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Risk assessment modelling of fecal shedding caused by extended-spectrum cephalosporin-resistant *Escherichia coli* transmitted through waste milk fed to dairy pre-weaned calves

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ABSTRACT

Waste milk feeding is a common practice in dairy operations. Regardless of the benefits of this practice to the dairy farmers, concerns from the potential dissemination of antimicrobial-resistant bacteria through the gut and subsequent shedding by calves into the environment are increasing. In this study, we employed Monte Carlo simulation to assess the risk of shedding extended-spectrum cephalosporin-resistant *Escherichia coli* (ESC-R *E. coli*) caused by waste milk feeding in pre-weaned calves using an exponential dose-response model fit to data for *E. coli* O157:H7 in cattle. Data from pertinent studies were included in our model to predict the risk of shedding. The median (5th and 95th percentiles) for the daily risk of shedding ESC-R *E. coli* by calves fed only contaminated waste milk was predicted to be 2.9×10^{-3} (2.1×10^{-3}, 3.7×10^{-3}), representing a median daily risk of 29 out of 10,000 calves shedding ESC-R *E. coli* due to exclusive feeding of waste milk containing ESC-R *E. coli*. This median value was reduced by 94% when accounting for the proportion of waste milk that does not contain ESC-R *E. coli*. The overall risk of shedding ESC-R *E. coli* through the pre-weaning period for farms that feed waste milk to calves was 5.7×10^{-3} (2.4×10^{-3}, 1.1×10^{-2}), representing 57 out of 10,000 calves. When accounting for the proportion of farms that do not feed waste milk, the pre-weaning period risk was reduced by 23%. By varying the prevalence of ESC-R *E. coli* in waste milk using values of 3, 1.5, and 1%, the daily risk of shedding decreased by factors of 50, 65, and 82%, respectively, which supports the reduction of contamination or discontinuation of feeding waste milk containing ESC-R *E. coli* as major mitigation measures to reduce the risk of shedding caused by ingestion of resistant bacteria. It is anticipated that the effects of antimicrobial residues in waste milk, which was not considered herein due to lack of data, would further increase risks. Although waste milk feeding to calves may be economically beneficial to the dairy farmers, there exists the risk of dissemination of ESC-resistant bacteria into the environment.

Key words: quantitative microbial risk assessment, antimicrobial resistance, AMR, Monte Carlo simulation, mathematical model

INTRODUCTION

Antimicrobial resistance attributed to veterinary use is a concern for both public health and food animal production systems; this is as a result of increasing levels of resistance in foodborne zoonotic bacteria and clinical pathogens (van den Bogaard and Stobberingh, 2000). In dairy production in some parts of the world, antimicrobials are commonly administered to dairy cows as an intramammary infusion or injectable for the treatment of mastitis and, during the dry-off, either as blanket or selective treatment for the prevention or treatment of mastitis (McEwen and Fedorka-Cray, 2002; Saini et al., 2012). Feeding milk from these cows to dairy calves is a common practice on many dairy farms (Duse et al., 2013). Such milk may contain multi-drug-resistant bacteria (Wray et al., 1990; Brunton et al., 2012) as well as antimicrobial residues (Pereira et al., 2014a), which may facilitate horizontal transfer of resistant genes and selection pressure on the calves' gastrointestinal bacteria, respectively. Sub-therapeutic antimicrobial exposure through waste milk feeding practices may explain to some extent the high prevalence of multi-drug-resistant bacteria such as *Escherichia coli* in pre-weaned calves (Aust et al., 2012). Economic considerations and convenience (Godden et al., 2005; Abb-Schwedler et al., 2014), environmental protection, and difficulty in disposal (Brunton et al., 2012) have been reported as some of the reasons for waste milk feeding in dairy farms.

