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Antimicrobial susceptibility patterns of *Escherichia coli* phylogenetic groups isolated from bovine clinical mastitis

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ABSTRACT

Determination of antimicrobial susceptibility (AMS) of *Escherichia coli* causing clinical mastitis (CM) according to the phylogenetic groups and its association with descriptors at the cow and herd level may help improve specific strategies for treatment and control of this pathogen in dairy herds. The aims of the present study were to (a) determine the frequency of phylogenetic groups of *E. coli* isolated from CM in dairy cows, and its association with cow-level descriptors (parity, lactation stage, CM severity, and affected quarter position), housing system, and season; and (b) determine and compare AMS among *E. coli* phylogenetic groups. A quadruplex PCR method was used to classify *E. coli* isolates into 1 of the 7 phylogenetic groups. Minimal inhibitory concentrations were determined for 10 antimicrobials, and survival analysis was performed to evaluate the AMS differences among *E. coli* phylogroups. Most *E. coli* isolates belonged to phylogroups A (52%) and B1 (38%). None of the cow- and herd-level descriptors were associated with the *E. coli* phylogenetic groups. Overall, *E. coli* isolates were mostly susceptible to ceftiofur (96.8%), sulfadimethoxine (75.5%), and cephalothin (74.5%). Based on the survival analysis, differences in AMS between phylogenetic groups of *E. coli* was observed only for cephalothin, in which strains of phylogroup A were inhibited at lower minimum inhibitory concentration than strains of phylogroup B1. Results of this study indicated low susceptibility of *E. coli* isolates identified from CM to most antimicrobials. In addition, differences in AMS can occur among *E. coli* phylogenetic groups, although they may be uncommon as they were limited to only one antimicrobial (i.e., cephalothin).

Key words: antimicrobial susceptibility, bovine intramammary infection, *Escherichia coli*, phylogeny

INTRODUCTION

Mastitis is one of the most important diseases affecting dairy cattle, and *Escherichia coli* is one of the main causes of clinical mastitis (**CM**) in dairy herds (Oliveira et al., 2013). *Escherichia coli* strains are mostly commensals; however, pathogenic types were also reported, which were clustered into different pathovars based on clinical data and specific virulence properties (Kempf et al., 2016). Recently, the term mammary pathogenic *E. coli* (**MPEC**) was proposed to classify pathovars associated with mastitis (Shpigel et al., 2008), although no specific virulence traits have been identified to classify these strains as specifically mastitis-related (Leimbach at al., 2017). Therefore, *E. coli* causing mastitis appears to be more associated with host and exposure factors, such as cows' health (and immune) status and environmental hygiene (Klaas and Zadoks, 2017), rather than a set of mammary pathogenic strains that affect risk of IMI. As has been shown for many other mastitis-causing pathogens, *E. coli* has a great intraspecific diversity and can be classified into different phylogenetic groups (Kempf et al., 2016).

Most studies describing the distribution of *E. coli* causing mastitis in phylogenetic groups were performed using the simple triplex PCR method that enables strains of *E. coli* to be assigned into one of the phylogroups A, B1, B2, or D (Clermont et al., 2000). Few reports described the use of the improved quadruplex PCR method in dairy cattle (Kempf et al., 2016), which allows the identification of 7 phylogenetic groups (A, B1, B2, C, D, E, and F; Clermont et al., 2013). Therefore, data on the frequency of groups C, E, and F causing CM are scarce in the indexed literature (Kempf et al., 2016). In addition, to our knowledge, studies describing the phylogeny of *E. coli* have not

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been evaluated in isolates of bovine CM from Brazil. The identification of *E. coli* phylogeny may improve the epidemiological understanding of this pathogen in dairy herds, especially in relation to the route of transmission, pathogenicity, virulence factors, and antimicrobial susceptibility (**AMS**).

