Science of the Total Environment 637–638 (2018) 657–664

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Clonal expansion of environmentally-adapted *Escherichia coli* contributes to propagation of antibiotic resistance genes in beef cattle feedlots

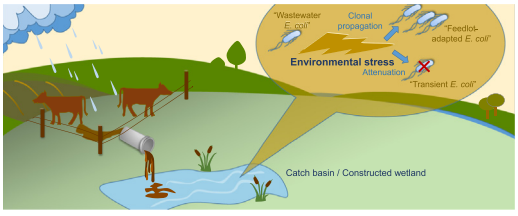
Lisa Tymensen [a,*], Rahat Zaheer [b], Shaun R. Cook [a], Kingsley K. Amoako [c], Noriko Goji [c], Ron Read [d], Calvin W. Booker [e], Sherry J. Hannon [e], Norman Neumann [f], Tim A. McAllister [b]

[a] Alberta Agriculture and Forestry, Irrigation and Farm Water Branch, Lethbridge, Alberta T1J 4V6, Canada
[b] Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada
[c] Canadian Food Inspection Agency, National Center for Animal Disease, Lethbridge, Alberta, Canada
[d] Microbiology, Immunology and Infectious Diseases, University of Calgary, Alberta T1Y 6J4, Canada
[e] Feedlot Health Management Services, Ltd., Okotoks, Alberta T1S 2A2, Canada
[f] School of Public Health, University of Alberta, 3-300 Edmonton Clinic Health Authority, 11405-87 Ave, Edmonton, Alberta T6G 1C9, Canada

HIGHLIGHTS

- Antibiotic resistance (AR) bacteria are emerging contaminants of feedlot wastewater.
- Clonal propagation is the major driver shaping AR *E. coli* populations in feedlots.
- Propagation of AR *E. coli* in wastewater is linked to environmental adaptation.
- Global stress response may play a key role in the fate of AR *E. coli* in wastewater.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 March 2018
Received in revised form 1 May 2018
Accepted 2 May 2018
Available online xxxx

Editor: Jay Gan

Keywords:
Wastewater
Constructed wetland
Catch basin
ARGs
Environmental adaptation
ESBLs

ABSTRACT

Livestock wastewater lagoons represent important environmental reservoirs of antibiotic resistance genes (ARGs), although factors contributing to their proliferation within these reservoirs remain poorly understood. Here, we characterized *Escherichia coli* from feedlot cattle feces and associated wastewater lagoons using CRISPR1 subtyping, and demonstrated that while generic *E. coli* were genetically diverse, populations were dominated by several 'feedlot-adapted' CRISPR types (CTs) that were widely distributed throughout the feedlot. Moreover, *E. coli* bearing beta-lactamase genes, which confer reduced susceptibility to third-generation cephalosporin's, predominantly belonged to these feedlot-adapted CTs. Remarkably, the genomic region containing the CRISPR1 allele was more frequently subject to genetic exchange among wastewater isolates compared to fecal isolates, implicating this region in environmental adaptation. This allele is proximal to the *mutS-rpoS-nlpD* region, which is involved in regulating recombination barriers and adaptive stress responses. There were no loss-of-function *mutS* or *rpoS* mutations or beneficial accessory genes present within the *mutS-rpoS-nlpD* region that would account for increased environmental fitness among feedlot-adapted isolates. However, comparative sequence analysis revealed that protein sequences within this region were conserved among most feedlot-adapted CTs, but not transient fecal CTs, and did not reflect phylogenetic relatedness, implying that adaptation to wastewater environments may be associated with genetic variation related to stress resistance. Collectively, our findings suggest adaptation of *E. coli* to feedlot environments may contribute to propagation of ARGs in wastewater lagoons.

