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Short Communication

Fluoroquinolone induction of phage-mediated gene transfer in multidrug-resistant *Salmonella*

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ABSTRACT

Fluoroquinolones are broad-spectrum antibiotics that inhibit bacterial DNA gyrase and topoisomerase activity, which can cause DNA damage and result in bacterial cell death. In response to DNA damage, bacteria induce an SOS response to stimulate DNA repair. However, the SOS response may also induce prophage with production of infectious virions. *Salmonella* strains typically contain multiple prophages, and certain strains including phage types DT120 and DT104 contain prophage that upon induction are capable of generalised transduction. In this study, strains of multidrug-resistant (MDR) *Salmonella enterica* serovar Typhimurium DT120 and DT104 were exposed to fluoroquinolones important for use in human and veterinary disease therapy to determine whether prophage(s) are induced that could facilitate phage-mediated gene transfer. Cultures of MDR *S.* Typhimurium DT120 and DT104 containing a kanamycin resistance plasmid were lysed after exposure to fluoroquinolones (ciprofloxacin, enrofloxacin and danofloxacin). Bacterial cell lysates were able to transfer the plasmid to a recipient kanamycin-susceptible *Salmonella* strain by generalised transduction. In addition, exposure of DT120 to ciprofloxacin induced the *recA* gene of the bacterial SOS response and genes encoded in a P22-like generalised transducing prophage. This research indicates that fluoroquinolone exposure of MDR *Salmonella* can facilitate horizontal gene transfer, suggesting that fluoroquinolone usage in human and veterinary medicine may have unintended consequences, including the induction of phage-mediated gene transfer from MDR *Salmonella*. 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.

1. Introduction

The use of antibiotics in human and veterinary medicine may have unintended consequences, including the selection of antimicrobial resistance against the antibiotic class that is being administered. However, antibiotic therapy may induce additional collateral effects such as stimulation of horizontal gene transfer, enhanced pathogen invasion, and shifts in the composition of the gastrointestinal microbiota that may predispose to diarrhoea [1–6].

Fluoroquinolones are broad-spectrum antibiotics that exhibit activity due to inhibition of the bacterial enzymes DNA gyrase and topoisomerase required for DNA synthesis [7]. Owing to their wide spectrum of activity against a variety of bacterial pathogens, fluoroquinolones are important for human clinical and veterinary therapy, including for community- and hospital-acquired pneumonia, urinary tract infections caused by *Escherichia coli* resistant to primary antibiotics, *Salmonella* bacteraemia, typhoid fever, infectious diarrhoea, meningococcal and anthrax prophylaxis, and respiratory and enteric diseases of cattle and swine. For various infections and prophylaxis, the typical dose range for human adults is 200–750 mg ciprofloxacin every 12 h for the duration of treatment. For treatment of infectious diseases in cattle and swine, the recommended dose range is 2.5–7.5 mg Baytril™ (enrofloxacin)/kg of body weight (bw) every 24 h for the duration of treatment. The recommended dose for treatment of bovine respiratory disease using Advocin™ (danofloxacin) is a one-time injection of 8 mg danofloxacin/kg bw, or two injections 48 h apart with 6 mg danofloxacin/kg bw.

Our prior work revealed that the agricultural antibiotic carbadox induces prophages that are integrated into the chromosome of *Salmonella enterica* serovar Typhimurium [2], and demonstrated that carbadox-induced prophage can mediate transduction into

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a recipient *Salmonella* strain. This induction of generalised transducing prophage present in *Salmonella* Typhimurium phage type DT120 and DT104 resulted in transfer both of chromosomal and plasmid DNA, including antibiotic resistance genes. In the current study, we investigated whether commonly used fluoroquinolones prescribed for human and veterinary clinical disease or prophylaxis could also induce generalised transducing prophage integrated in multidrug-resistant (MDR) *S.* Typhimurium DT120 and DT104. We demonstrate that fluoroquinolones can induce phage-mediated gene transfer of a native *Salmonella* plasmid that encodes resistance to the antibiotic kanamycin.

2. Materials and methods

2.1. Bacterial strains

S. Typhimurium strains LT2, DT120-305, DT104-530, DT104-745, and BBS 243 and the protocol for carbadox-induced phage transduction were previously described [2]. BBS 1162 is a derivative of *S.* Typhimurium DT120-305, and BBS 1165 is a derivative of *S.* Typhimurium DT104-530 that contain a native plasmid conferring kanamycin resistance that was transduced from DT104-745 using a carbadox-induced lysate. BBS 1170 is a *S.* Typhimurium LT2 derivative containing the plasmid conferring kanamycin resistance that was transduced from BBS 1162 following ciprofloxacin induction (see below).

