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 et 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

plate after 48 h of incubation. On the other hand, a milk sample was defined as culture positive if at least one colony of *E. coli* was observed in the streaking field of the agar plate (Dohoo et al., 2011). A milk sample was defined as contaminated if >2 different colony types were present in the streaked field of the agar plate.

In addition, all isolates identified as *E. coli* by microbiological culturing were confirmed using MALDI-TOF MS according to the method described by Barreiro et al. (2010). Only *E. coli* isolates identified with MALDI scores identification at species level (>2.0) were selected for this study. After microbiological culturing, a total of 177 *E. coli* isolates were stored at -80°C in vials containing 2 mL of brain heart infusion (Becton, Dickinson and Company/BBL, Le Pont Claix, France) broth supplemented with 10% glycerin. For the present study, 100 *E. coli* isolates were selected after meeting the following inclusion criteria: (1) isolated from a new case of CM (occurring in the same quarter of the same cow at least 14 d apart); (2) identified from cultures with identification of a single pathogen instead of mixed cultures; and (3) having a pure growth (without contamination) in the re-cultivation performed before phylotyping.

Selection of isolates was performed with the intention to include in the study strains identified in all herds. Thus, all strains were selected for herds with less than 12 isolates; whereas, for herds with ≥ 12 isolates, strains were randomly selected using the RAND function of Excel software (2010, Microsoft Office Corporation, Redmond, WA).

DNA Extraction

All *E. coli* isolates were cultured overnight on blood agar plates at 37°C under aerobic conditions. The DNA extraction of *E. coli* isolates was performed using an adaptation of the method described by Fan et al. (1995). In our study, PBS (pH 7.2) was substituted with Tris-EDTA buffer (10 mM Tris HCl, 5 mM EDTA, pH = 8.0). After DNA extraction, the lysates were kept frozen (-20°C) until phylogenetic grouping.

Phylogenetic Group Determination

Escherichia coli isolates were assigned to 1 of the 7 phylogroups (A, B1, B2, C, D, E, or F) based on amplification of *chuA*, *yjaA*, *arpA*, and *trpA* genes and the TSPE4.C2 DNA fragment by PCR, according to Clermont et al. (2013). The reference *E. coli* strain ATCC 25922 was used as quality control throughout the procedure.

All PCR reactions were carried out in a 20 μL final volume that contained the following: 100 ng of DNA,

0.2 mM of each deoxynucleotide triphosphate, $1\times$ Green GoTaq Reaction Buffer (Promega), 2 U of GoTaq DNA polymerase (Promega), and 1 μL of each primer, except for the primers of *arpA* gene in the quadruplex analysis, of which 2 μL was used. The PCR reactions were performed under the following conditions: denaturation for 4 min at 94°C , 30 cycles of 20 s at 94°C , 20 s at 57°C (group E) or 59°C (quadruplex and group C), and 25 s at 72°C , and a final extension step of 5 min at 72°C (Clermont et al., 2013).

Ten microliters of the amplified product was electrophoresed in 1.5% agarose gel with $1\times$ Tris-borate-EDTA buffer stained with 0.1 $\mu\text{g}/\mu\text{L}$ of ethidium bromide. Images of gel were taken under UV light using a photo-documentation system. A 100-bp DNA ladder (Thermo Fisher Scientific Inc., Pittsburgh, PA) was used to evaluate whether specific bands were present and to compare size of the bands generated in the electrophoresis.

Antimicrobial Susceptibility Testing

The MIC of selected antimicrobials against *E. coli* isolates was tested using the mastitis panel of a commercial broth microdilution test (CMV1AMAF, Sensititer, TREK Diagnostic Systems Inc., Cleveland, OH), following the manufacturer's guidelines. For each batch of microorganism tested, the CLSI (2013)-recommended quality control strains of *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were also evaluated. The antimicrobial agents and dilution ranges tested for each are presented in Table 1.

The concentrations of antimicrobial that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of *E. coli* isolates were calculated for each antimicrobial. Where interpretive cut-points for MIC have been established by CLSI (2013) for *E. coli* and antimicrobials evaluated, these were used to classify each isolate as susceptible, intermediate, or resistant. Isolates classified as intermediate were interpreted as resistant.

Table 1. Antimicrobial agents and their dilution ranges used in the susceptibility test of *Escherichia coli* isolates identified from clinical mastitis (CM)

Antimicrobial agent	MIC dilution range ($\mu\text{g}/\text{mL}$)
Penicillin	0.12–8.0
Ampicillin	0.12–8.0
Oxacillin	2.0–4.0
Cephalothin	2.0–16.0
Ceftiofur	0.5–4.0
Penicillin + novobiocin	1.0/2.0–8.0/16.0
Erythromycin	0.25–4.0
Pirlimycin	0.5–4.0
Tetracycline	1.0–8.0
Sulfadimethoxine	32.0–256.0

Data Analysis

Escherichia coli isolates were categorized according to the CM case and characteristics at the cow and herd level as follows: stage of lactation (≤ 100 , 101–200, or ≥ 201 d after calving); parity (primiparous or multiparous); quarter position (front or hind); CM severity (mild, changes only in the milk appearance; moderate, presence of abnormal milk accompanied by changes in the udder, or severe, combination of abnormal milk, with signs of inflammation in the udder and systemic signs; Wenz et al., 2006); season of CM occurrence in the southeastern region of Brazil (rainy: October–March; or dry: April–September; Oliveira et al., 2015); and housing system of the farm where the CM case was identified (compost-bedded pack barn, freestall, paddocks). The paddock housing system was characterized as an open area surrounded by fences or rails and without pasture for grazing.