* Corresponding author.
E-mail address: lisa.tymensen@gov.ab.ca (L. Tymensen).

https://doi.org/10.1016/j.scitotenv.2018.05.021
0048-9697/

1. Introduction

Propagation of antibiotic resistance genes (ARGs) in environmental reservoirs is an emerging global threat to human health due to the potential for promoting antimicrobial resistance (AMR) among medically important bacterial pathogens (Pruden et al., 2006). Of such reservoirs, livestock wastewater lagoons have been receiving increased attention with regard to their capacity to paradoxically eliminate ARGs present in receiving wastewaters but also serve as environments where ARGs persist and can multiply (Barkovskii et al., 2012; Cheng et al., 2013). Concerning beef cattle and associated environments, most of the focus continues to be devoted to describing the different ARGs present (Agga et al., 2015; Noyes et al., 2016), although there is growing recognition of the need to understand the mechanisms responsible for ARG persistence in these environments (Suzuki et al., 2017).

Ceftiofur, a third-generation cephalosporin (3GC) approved for use in beef cattle production, is classified as critically important by the World Health Organization (AGISAR, 2016). Development of resistance to ceftiofur is particularly concerning due to associated cross-resistance to ceftriaxone, which is used in human medicine. Numerous ARGs conferring reduced susceptibility to 3GCs have been identified among *Escherichia coli* from feedlot cattle, including the beta-lactamase genes $bla_{\text{CMY-2}}$ and $bla_{\text{CTX-M}}$ (Cormier et al., 2016; Schmid et al., 2013; Schmidt et al., 2013), which are often plasmid-encoded. While plasmids help promote ARG dissemination, some like IncA/C plasmids, can also impart a metabolic burden on their bacterial host, and consequently it has been proposed that continuous antibiotic selection pressure may be required for their long-term maintenance (>15 days) among cattle (Subbiah et al., 2011). Nonetheless, it has been shown that 3GC resistant *E. coli* can persist in wastewater in the absence of antibiotic selective pressure (Jorgensen et al., 2017; Vivant et al., 2016). Likewise, it has been recently reported that 3GC resistant *E. coli* were capable of propagating in beef feedlot catch basins and a constructed wetland for many weeks under drought conditions that curtailed wastewater input into the lagoons (Tymensen et al., 2017). Such observations hint at the presence of compensatory mechanisms that can ameliorate the fitness burden associated with AMR and promote environmental persistence (Bjorkman et al., 2000).

Bacteria can rapidly adapt to new ecological niches through different mechanisms that include acquiring beneficial accessory genes via horizontal gene transfer (HGT) (Ochman et al., 2000) or altering the regulation of environmental stress responses. The RpoS sigma factor regulates the general stress response and is involved in the trade-off between bacterial self-preservation and nutritional competence (SPANC) (Ferenci and Spira, 2007). Environmental conditions such as low temperatures and nutrient limitation during stationary phase induce high levels of RpoS, which promotes increased stress resistance but diminished nutritional competence. Tremendous variation in adaptive stress responses among different *E. coli* strains within natural populations has been observed, and is often associated with *rpoS* polymorphisms (Chiang et al., 2011; Ferenci et al., 2011).

Besides its role in SPANC, RpoS also modulates stress-induced mutation rates (Galhardo et al., 2007; Saint-Ruf and Matic, 2006). Under stressful conditions, RpoS reduces the expression of the adjacent methyl-directed mismatch repair gene, *mutS*, which subsequently relaxes recombination barriers and increases the potential for HGT. The *mutS-rpoS-nlpD* genomic region is thus known as a 'hotspot of recombination' (Brown et al., 2001), and exhibits a mosaic structure due to loss or acquisition of accessory genes from non-parental lineages (Ewers et al., 2014). In addition, nonsense mutations of the *mutS* result in hypermutability which can accelerate environmental adaptation and represents an important mechanism for the emergence of AMR (Chopra et al., 2003). By affecting both SPANC balance and mutation rates, genetic variation within the *mutS-rpoS-nlpD* genomic region can generate strains with greater competitive fitness for specific environments (Saint-Ruf and Matic, 2006), and thus plays a pivotal role in the adaptation of bacteria to new ecological niches.

