J. Dairy Sci. 100:1–10
https://doi.org/10.3168/jds.2016-11839

Prevalence and risk factors for extended-spectrum beta-lactamase or AmpC-producing *Escherichia coli* in organic dairy herds in the Netherlands

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ABSTRACT

Extended-spectrum β-lactamase and AmpC-producing *Escherichia coli* (ESBL/AmpC) are an emerging problem and are hypothesized to be associated with antimicrobial use (AMU), and more specifically with the use of third- and fourth-generation cephalosporins. Whether ESBL/AmpC also occur in organic dairy herds, which have restricted AMU, is not known. Additionally, it is unknown whether, in addition to restricted AMU, other factors in organic herd management are associated with ESBL/AmpC herd status. The aim of this study was to estimate the prevalence of ESBL/AmpC in organic dairy herds in the Netherlands. Subsequently, the relationships between the ESBL/AmpC herd status and AMU and between ESBL/AmpC herd status and farmers' management were assessed in organic dairy herds. For this study, 90 randomly selected, officially registered organic dairy herds were included. The ESBL/AmpC herd status was determined based on the bacteriological culture result of a slurry sample. The sensitivity of testing slurry samples for ESBL/AmpC herd status is less than 100% for detecting herds with a low ESBL/AmpC prevalence. For that reason, herds that tested positive for ESBL/AmpC in slurry were defined as positive and herds with negative slurry samples were defined as unsuspected. A comprehensive questionnaire on management practices was conducted and records on specified antimicrobials that were provided to these herds by the veterinary service providers were obtained. From the data on antimicrobial supplies by the veterinarian, the animal daily defined dose of antimicrobials per farm per year ($DDDA_F$) was calculated. Descriptive statistics were used to describe the relation between the ESBL/AmpC herd status and $DDDA_F$. Multivariable logistic regression models were used to evaluate management factors associated with the ESBL/AmpC herd status. We found ESBL/AmpC in 12 of the 90 (13%; 95% confidence interval = 7–22%) slurry samples from organic dairy herds. The median $DDDA_F$ in organic dairy herds was 0.5, which was not significantly different between ESBL/AmpC-positive and -unsuspected dairy herds. No association could be found between the use of different types of antimicrobials, such as third- and fourth-generation cephalosporins, and ESBL/AmpC herd status. Factors that were associated with higher odds of being ESBL/AmpC-positive were pig farms located within a 2-km radius of the barn, applying parental treatment for clinical mastitis, and providing milk replacer to the female calves after colostrum intake. The prevalence of ESBL/AmpC in organic dairy herds appeared lower than the prevalence in previous studies conducted in conventional dairy herds. Apparently, ESBL/AmpC are also present in herds with low AMU; this indicates that other factors than AMU are also associated with ESBL/AmpC herd status.

Key words: dairy cattle, organic farming, antimicrobial use, extended-spectrum β-lactamase, antimicrobial resistance

INTRODUCTION

Antimicrobial resistance is an important problem in both veterinary and human medicine (Marshall and Levy, 2011). In Dutch livestock, antimicrobial resistance is mainly associated with *Escherichia coli*, *Campylobacter*, and *Salmonella* bacteria, and resistance in dairy cattle is considered to be low (MARAN, 2015). In recent years, many studies described the emergence of *E. coli* producing extended-spectrum β-lactamase (**ESBL**) and AmpC type β-lactamases (**AmpC**; Teale et al., 2005; Reist et al., 2013; Stefani et al., 2014). These ESBL/AmpC-producing *E. coli* (**ESBL/AmpC**) have been demonstrated to be related to resistance against

Received August 6, 2016.
Accepted October 7, 2016.
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third- and fourth-generation cephalosporins in humans and animals (Li et al., 2007; Snow et al., 2012; Gonggrijp et al., 2016), which are defined as critically important antimicrobials by the World Health Organization (www.who.int).