The following logistic regression model was used to determine if descriptors at the cow level (stage of lactation, parity, quarter-position, CM severity), housing style, and season were associated with phylogenetic groups:

$$\begin{aligned} \text{logit (phylogroup)} = & \text{intercept} + \text{housing} \\ & + \text{season} + \text{DIM} + \text{parity} + \text{position} + \text{severity} \\ & + \text{herd (random)} + e, \end{aligned}$$

where phylogroup is the result of phylogenetic analysis (A or B1); housing (compost-bedded pack barn, freestall or paddocks); season (rainy or dry); DIM is the stage of lactation (≤ 100 , 101–200, or ≥ 201 d after calving); parity is related to the number of lactations (primiparous or multiparous); position is the position (front or hind) of the affected quarter; severity (mild, moderate, or severe); and e is the random residual error. Herds were included in the model as random effect. Analyses were carried out using PROC GLIMMIX of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Survival analysis using the PROC LIFETEST of SAS (version 9.4, SAS Institute Inc.) was completed to determine differences between AMS among *E. coli* phylogroups (Cortinhas et al., 2013). The concentrations of antimicrobials contained in the commercial broth microdilution test were used as the “time” variable, and the inhibition of bacterial growth was used as the event. Isolates that presented growth at the highest tested concentration were censored by the statistical model. Kaplan-Meier survival curves of the phylogenetic groups were performed for each studied antimicrobial. The null hypothesis of no differences (homogeneity of

survival curves) in the survivor functions of the strata (phylogenetic groups) was evaluated using log-rank and Wilcoxon tests.

To control for the potential effect of herd-level factors (e.g., drug use practices) on the AMS of *E. coli* isolates, a regression analysis of survival data based on the Cox proportional hazards model was also completed to compare AMS among phylogroups using the PROC PHREG of SAS (version 9.4, SAS Institute Inc.). The shared frailty model approach was used to adjust for within-cluster (herd) correlation. The model fit was

$$\begin{aligned} \text{MIC} \times \text{censor (0)} = & \text{phylogroup} \\ & + \text{herd (random)} + e, \end{aligned}$$

where MIC = concentration of antimicrobials contained in the commercial broth microdilution test that inhibited the bacterial growth; censor = 0 indicated that the isolate presented bacterial growth at the highest tested concentration; phylogroup = phylogenetic group to which the isolates belonged; herd (random) = herd from which the isolate was identified (included in the model as random effect), and e was the error term.

For all statistical analysis, significance was defined at $P \leq 0.05$.

RESULTS

Phylotyping Profile and Association with Herd- and Cow-Level Descriptors

Descriptive data of herds from which *E. coli* isolates were identified are presented in Table 2. *Escherichia coli* isolates were identified from cases of CM in 19 of 20 dairy herds evaluated in an epidemiological study on the characterization of CM (Tomazi, 2017). One herd (named herd N) had no isolation of *E. coli* during the study period. The overall mean herd size was 227 (SD = 333) lactating cows. On average, each herd was monitored for a period of 12.6 mo (SD = 1.4; ranging from 8 to 15 mo). The sample collection period among farms varied according to the willingness of the owners to remain in the study.

A total of 77 cryopreserved isolates that were recultivated for this study were not selected because of the following reasons: no growth during recultivation ($n = 26$), MALDI score ≤ 2.0 ($n = 16$), and contamination of the cryopreserved tube ($n = 8$). In addition, other 27 isolates from 3 herds were not selected during the randomization procedure: herd A ($n = 13$), herd I ($n = 7$), and herd K ($n = 7$).

Therefore, 100 *E. coli* isolates were selected and assigned to one of the phylogroups described by Clermont et al. (2013). Most isolates belonged to phylogroups A (n = 52; 52%) and B1 (n = 38; 38%). In addition, a total of 10 isolates (10%) belonged to one of the other less frequent phylogenetic groups: B2 (n = 2), C (n = 4), D (n = 3), and E (n = 1, Table 2).

Phylogroup A was identified in 14 of 19 (73.7%) herds, whereas group B1 was identified in 15 (78.9%) herds. Phylogenetic groups identified at lower frequency were isolated from 1 (groups B2 and E), 2 (group D), and 3 herds (group C, Table 2).

Association analysis between phylogenetic groups and descriptors related to the cows and herds were performed only for the 2 most frequently identified phylogroups (A and B1). No association ($P > 0.05$) was observed in logistic regression model between phylogenetic groups and evaluated descriptors (i.e., housing type, season, stage of lactation, parity, quarter position, and CM severity; Table 3).

Association of the other identified phylogroups (B2, C, D, and E) and the outcome variables were not statistically evaluated because of the low frequency of isolates (n ≤ 10) assigned to those groups. However,

the distribution of isolates within the categories of the evaluated variables is presented in Table 4.

Overall Antimicrobial Susceptibility Testing

In total, 94 isolates previously submitted for phylogenetic analysis had results of MIC (Table 5). Of isolates that did not have MIC results (n = 6), one was contaminated (i.e., presence of >2 morphologically different colonies) during re-inoculation of cryopreservation tube on blood agar plate, and 5 had no growth in the positive control wells of the Sensititer panel; these isolates were excluded from the analysis.

