmonella enter-
ica serotype Typhimurium DT104. J. Clin. Microbiol. 42, 1807–1812. doi: 10.1128/JCM.42.4.1807-1812.2004

Varma, J. K., Greene, K. D., Ovitt, J., Barrett, T. J., Medalla, F., and Angulo, F. J. (2005a). Hospitalization and antimicrobial resistance in Salmonella out-
breaks, 1984–2002. Emerg. Infect. Dis. 11, 943–946. doi: 10.3201/eid1106.041231

Varma, J. K., Molbak, K., Barrett, T. J., Beebe, J. L., Jones, T. E., Rabatysk-Ehr, T., et al. (2005b). Antimicrobial-resistant nontyphoidal Salmonella is associ-
ated with excess bloodstream infections and hospitalizations. J. Infect. Dis. 191, 554–561. doi: 10.1086/427263

Vogel, H. J., and Bonner, D. M. (1956). Acetylornithinase of Escherichia coli: partial purification and some properties. J. Biol. Chem. 218, 97–106.

Waldor, M. K., and Mekalanos, J. J. (1996). Lysogenic conversion by a filamen-
tous phage encoding cholera toxin. Science 272, 1910–1914. doi: 10.1126/sci-
ence.272.5720.1910

Wang, S., Zhao, W., Raza, A., Friendship, R., Johnson, R., Kostrzynska, M., et al. (2010). Prevalence of Salmonella infecting bacteriophages associated with Ontario pig farms and the holding area of a high capacity pork processing facility. J. Sci. Food Agric. 90, 2318–2325. doi: 10.1002/jsfa.4090

Weigle, J. (1966). Assembly of plaque lambda in vitro. Proc. Natl. Acad. Sci. U.S.A. 63, 1462–1466. doi: 10.1073/pnas.63.5.1462

Winston, F., Botstein, D., and Miller, J. H. (1979). Characterization of amber and ochre suppressors in Salmonella typhimurium. J. Bacteriol. 137, 433–439.

Yim, G., McClure, J., Surette, M. G., and Davies, J. E. (2011). Modulation of Salmonella gene expression by subinhibitory concentrations of quinolones. J. Antimicrob. Chemother. 64, 755–769.

Zinder, N. D., and Lederberg, J. (1952). Genetic exchange in Salmonella. J. Bacteriol. 64, 679–699.

Conflict of Interest Statement: The authors declare that the research was con-
ducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 24 September 2013; paper pending published: 02 November 2013; accepted: 23 January 2014; published online: 11 February 2014.

Citation: Pearson BL, Allen HK, Brunelle BW, Lee IS, Casjens SR and Stanton TB (2014) The agricultural antibiotic carbadox induces phage-mediated gene transfer in Salmonella. Front. Microbiol. 5:52. doi: 10.3389/fmicb.2014.00052

This article was submitted to Evolutionary and Genomic Microbiology, a section of the journal Frontiers in Microbiology.

Copyright © 2014 Pearson, Allen, Brunelle, Lee, Casjens and Stanton. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.