High prevalence of ESBL-producing *E. coli* in animals have been demonstrated in many studies and vary between countries and animal species (Wu et al., 2013; Valentin et al., 2014). In cattle, studies focused on ESBL/AmpC in veal, dairy, and beef cattle and showed prevalence ranging between 1 to 32.8% on an animal level and 35.4 to 86.7% on a herd level (Snow et al., 2012; Hordijk et al., 2013a; Schmid et al., 2013; Wu et al., 2013; Carmo et al., 2014; Dorado-García et al., 2016; Gonggrijp et al., 2016); these studies, however, included only conventionally farmed cattle. Although, hospital submission, travel, and human-human contact are considered important contamination routes for humans, pets and livestock are considered relevant sources for the colonization of humans with ESBL (Meyer et al., 2012; Wu et al., 2013; von Wintersdorff et al., 2014; Sharp et al., 2014).

In European registered organic dairy herds, a restricted antimicrobial usage (**AMU**) policy is applied that prohibits AMU unless it is prescribed by a veterinarian and with a maximum frequency of 3 treatments per cow per year (European Union regulation 834/2007 and 889/2008; EC, 2007, 2008). Because of these restrictions, the use of third- and fourth-generation cephalosporins in organic herds generally is very limited. As the use of this type of antibiotic is known to be associated with the ESBL/AmpC herd status, it is hypothesized that the ESBL/AmpC herd prevalence in organic dairy herds is lower than in conventional dairy herds. Currently, no information exists that confirms or rejects this hypothesis. To our knowledge, only one study has evaluated ESBL/AmpC in an organic herd. Dolejska et al. (2011) found none of the 154 fecal samples (prevalence <1%) from a single organic dairy herd to test positive for ESBL/AmpC compared with 39% of the cattle in a single conventional dairy herd in the Czech Republic. Those authors suggested an association between AMU, especially cephalosporin use, and ESBL/AmpC herd status. Whether ESBL/AmpC occurs in organic dairy herds and to what extent is unknown. In addition, the relationship between ESBL/AmpC herd status in organic dairy herds and AMU, and whether other factors are associated with ESBL/AmpC herd status, is unknown.

The aim of the current study was to estimate the prevalence of ESBL/AmpC in organic dairy herds in the Netherlands. In addition, the relationships between the ESBL/AmpC herd status and AMU and the ESBL/AmpC herd status and farmers' management in organic dairy herds were evaluated.

MATERIALS AND METHODS

Definition of ESBL/AmpC Herd Status

In our study, slurry sample levels were evaluated instead of individual feces samples to assign an ESBL/AmpC herd status in accordance to Gonggrijp et al. (2016). The slurry samples could either contain fresh manure gathered from the scraper or, if no scraper was present, from 5 different places on the slatted floor. Because the slurry may not be sensitive enough to detect a low ESBL/AmpC prevalence within the herd, herds with ESBL/AmpC-negative slurry samples were described as unsuspected herds. Herds that tested positive for ESBL/AmpC in the slurry sample were described as positive herds.

Study Population

For this cross-sectional study, all 400 Dutch organic dairy farms that are officially registered in the SKAL database (control organization for organic farming, Zwolle, the Netherlands) were put in a random sequence and were contacted in that order by phone. All milking cows of the first 90 organic farmers that agreed to participate were included in the study and are described as herds in the remainder of this paper. These herds represent organic herds and have a low AMU as compared with conventional herds. According to European Union regulation (EC, 2007, 2008), these herds are obliged to apply a very restrictive AMU policy.

Based on calculations in Win-episcope 2.0 (Thrusfield et al., 2001), we estimated that with this population size it was possible to obtain a precise estimate (accepted error of 6%) of the herd prevalence of ESBL/AmpC in organic dairy herds (assuming 95% confidence, 80% power, and an expected herd prevalence of <10%). In addition, we estimated that this population size enabled the possibility to detect risk factors for an ESBL/AmpC-positive herd status with an odds ratio of 3.5 or higher (assuming 95% confidence, 80% power, and an expected prevalence of the risk factors of control herds of 20%).

Sampling and Laboratory Analyses

All herds were sampled between September 1 and December 31, 2011. During the visits, a transport swab with Amies medium (Beldico, Duiven, the Netherlands) was used to swab the scraper at different places. When no scraper was present, slurry samples from 5 randomly