For ampicillin, the MIC₅₀ was 2 µg/mL and the MIC₉₀ was not able to be determined, as >10% of isolates had growth at the highest antimicrobial concentration present in the panel. The strains were highly susceptible to ceftiofur, and both MIC₅₀ and MIC₉₀ were 0.5 µg/mL; only one isolate was not inhibited at the highest concentration of ceftiofur. For cephalothin, the overall MIC₅₀ was 8 µg/mL, whereas the MIC₉₀ was 16 µg/mL. For sulfadimethoxine, 76.6% of isolates were susceptible at ≤256 µg/mL (MIC₅₀), whereas 23.4% were not susceptible at the highest concentration contained in the

Table 2. Descriptive herd-level data, frequency of clinical mastitis (CM; overall and pathogen-specific), and frequency of phylogenetic groups of *Escherichia coli* isolates (n = 100) selected from CM in 19 dairy herds of southeastern Brazil

Herd	Lactating cows ¹	Period in the study	Frequency of CM ²	<i>E. coli</i> CM ³	Cryopreserved isolates ⁴	Selected isolates ⁵	Frequency of isolates assigned to the phylogenetic groups (no.)					
							A	B1	B2	C	D	E
A	1,470 (52)	Jul. 2014–Jul. 2015	442	44	30	12	6	5	—	1	—	—
B	184 (25)	Apr. 2014–Apr. 2015	179	8	3	3	—	1	—	2	—	—
C	68 (5)	May 2014–Apr. 2015	36	3	3	3	—	3	—	—	—	—
D	165 (11)	Apr. 2014–Apr. 2015	225	9	8	5	3	2	—	—	—	—
E	371 (24)	Apr. 2014–Apr. 2015	627	12	5	4	3	1	—	—	—	—
F	253 (13)	Mar. 2014–Apr. 2015	874	17	9	6	5	1	—	—	—	—
G	77 (15)	Apr. 2014–Nov. 2014	43	3	2	2	1	1	—	—	—	—
H	71 (9)	Feb. 2015–Jan. 2016	98	4	4	2	—	2	—	—	—	—
I	167 (11)	May 2014–Apr. 2015	383	22	19	12	5	5	—	—	2	—
J	120 (10)	Apr. 2014–Apr. 2015	72	3	2	1	1	—	—	—	—	—
K	313 (7)	Mar. 2014–Apr. 2015	314	35	23	12	3	6	2	—	—	1
L	194 (7)	May 2014–Apr. 2015	288	24	19	11	11	—	—	—	—	—
M	586 (17)	Dec. 2014–Dec. 2015	1,395	61	25	10	7	3	—	—	—	—
O	46 (3)	Apr. 2014–Mar. 2015	48	3	2	1	—	1	—	—	—	—
P	36 (1)	Apr. 2014–Apr. 2015	69	6	4	3	3	—	—	—	—	—
Q	75 (12)	May 2014–Apr. 2015	220	10	8	6	2	3	—	1	—	—
R	22 (3)	Apr. 2014–Mar. 2015	16	1	1	1	1	—	—	—	—	—
S	55 (7)	Jun. 2014–May 2015	52	8	7	5	1	3	—	—	1	—
T	46 (4)	Apr. 2014–Apr. 2015	46	3	3	1	—	1	—	—	—	—
Overall	227 (333)	Mar. 2014–Jan. 2016	5,427	276	177	100	52	38	2	4	3	1

¹Herd size: average number of lactating cows (SD in parentheses) per herd during the study period.

²Frequency (no.) of CM cases identified during the study period regardless of the causing pathogen.

³Frequency (no.) of CM cases with identification of *Escherichia coli* in the bacteriological culture.

⁴Frequency (no.) of isolates cryopreserved during the study period.

⁵Frequency (no.) of isolates selected for phylogenetic analysis and antimicrobial susceptibility testing.

Table 3. Results of a generalized linear mixed model evaluating the association between phylogenetic groups A (n = 52) and B1 (n = 38) of *Escherichia coli* identified from clinical mastitis (CM) in 19 dairy herds of southeastern Brazil and descriptors at the cow level, housing type, and season

Variable	Category	Phylogenetic group				F-value	P-value
		A		B1			
		No.	%	No.	%		
Housing (n = 90)	Freestall	19	54.3	16	45.7	1.06	0.35
	CBPB ¹	8	44.4	10	55.6		
	Paddocks ²	25	67.6	12	32.4		
Season ³ (n = 90)	Rainy	42	60.9	27	39.1	3.61	0.06
	Dry	10	47.6	11	52.4		
Stage of lactation ⁴ (n = 89)	0–100	14	58.3	10	41.7	0.30	0.74
	101–200	17	60.7	11	39.3		
	>200	20	54.1	17	46.0		
Parity (n = 88)	Primiparous	11	84.6	2	15.4	1.92	0.16
	Multiparous	40	53.3	35	46.7		
Quarter position (n = 90)	Front	21	51.2	20	48.8	1.02	0.32
	Hind	31	63.3	18	36.7		
CM severity ⁵ (n = 85)	Mild	24	66.7	12	33.3	1.90	0.16
	Moderate	22	61.1	14	38.9		
	Severe	5	38.5	8	61.5		

¹CBPB = compost-bedded pack barn.

²The paddock housing system was characterized as an open area surrounded by fences or rails and without pasture for grazing.