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Extended-spectrum cephalosporins (**ESC**) such as ceftiofur and cefquinome are commonly used in dairy production as intramammary preparation for treatment purposes against mastitis (Saini et al., 2012; Brunton et al., 2014; Randall et al., 2014). Therapeutic effectiveness and short withdrawal times make this class of antimicrobials increasingly important in dairy farming (Snow et al., 2012). However, emergence of antimicrobial resistance due to selection pressure by production of extended-spectrum β-lactamases (**ESBL**) and other β-lactamases has been documented (Daniels et al., 2009; Cormier et al., 2015). Different studies have reported on the detection of cephalosporin residues (Brunton et al., 2014; Pereira et al., 2014a; Randall et al., 2014), ESC-resistant (**ESC-R**) bacteria, and β-lactamase resistance genes in waste milk (Brunton et al., 2014; Randall et al., 2014) as well as relationships between feeding waste milk containing cephalosporin residues to calves and fecal shedding of ESBL-producing *E. coli* (Pereira et al., 2014b). A recent report by the European Food Safety Authority examined the risk from feeding waste milk containing antimicrobial residues to calves using a qualitative approach and concluded that consumption of waste milk containing antimicrobial residues leads to subsequent shedding of resistant bacteria, and that this process occurs in practice [EFSA Panel on Biological Hazards (BIOHAZ) et al., 2017].

Thus far, limited information is available establishing the relationship between feeding waste milk containing resistant bacteria and fecal shedding in calves. Quantitative microbial risk assessment (**QMRA**) has rarely been applied to study this relationship. Available studies include a previous risk assessment in the United Kingdom that reported that 60% of calves in 5,000 simulated farms were shedding ESC-R *E. coli* caused by antibiotic residues and ESBL *E. coli* in waste milk (Simmons et al., 2015). Quantitative microbial risk assessment is a scientific tool that quantifies the level of exposure and the subsequent risk due to a specific bacterial pathogen of concern (Lammerding and Fazil, 2000; Snary, 2004). This assessment involves 4 science-based investigation steps: hazard identification, exposure assessment, hazard characterization, and risk characterization (Lammerding and Fazil, 2000). The output of the risk assessment process represents the probability and effect of exposure to a hazard or adverse event. The QMRA process is useful to direct risk management efforts by examining the effects of model inputs on results through sensitivity analysis, modeling the effects of interventions, and identifying data gaps for further research efforts. The aim of our study was to adopt the QMRA approach to quantify the general risk of fecal shedding of ESC-R *E. coli* from feeding of waste milk containing ESC-R *E. coli* to pre-weaned calves using Monte Carlo simulation.

MATERIALS AND METHODS

Risk Assessment Model

The scientific literature was searched in Google Scholar (https://scholar.google.ca/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) databases using the key words of each of the input parameter definitions in Table 1, and data were extracted from the relevant articles. The risk assessment was not tailored to any single region (e.g., country, continent), resulting in the generation of a generic model (i.e., not geographically specific). Whenever possible, input data used in the model were represented by distributions rather than discrete point estimates. The variability for prevalence data inputs was incorporated using β distributions, where possible. However, where only few observations were available for input variables, uniform or triangular distributions were used to account for the uncertainty associated with the input data. Using the software @RISK (Palisade Corporation, New York, NY) as an add-on package in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA), Monte Carlo simulation using Latin hypercube sampling was performed using 10,000 iterations. The model outputs a probabilistic distribution of the risk of fecal shedding of ESC-R *E. coli* due to waste milk feeding in dairy calves. Sensitivity analysis was also carried out to explore different scenarios and their effect on the risk of shedding using Spearman rank correlation coefficients.

Hazard Identification

Waste milk feeding is a common practice widely used in dairy operations [EFSA Panel on Biological Hazards (BIOHAZ) et al., 2017]. Regardless of the benefits of this practice to dairy farmers, concerns from the potential for development of antimicrobial resistance and subsequent shedding are increasing due to (1) antimicrobial residues and selective pressure on gastrointestinal microflora of calves, and (2) transmission as well as lateral transfer of resistant bacteria within the gastrointestinal tract. Apart from the increased risk of antimicrobial resistance selection among the intestinal microflora due to antimicrobial residues in waste milk, contamination of waste milk with infectious pathogens and multi-drug-resistant bacteria due to pooling of waste milk from different sources has been documented (Wray et al., 1990; Aust et al., 2013). The introduction, multiplication, and establishment of multi-drug-