In this work, we examined the role of environmental adaptation in promoting propagation of antimicrobial resistant bacteria in beef cattle feedlots. We hypothesized that environmental adaptation is a contributing factor in the propagation of ARGs within feedlots, and may be attributed to genetic variation related to stress responses. Our first objective was to identify feedlot-adapted *E. coli* from an existing collection of generic (i.e., not subject to selective isolation) and 3GC resistant isolates that were obtained from beef cattle feces and associated wastewater lagoons (Tymensen et al., 2017). To this end, we used CRISPR subtyping to compare the distribution of different CRISPR types (CTs) between cattle feces and wastewater. Under the assumption that natural selection favors *E. coli* strains with superior fitness in specific environments, feedlot-adapted CTs were defined as those that were abundant and widely disseminated throughout the feedlot (i.e., isolated from both cattle feces and wastewater on multiple sampling occasions across a two year period). In contrast, transient CTs, were defined as those that did not appear to persist in the feedlot, and were isolated only from cattle feces, typically on only one sampling occasion. Our second objective was to investigate the fine scale genetic structure of the MutS-RpoS-NlpD region from representative feedlot-adapted isolates, and compare this region with that from transient fecal isolates. Initially, we examined amino acid sequences of various proteins within this region, with emphasis on MutS and RpoS, since polymorphisms in either could affect environmental fitness by promoting hypermutability or changes in SPANC balance, respectively. We also looked for the presence of environmentally adaptive accessory genes and examined genetic variability among all proteins within the MutS-RpoS-NlpD region.

2. Material and methods

2.1. E. coli isolates

Generic and 3GC resistant *E. coli* isolates were obtained from a previously described culture collection of fecal and wastewater strains that were isolated from a feedlot in Alberta, Canada from April to October of 2014 and 2015 (Tymensen et al., 2017). Briefly, *E. coli* were isolated from pen fecal composite samples by directly plating of fecal suspensions (diluted 1:10 v/v in PBS) onto MacConkey agar (BD Canada, Inc., Mississauga, ON, Canada). Fecal suspensions were also enriched in EC broth (BD Canada) containing 2 μg mL^{-1} cefotaxime followed by selective isolation on MacConkey agar containing 1 μg mL^{-1} ceftriaxone. Isolation of *E. coli* from wastewater (i.e., two catch basins that drained feedlot pens and a constructed wetland that occasionally received wastewater from the catch basins) was done using membrane filtration onto modified mTEC (BD Canada) agar (with and without 1 μg mL^{-1} ceftriaxone) according to US EPA Method 1603 (USEPA, 2006).

Only a subset of isolates from the original collection, that belonged to a single monophyletic clade within phylogroup B1, previously defined as 'clade ET-1' (Walk et al., 2007), was used. This was done to ensure valid genetic comparisons between feedlot-adapted and transient *E. coli*. Clade ET-1 isolates are characterized by the presence of the clpX6 allele (Tymensen, 2016), which is used for phylogeny inference according to the Whittam multilocus sequence typing scheme (MLST) (Qi et al., 2004). The ET-1 clade is generally analogous to Clonal Complex (CC) 155 of the Achtman MLST scheme (Wirth et al., 2006). In the previous study, phylogroup B1 *E. coli* predominated in cattle feces and feedlot environments, with clade ET-1 collectively accounting for approximately one fifth of all *E. coli* isolated (i.e., 329 of 1557 isolates). Specifically, the original isolate collection consisted of 389 fecal isolates plus 824 wastewater isolates, of which 66 fecal and 199 wastewater isolates were from clade ET-1. The collection also included 249 3GC resistant fecal isolates and 95 3GC resistant wastewater isolates, of which 51 fecal and 13 wastewater isolates were from clade ET-1. All clade ET-1 isolates, except one wastewater isolate which failed to grow, were included in the current study (Table 1).