³Season categories were formed by the association of the 2 characteristic seasons in southeast Brazil [rainy (October–March); or dry (April–September)]; Oliveira et al., 2015.

⁴Stage of lactation categorized according to DIM.

⁵CM severity: (mild) changes only in the milk appearance; (moderate) presence of abnormal milk accompanied by changes in the udder; or (severe) combination of abnormal milk, with signs of inflammation in the udder and systemic sign.

Table 4. Distribution of less frequent phylogroups of *Escherichia coli* (n = 10) identified from clinical mastitis (CM) cases in 19 dairy herds of southeastern Brazil, stratified by variables at the cow-level, housing type, and season

Variable	Categories	Phylogenetic group			
		B2	C	D	E
Housing (n = 10)	Freestall	2	1	—	1
	CBPB ¹	—	3	2	—
	Paddocks ²	—	—	1	—
Season ³ (n = 10)	Rainy	2	4	3	1
	Dry	—	—	—	—
Stage of lactation ⁴ (n = 9)	0–100	—	1	1	—
	101–200	—	2	1	—
	>200	2	1	1	—
Parity (n = 9)	Primiparous	—	2	1	—
	Multiparous	2	2	2	—
Quarter position (n = 10)	Front	2	—	—	1
	Hind	—	4	3	—
CM severity ⁵ (n = 9)	Mild	1	1	—	—
	Moderate	—	2	3	1
	Severe	1	—	—	—

¹CBPB = compost-bedded pack barn.

²The paddock housing system was characterized as an open area surrounded by fences or rails and without pasture for grazing.

³Season categories were formed by the association of the 2 characteristic seasons in southeast Brazil [rainy (October–March); or dry (April–September)]; Oliveira et al., 2015.

⁴Stage of lactation categorized according to days in milk.

⁵CM severity: (mild) changes only in the milk appearance, (moderate) presence of abnormal milk accompanied by changes in the udder; or (severe) combination of abnormal milk, with signs of inflammation in the udder and systemic sign.

Table 5. Overall frequency (%) of *Escherichia coli* isolates (n = 94) that had 50% (MIC₅₀) and 90% (MIC₉₀) of bacterial growth inhibited at each antimicrobial concentration

Antimicrobial	Frequency (%) of isolates at each indicated MIC ¹ (μg/mL)												NI ²	MIC ₅₀ ³	MIC ₉₀ ⁴
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256			
Ampicillin	1.1	2.1	4.3	5.3	41.5	29.8	1.1	—	—	—	—	—	14.8	2	>8
Ceftiofur	—	—	92.6	2.1	2.1	2.1	—	—	—	—	—	—	1.1	0.5	0.5
Cephalothin	—	—	—	—	4.3	13.8	57.5	17.0	—	—	—	—	7.4	8	16
Erythromycin	—	1.1	2.1	3.2	0	1.1	—	—	—	—	—	—	92.6	>4	>4
Oxacillin	—	—	—	—	19.1	2.1	—	—	—	—	—	—	78.7	>4	>4
Penicillin + novobiocin	—	—	—	0	1.1	1.1	27.7	—	—	—	—	—	70.2	>8/16	>8/16
Penicillin	1.1	0	0	0	1.1	2.1	2.1	—	—	—	—	—	93.6	>8	>8
Pirlimycin	—	—	6.4	0	0	2.1	—	—	—	—	—	—	91.5	>4	>4
Sulfadimethoxine	—	—	—	—	—	—	—	—	25.5	34.0	12.8	4.3	23.4	64	>256
Tetracycline	—	—	—	6.4	18.1	41.5	4.3	—	—	—	—	—	29.8	4	>8

¹The light gray shading represents the susceptible zone, and the darker gray shading represents the resistant zone. A dash (—) indicates that the antimicrobial concentration was not tested. Results were interpreted according to the CLSI (2013). Interpretative criteria were based on human data (cephalothin, sulfadimethoxine, and tetracycline), dog data (ampicillin), and bovine mastitis (ceftiofur). The resistant category included those isolates categorized as either intermediate or resistant. Antimicrobials without shading have no interpretive criteria available for *Escherichia coli*.

²NI = not inhibited (growth at highest concentration tested).

³MIC (μg/mL) that inhibited 50% (MIC₅₀) of the isolates.

⁴MIC (μg/mL) that inhibited 90% (MIC₉₀) of the isolates.

test. The overall MIC₅₀ for tetracycline was 4 μg/mL, whereas 29.8% of the isolates were not inhibited at the highest concentration present in the test.

For erythromycin, oxacillin, penicillin, penicillin/novobiocin, and pirlimycin, more than 70% of strains were not inhibited at the highest antimicrobial concentration contained in the panel (Table 5).

Antimicrobial Susceptibility Testing of Phylogenetic Groups A and B1

The frequency distribution of MIC results (MIC₅₀ and MIC₉₀), according to the phylogenetic groups A and B1 of *E. coli* isolates, are shown in Table 6. The MIC₅₀ of ampicillin for *E. coli* isolates of both phylogroups (A and B1) was 2 μg/mL. The MIC₉₀ for the same antimicrobial was 4 μg/mL for isolates of phylogroup A; however, the MIC₉₀ for isolates of phylogroup B1 was not determined because more than 10% of tested isolates were not inhibited at the highest concentration of ampicillin present in the panel. In total, 10% of isolates assigned to phylogroup A, and 14% of isolates of phylogroup B1 were not inhibited at the highest concentration of ampicillin. Only 2% of isolates from phylogroup A and 5.8% from the phylogroup B1 were susceptible to ampicillin.