2.2. Generalised transduction following fluoroquinolone induction

Overnight cultures were diluted 1:100 in 3 mL of Luria–Bertani (LB) medium [10 g/L tryptone (BD Diagnostics, Sparks, MD), 5 g/L yeast extract (BD Diagnostics) and 5 g/L NaCl (Fisher Scientific, Fairlawn, NJ)] with 0.4% glucose (Fisher Scientific) in a 13-mm tube (Kimble Glass, Vineland, NJ) and were incubated at 37 °C with shaking at 180 rpm. To determine the antibiotic concentration producing optimal bacterial lysis, two-fold dilutions of each antibiotic ranging from 0.025 μg/mL to 1 μg/mL were evaluated. Fluoroquinolone antibiotics were added at an optical density at 600 nm (OD_{600}) of 0.2 to the indicated cultures using a final concentration of 0.1 μg/mL ciprofloxacin (Sigma-Aldrich, St Louis, MO) and 0.5 μg/mL of either enrofloxacin (Bayer HealthCare LLC, Shawnee Mission, KS) or danofloxacin (Pfizer Animal Health, New York, NY). Cultures were monitored for bacterial cellular lysis, and bacterial supernatants were harvested 4–5 h following antibiotic addition as previously described [2]. Transductions were performed in a microcentrifuge tube (Eppendorf, Hauppauge, NY) using a ratio of bacterial supernatant (DNA donor) to recipient bacterial cells of 1:10 for antibiotic-induced cultures and 1:1 for control cultures without antibiotic addition. Transductions were incubated at 37 °C for 30 min followed by centrifugation at 25 000×*g* for 1 min. Supernatants were discarded and the cell pellets suspended in 1 mL of phosphate-buffered saline (PBS) and vortexed. Transductions were further incubated for ca. 2.5 h followed by centrifugation. Supernatants were discarded and the cell pellets were suspended in 225 μL of PBS and vortexed. Aliquots of the transductions (100 μL/plate) were spread onto two plates with LB medium containing kanamycin (50 μg/mL) (Fisher Scientific) and were incubated at 37 °C overnight. Exposure of bacterial cultures to fluoroquinolones and transductions were performed a minimum of three times.

2.3. Gene expression analysis following ciprofloxacin induction

Overnight cultures were diluted 1:200 in 50 mL of LB 0.4% glucose in a 250-mL flask (Corning Pyrex, Tewksbury, MA) and were incubated at 37 °C with shaking at 180 rpm. Ciprofloxacin (0.1 μg/mL final) was added at OD_{600} = 0.2 to the indicated cultures; control samples without antibiotic addition were also used. Samples were taken for RNA extraction at 0, 60, and 120 min ± ciprofloxacin by adding 500 μL of bacterial culture to 1 mL of RNAprotect Bacteria Reagent (QIAGEN, Germantown, MD) in a 2 mL RNase-free microcentrifuge tube (Ambion, Austin, TX). Following 5 min of incubation at room temperature, each tube was centrifuged at 5000×*g* for 10 min. Supernatants were discarded and the tubes containing the bacterial pellets were frozen at–80 °C until RNA extraction. These experiments were performed in triplicate. RNA isolation and real-time gene expression assays were performed as previously described [3]. Real-time PCR was used to evaluate expression differences between pre-antibiotic addition (0 min) and post-antibiotic addition (60 min and 120 min) using primer sets for the following three genes: *recA* (BWB-319, 5′-CGACGAAAACAAACAGAAAGC-3′; BWB-320, 5′-CATCCATAGAACGGTCTTCAC-3′); *abc2* (BWB-333, 5′-AAAAGCAGAACGGAAGTACC-3′; BWB-334, 5′-TGCTGTTTCGTTTATTCGG-3′); and *kil* (BWB-325, 5′-GTTCAGCAAGGAAACAGG-3′; BWB-326, 5′-CCACTTATCCCAACCAAG-3′). Expression data were $\log_2$ transformed, and statistical differences between all sets of time points and antibiotic conditions for each gene were assessed by analysis of variance (ANOVA) with Tukey's post-test using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA). A *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Fluoroquinolones induce phage-mediated gene transfer

The agricultural antibiotic carbadox was previously demonstrated to induce phage-mediated gene transfer in MDR *S.* Typhimurium DT104 and DT120 isolates [2]. In the current study, three fluoroquinolones (ciprofloxacin, enrofloxacin and danofloxacin) were investigated to determine whether exposure to any of these antibiotics would induce endogenous prophage in *S.* Typhimurium DT104 and DT120 resulting in bacterial cellular lysis. Ciprofloxacin is indicated for treating bacterial diseases in humans, whereas enrofloxacin and danofloxacin are used in veterinary medicine. Following exposure to the three fluoroquinolones, the bacterial culture densities of BBS 1162 (DT120), BBS 1165 (DT104) and BBS 1170 (LT2 derivative) precipitously decreased ca. 2 h after antibiotic addition (data not shown); the decline in culture density was similar to *S.* Typhimurium exposed to carbadox [2]. The three strains contain a plasmid that is native to MDR *S.* Typhimurium DT104-745 and confers kanamycin resistance [2]. Phage transductions were performed with the fluoroquinolone-induced phage lysates from the three strains into a kanamycin-sensitive recipient (BBS 243). For BBS 1162 and BBS 1165, all three antibiotics (ciprofloxacin, enrofloxacin, and danofloxacin) stimulated generalised transduction of the kanamycin resistance plasmid into BBS 243 (Table 1). This indicates that these fluoroquinolones enhance horizontal gene transfer from MDR *S.* Typhimurium to a susceptible bacterial host strain. In contrast, fluoroquinolone exposure of BBS 1170 did not cause antibiotic-induced generalised transduction. This inability to perform generalised transduction was expected since it is known that although LT2 harbours several prophages in its genome [8], it does not contain a prophage that confers generalised transductions such as bacteriophage P22.

3.2. Fluoroquinolones increase prophage gene expression

Prophage induction is often due to activation of the bacterial SOS response that includes an increase in expression of the *recA* gene. Induction of phage-mediated gene transfer following