The majority of *E. coli* strains causing bovine mastitis were reported to belong to phylogroups A and B1 (Liu et al., 2014; Keane, 2016). In addition, virulence genes were more often detected in certain phylogroups than others (Suojala et al., 2011; Liu et al., 2014; Zhang et al., 2018). Therefore, considering the potential diversity of genetic properties (e.g., virulence genes) among phylogroups, it can be hypothesized that different groups could be more or less frequent in dairy herds according to characteristics at the cow-level, such as parity, stage of lactation, or even with characteristics that may affect CM occurrence in the herd, such as housing system and season of the year. Furthermore, different *E. coli* strains can cause CM with signs varying from mild to severe, and considering that some virulence factors can be more present in certain phylogenetic group than others, it can be hypothesized that the phylogenetic group may be associated with CM severity.

Clinical mastitis caused by *E. coli* is mostly of short duration, and the cow's immune system is generally competent to spontaneously eliminate mild and moderate infections (Burvenich et al., 2003). Therefore, antimicrobial treatment might not be necessary for most *E. coli* CM cases. However, even though interest in the use of management strategies for selective therapy such as on-farm culture is growing in dairy herds (Lago et al., 2011), most farms still do not identify the pathogen causing CM before treatment. Therefore, blanket antimicrobial therapy remains the main strategy for CM treatment in dairy herds.

The abusive and nonjudicious use of antimicrobials in dairy herds was associated with increased risk of antimicrobial resistance, which has been reported as a significant cause of mastitis treatment failure (Suojala et al., 2013). However, the association between the phylogeny of *E. coli* causing CM and AMS needs further explanation. Whereas some studies reported an association of AMS and specific phylogenetic groups (Liu et al., 2014), others found no significant association (Keane, 2016). Since blanket treatments of CM are still largely used in dairy herds, a better understanding of the susceptibility of *E. coli* can be used to monitor the efficiency of antimicrobials used for the treatment of IMI caused by this pathogen. Furthermore, the evaluation of *E. coli* susceptibility at the phylogenetic group-level can also serve as a basis for further studies evaluating potential resistance traits of this pathogen causing mastitis.

The objectives of this study were to (1) determine the frequency of phylogenetic groups of *E. coli* strains isolated from CM in dairy cows and its association with the following variables: cow-level descriptors (DIM, parity, position of affected quarter, and CM severity score), housing system and season; and (2) determine and compare AMS among the most prevalent *E. coli* phylogenetic groups identified from CM.

MATERIALS AND METHODS

Herd Selection and Bacterial Isolation, Identification, and Storage

Isolates were selected from a collection of *E. coli* previously identified by microbiological culture during the data collection of a study on epidemiological characterization of CM in 20 dairy herds of southeastern Brazil (Tomazi, 2017). Herds were conveniently selected based on a client list of the Qualileite Lab (Mastitis and Milk Quality Research Laboratory at University of Sao Paulo, Brazil). Clinical mastitis was identified at the quarter-level upon routine examination of fore-stripped milk immediately before milking by trained farm personnel, and aseptic milk samples were collected from the affected quarter(s) according to guidelines of NMC (1999). After collection, milk samples were frozen and sent in batches to the microbiology laboratory on ice packs for culture or on-farm stored (at approximately −20°C) until retrieval by researchers. Farm visits by researchers were performed every 14 to 30 d.

During the study period (March 2014 to January 2016), 276 *E. coli* isolates were identified by microbiological culture of 4,212 individual quarter milk samples collected from 2,637 cows with CM (Tomazi, 2017). Briefly, a 0.01-mL sterile loop was used to plate milk samples onto trypticase soy agar plates (BBL-Becton Dickinson and Co., LePoint de Claix, France) enriched with 5% bovine blood, and plates were incubated aerobically at 37°C. Phenotypic features were examined at 24 and 48 h after incubation and specific biochemical testing was performed to determine bacterial genus, species, or both. All isolates were submitted for KOH (potassium hydroxide) test. Isolates with positive test reaction were suggestive of gram-negative microorganisms and then were presumptively identified by colony morphology on MacConkey's agar. Identification of *E. coli* isolates was performed using a set of biochemical reactions: sucrose and glucose fermentation, hydrolysis of urea, gas production, motility capacity, indole production, H_2S production, L-tryptophan deaminase, and lysine reaction.

A milk sample was defined as culture negative if no colonies were observed on the streaking field of the agar