For ceftiofur, the MIC₅₀ and MIC₉₀ was observed at the lowest concentration (0.5 μg/mL) for both phylogenetic groups (A and B1). Only 2% (1 of 50) of isolates from phylogroup A and 5.8% (2 of 34) from

phylogroup B1 were not susceptible to ceftiofur. The MIC₅₀ of cephalothin (8 μg/mL) was also similar among phylogroups A and B1; however, the MIC₉₀ was 16 μg/mL for isolates assigned to phylogroup A and >16 μg/mL for isolates assigned to phylogroup B1. A total of 86% of isolates belonging to phylogroup A and 61.8% of isolates from phylogroup B1 were susceptible to cephalothin (Table 6).

The MIC of sulfadimethoxine to inhibit 50% of isolates from both phylogroups A and B1 was 64 μg/mL. On the other hand, estimation of MIC₉₀ for sulfadimethoxine was not possible because more than 10% of tested isolates were resistant to this antimicrobial at the highest concentration present in the commercial panel. A total of 80% of isolates assigned to phylogroup A and 76.5% of isolates of phylogroup B1 were susceptible to sulfadimethoxine.

A total of 66% of isolates from phylogroup A and 67.6% from phylogroup B1 were susceptible to tetracycline. For this same antimicrobial, the MIC₅₀ was similar for both groups (4 μg/mL), whereas the MIC₉₀ was greater than the highest antimicrobial concentration (8 μg/mL).

For antimicrobials with no expected antimicrobial activity against *E. coli*, the isolates of both phylogroups A and B1 were resistant to the highest antimicrobial concentration contained in the panel in more than 70% of the tests for oxacillin and penicillin-novobiocin, and in more than 90% for erythromycin, penicillin, and pirlimycin.

Table 6. Frequency (%) of *Escherichia coli* isolates according to phylogenetic groups A (n = 50) and B1 (n = 34) that had 50% (MIC₅₀) and 90% (MIC₉₀) of bacterial growth inhibited at each antimicrobial concentration

Antimicrobial	Phylo-group	Frequency (%) of isolates at each indicated MIC ¹ (µg/mL)													NI ²	MIC ₅₀ ³	MIC ₉₀ ³
		0.12	0.25	0.5	1	2	4	8	16	32	64	128	256				
Ampicillin	A	0	2.0	0	8.0	44.0	36.0	0	—	—	—	—	—	10.0	2	4	
	B1	2.9	2.9	8.8	2.9	35.3	29.4	2.9	—	—	—	—	—	14.7	2	>8	
Ceftiofur	A	—	—	94.0	2.0	2.0	2.0	—	—	—	—	—	—	0	0.5	0.5	
	B1	—	—	91.2	2.9	0	2.9	—	—	—	—	—	—	2.9	0.5	0.5	
Cephalothin	A	—	—	—	—	8.0	20.0	58.0	10.0	—	—	—	—	4	8	16	
	B1	—	—	—	—	0	2.9	58.8	26.5	—	—	—	—	11.8	8	>16	
Erythromycin	A	—	2.0	2.0	4.0	0	0	—	—	—	—	—	—	92	>4	>4	
	B1	—	0	0	2.9	0	0	—	—	—	—	—	—	97.1	>4	>4	
Oxacillin	A	—	—	—	—	16.0	0	—	—	—	—	—	—	84.0	>4	>4	
	B1	—	—	—	—	20.6	5.9	—	—	—	—	—	—	73.5	>4	>4	
Penicillin + novobiocin	A	—	—	—	0	2.0	0	26.0	—	—	—	—	—	72.0	>8/16	>8/16	
	B1	—	—	—	0	0	0	29.4	—	—	—	—	—	70.6	>8/16	>8/16	
Penicillin	A	0	0	0	0	2.0	4.0	2.0	—	—	—	—	—	92.0	>8	>8	
	B1	2.9	0	0	0	0	0	2.9	—	—	—	—	—	94.1	>8	>8	
Pirlimycin	A	—	—	4.0	0	0	2.0	—	—	—	—	—	—	94.0	>4	>4	
	B1	—	—	5.9	0	0	2.9	—	—	—	—	—	—	91.2	>4	>4	
Sulfadimethoxine	A	—	—	—	—	—	—	—	—	30.0	36.0	10.0	4.0	20.0	64	>256	
	B1	—	—	—	—	—	—	—	—	20.6	38.2	14.7	2.9	23.5	64	>256	
Tetracycline	A	—	—	—	8.0	20.0	38.0	4.0	—	—	—	—	—	30.0	4	>8	
	B1	—	—	—	0	20.6	47.1	2.9	—	—	—	—	—	29.4	4	>8	

¹The light gray shading represents the susceptible zone, and the darker gray shading represents the resistant zone. A dash (—) indicates that the antimicrobial concentration was not tested. Results were interpreted according to the CLSI (2013). Interpretative criteria were based on human data (cephalothin, sulfadimethoxine, and tetracycline), dog data (ampicillin), and bovine mastitis (ceftiofur). The resistant category included those isolates categorized as either intermediate or resistant. Antimicrobials without shading have no interpretative criteria available for *Escherichia coli*.

²NT = not inhibited (growth at highest concentration tested).

³MIC (µg/mL) that inhibited 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates.

Table 7. Results of survival function analysis and Cox proportional hazards model comparing the antimicrobial susceptibility of *Escherichia coli* isolated from clinical mastitis cases identified in 19 dairy herds and stratified by the phylogeny groups A (n = 50) and B1 (n = 34)

Antimicrobial	PG ¹	Censor ²	Survival function		Cox proportional hazards model ⁵				
			Log-rank ³	Wilcoxon ⁴	Estimate	SE	Hazard ratio	95% CI	P-value
Ampicillin	A	5	0.92	0.93	0.18	0.25	1.20	0.73, 1.98	0.48
	B1	5							
Ceftiofur	A	0	0.35	0.60	0.69	0.96	2.00	0.30, 13.15	0.45
	B1	1							
Cephalothin	A	2	0.002	0.0007	0.47	0.24	1.60	1.01, 2.54	0.04
	B1	4							
Erythromycin	A	46	0.33	0.33	0.42	1.24	1.52	0.13, 17.29	0.73
	B1	33							
Oxacillin	A	42	0.25	0.29	−0.51	0.49	0.60	0.23, 1.56	0.29
	B1	25							
Penicillin/novobiocin	A	36	0.93	0.94	0.09	0.43	1.09	0.47, 2.53	0.82
	B1	24							
Penicillin	A	46	0.72	0.72	1.13	1.14	3.09	0.33, 28.90	0.31
	B1	32							
Pirlimycin	A	47	0.62	0.62	−0.41	1.41	0.67	0.04, 10.66	0.77
	B1	31							
Sulfadimethoxine	A	10	0.49	0.39	0.14	0.25	1.15	0.70, 1.88	0.58
	B1	8							
Tetracycline	A	15	0.73	0.65	0.07	0.27	1.07	0.64, 1.81	0.79
	B1	10							

¹PG = phylogenetic group.²Frequency of isolates censored (observed growth at the highest antimicrobial drug concentration of the used test).³Log-rank test for equality of strata at higher antimicrobial concentrations.⁴Wilcoxon test for equality of strata at lower antimicrobial concentrations.⁵Herd was included in the model as a random effect.

Survival Function Analysis

Antimicrobial susceptibility among phylogenetic groups A and B1 were compared using Kaplan-Meier survival curves and Cox proportional hazards model (Table 7). Homogeneous survival curves were observed for all evaluated antimicrobials (log-rank test >0.25; Wilcoxon >0.29), except for cephalothin (log-rank test = 0.002; Wilcoxon = 0.0007). Survival curves showed heterogeneity among phylogenetic groups for cephalothin, indicating that isolates of phylogroup A were inhibited at lower concentration of this antimicrobial than isolates of phylogroup B1 (Figure 1). Similarly, cephalothin was the only antimicrobial showing difference in AMS among phylogroups A and B1 when the Cox proportional hazards model was used (Table 7).

DISCUSSION

To our knowledge, this is the first study evaluating the association of phylogenetic groups and AMS of *E. coli* isolated from bovine CM in Brazil. Phylogenetic studies may provide insights into the epidemiology of *E. coli* associated with bovine mastitis and may indicate specific host-pathogen relationships (Tenaillon et al., 2010). However, our data showed no association between *E. coli* phylogenetic groups and cow-level de-

scriptors, housing system, or CM severity. Furthermore, differences in AMS may be uncommon as they were limited to only one antimicrobial (i.e., cephalothin). Nevertheless, our results on AMS of *E. coli* causing

Figure 1. Heterogeneous Kaplan-Meier survival curves (log-rank test = 0.002; Wilcoxon test = 0.0007) for *Escherichia coli* isolated from clinical mastitis in 19 dairy herds, according to cephalothin susceptibility testing and stratified by phylogenetic groups A (n = 50) or B1 (n = 34). Censored data are indicated as a cross on the right end of the lines.

mastitis can be used as a basis for further pharmaco-epidemiological studies to monitor the emergence of potential resistant strains.

In our study, 90% of *E. coli* isolates were assigned to phylogroups A (58%) or B1 (32%), and a small proportion (10%) of isolates were assigned to phylogroups B2 (2.0%), C (4.0%), D (3.0%), and E (1.0%), whereas no isolates were identified as belonging to group F. High frequency of phylogroups A and B1 were also observed in other studies evaluating the phylogeny of *E. coli* isolated from CM (Liu et al., 2014; Keane, 2016; Zhang et al., 2018). In a recent study conducted in China, of 79 *E. coli* isolates from bovine mastitis, 59.5% belonged to phylogenetic group A, whereas 27.8% were assigned to phylogroup B1 (Zhang et al., 2018). Similarly, in a study conducted in Ireland, 54% (20/37) of *E. coli* isolates recovered from CM were assigned to phylogroup A and 32% (12/37) to group B1 (Keane, 2016). Phylogenetic groups A and B1 were mainly associated with commensal strains of *E. coli* in dairy cattle (Son et al., 2009), which may explain the highest prevalence (>85%) of these groups among isolates recovered from bovine IMI in our study and other studies.

Goldstone et al. (2016) described that *E. coli* assigned to phylogroup A (the most common phylogroup causing mastitis) have some unique characteristics, such as genomic content and phylogenetic similarity, indicating that some functional products of the genes may be essential for *E. coli* causing IMI. Moreover, *E. coli* associated with mastitis still cannot be distinguished from bovine commensal *E. coli* based on the presence of virulence-associated factors, such as is performed for intestinal pathogenic *E. coli*. However, it is known that the presence of few putative genes in mastitis-associated *E. coli* are important for gastrointestinal colonization (Leimbach et al., 2017). Therefore, because *E. coli* causing mastitis are mostly associated with environmental sources, the control of this pathogen should focus on management and hygiene practices aiming to reduce exposure of teat ends to intestinal pathogenic strains. A study comparing phylotypes among fecal commensal *E. coli* isolated on the same farms (and time) where the isolates from CM were obtained could improve epidemiological understanding of *E. coli* phylogroups causing mastitis.

Although there was high prevalence of isolates belonging to phylogroups A and B1, other less frequently isolated phylogroups (B2, C, D, and E) were also identified in our study, which indicates their potential to cause CM in dairy cows. Phylogroups B2 and D were also described in other reports evaluating *E. coli* isolated from IMI. Isolates belonging to group B2 had also low frequency (1.4%; 2/144 isolates) in a Finnish study evaluating isolates recovered from CM (Suojala et al.,

2011), and in a Chinese study (4.3%; 2/46) evaluating multi-drug-resistant extended-spectrum β -lactamase-producing *E. coli* isolated from bovine mastitis (Ali et al., 2017). Similarly, another study found that only 2 of 30 *E. coli* isolates pertained to phylogroup B2 (Dogan et al., 2012). On the other hand, phylogroup D was reported in higher frequency in studies about mastitis, described in 11.1% (second most frequent phylogroup) of isolates in the study of Suojala et al. (2011); in 16.4% of isolates recovered from CM in dairy herds of Iran (Ghanbargpour and Oswald, 2010); in 14% of isolates in an Irish study (Keane, 2016), and in 21.7% of extended-spectrum β -lactamase-producing *E. coli* in the study conducted in China (Ali et al., 2017).

Some phylogroups identified in our study were not described (e.g., phylogroup C), or had low frequency in other studies evaluating their relationship with IMI in dairy cows (e.g., phylogroup E; Kempf et al., 2016). Because many phylogenetic studies were based on the initial rapid determination scheme purposed by Clermont et al. (2000), which only permits distinguishing isolates among 4 phylogenetic groups (A, B1, B2, and D), the frequency of groups C and E may have been underestimated in previous studies. Using the quadruplex PCR method, it is possible to assign *E. coli* into 8 phylogroups: 7 belonging to *E. coli sensu stricto* (A, B1, B2, C, D, E, F), whereas the eighth is the *Escherichia* cryptic clade I (Clermont et al., 2013).

Previous studies have shown that mastitis-associated strains were found across 5 (A, B1, B2, D, and E) of 7 *E. coli sensu stricto* groups (Kempf et al., 2016). However, to the best of our knowledge, this is the first study reporting *E. coli* of phylogroup C as the cause of CM. In other studies, strains belonging to phylogroup C were associated with intestinal and extra-intestinal infections in humans (Clermont et al., 2011), and with fecal samples collected from diarrheal calves (Souto et al., 2017). Only 4 isolates were assigned to phylogroup C in our study and few isolates were assigned to phylogroups B2, D, and E. Therefore, further studies with a larger number of strains could better clarify whether these less frequently identified phylogroups are only opportunistic pathogens or are emerging phylogroups associated with CM in dairy cows.

None of the factors or descriptors evaluated in our study (i.e., housing system, season, quarter position, stage of lactation, parity, CM severity) were associated with the phylogenetic groups. Therefore, our results did not allow us to reject the null hypothesis that phylogroup (A or B1) is independent of evaluated variables. As both phylogroups A and B1 were reported to have specific virulence factors (Liu et al., 2014), it could be speculated that differences in mastitis severity could be attributed to the phylogenetic group; how-

ever, no differences of CM severity were found among the phylogroups in our study. Likewise, another study found no association among phylogenetic groups with clinical signs of dairy cows with acute CM (Suojala et al., 2011). Furthermore, a review on *E. coli* CM reported that clinical signs are mainly determined by cow factors such as immunological status or hormonal and metabolic alterations, rather than specific features of the bacterial strain (Burvenich et al., 2003). Conversely, immune response and mammary pathogenesis can vary according to *E. coli* strains causing IMI. Differences in the intensity and duration of cellular (e.g., SCC) and other mammary immune responses (e.g., type of cytokine secreted) was observed in a challenge study comparing 3 distinct MPEC isolated from 3 forms of mastitis (peracute, persistent, and acute; Blum et al., 2017).

Escherichia coli isolates in our study were submitted for analysis of AMS for determination of MIC₅₀ and MIC₉₀, and a high frequency of isolates were considered resistant to most of antimicrobials tested. Overall, more than 96% of strains were resistant to ampicillin, and more than 23% were considered resistant to cephalothin, sulfadimethoxine, or tetracycline. It is important to mention that a direct comparison between studies cannot be easily performed, because several factors can affect the susceptibility results, such as methodology used for susceptibility testing (disk diffusion vs. broth dilution method), concentration of antimicrobials used in MIC analysis, and differences in the interpretive criteria used for categorizing isolates as susceptible or resistant.

Only 2 reports were found describing the same commercial panel as used in our study for AMS testing of *E. coli* strains (Rajala-Schultz et al., 2004; Hoe and Ruegg, 2005). In comparison to our study, Rajala-Schultz et al. (2004) reported lower frequency of resistance to ampicillin (44.4 vs. 96.8% in our study), but higher resistance frequency to the rest of the antimicrobials tested. Although a higher resistance to ampicillin was observed in our study, the MIC₅₀ was lower (2 µg/mL) in comparison to the aforementioned report (4 µg/mL). Lower MIC₅₀ were also observed in our study for sulfadimethoxine (64 vs. >256 µg/mL) and tetracycline (4 vs. 8 µg/mL) in comparison to the results found by Rajala-Schultz et al. (2004), and similar MIC₅₀ were observed between studies for ceftiofur (0.5 µg/mL) and cephalothin (8 µg/mL). Furthermore, our results showed lower MIC₉₀ for ceftiofur (0.5 µg/mL) and cephalothin (16 µg/mL) than observed in the study of Rajala-Schultz et al. (2004), in which the MIC₉₀ was 2.0 µg/mL for ceftiofur and >16 µg/mL for cephalothin.

Hoe and Ruegg (2005) evaluated in vitro AMS of *E. coli* isolated from cows with CM and reported an

overall susceptibility of *E. coli* similar to our results. However, 91% of isolates were reported as susceptible to ampicillin in the study, whereas only 3.2% of isolates was susceptible to this antimicrobial in our study. The difference in MIC results for ampicillin between studies is due to different interpretive criteria for classification of the isolates as susceptible or resistant. We used 0.25 µg/mL as cut-off (criteria used for dog's skin and soft tissue), while a higher limit (8.0 µg/mL) was used in the study of Hoe and Ruegg (2005), which was based on human criteria. Adopting the same interpretative criteria as used by Hoe and Ruegg (2005), 85.1% (80/94) of *E. coli* isolates would be considered susceptible to ampicillin in our study.

High proportions of antimicrobial resistance (>70%) were also observed for erythromycin, oxacillin, penicillin, penicillin associated with novobiocin, and pirlimycin. However, these antimicrobials are not expected to have activity against gram-negative bacteria, and no interpretive criteria are available for susceptibility analysis of *E. coli* strains. Similar outcomes were observed in other studies (Rajala-Schultz et al., 2004; Hoe and Ruegg, 2005). Future studies evaluating AMS of mastitis-causing *E. coli* may consider using panels with antimicrobials that have a more specific spectrum of activity against gram-negative organisms. Nevertheless, our results about AMS are still relevant considering global concerns about the rational use of antimicrobials. Blanket protocols (instead of culture based) are still widely used for CM treatment in dairy herds; a high frequency of *E. coli* CM cases have been treated with antimicrobials included in the panel evaluated in this study, or with other formulations with greater activity against gram-positive organisms.

Most strains (96.8%) were susceptible to ceftiofur and comparable results were reported in other studies. Bengtsson et al. (2009) reported that ceftiofur at a concentration of 0.5 µg/mL inhibited 96.3% of 163 *E. coli* strains isolated from acute CM in dairy cows. In another study, 100% of *E. coli* strains isolated from CM were susceptible to ceftiofur at a concentration of 0.5 µg/mL (Thomas et al., 2015). Ceftiofur is a third-generation cephalosporin that acts by inhibiting bacterial cell wall synthesis of both gram-negative and gram-positive bacteria (Schukken et al., 2011). Because this antimicrobial was marketed to be administered via the intramammary route, it has been used extensively for CM treatment in dairy cattle (Saini et al., 2012; Oliveira and Ruegg, 2014).

Kaplan-Meier curves and survival analysis were used in our study to determine whether phylogroups A and B1 had different MIC for evaluated antimicrobials. In general, it is assumed in survival analysis that events may occur over a continuous scale, such as time (Cor-

tinhas et al., 2013). In our study, we assumed that MIC determined by broth microdilution was the continuous variable, which allowed us to compare the shape of survival curves of the susceptibility test. An important feature of this method is the possibility of inclusion in the analysis of those isolates that were not inhibited at the highest concentration. On the other hand, a drawback of this method is the inability to analyze survival data with clustered events. In our study, herd-level factors (e.g., overuse of certain antimicrobials) could affect AMS of *E. coli* isolates. Therefore, not considering the herd effect in the data analysis may bias the outcomes by violation of the assumption of independence of the observations. Hence, in addition to the estimation of the Kaplan-Meier curves, we performed a regression analysis of survival data based on the Cox proportional hazards model. This method allowed us to compare AMS between phylogroups A and B1 by controlling the potential effect of herds on the outcomes.

In both survival methods, a difference in AMS among phylogroups was observed only for cephalothin, in which strains belonging to phylogroup A were inhibited at lower antimicrobial concentrations than strains assigned to phylogroup B1. For the remaining antimicrobials, the curves comparing AMS were homogeneous between phylogroups A and B1. Studies evaluating the relationship of phylogenetic groups and susceptibility to antimicrobials are scant in the indexed literature (Liu et al., 2014; Keane, 2016). Furthermore, studies using survival analysis to compare the susceptibility of antimicrobials among phylogenetic groups were not found, which makes it difficult to compare our results with those in other studies.

A recent study conducted in Ireland also reported a high prevalence of strains belonging to phylogroups A and B1, although no relationship between antimicrobial resistance and phylogenetic group was observed. In another study, 70 *E. coli* isolates recovered from clinical and subclinical mastitis were characterized with respect to their phylogenetic groups, virulence factors, and AMS (Liu et al., 2014). The results of this study showed that 67.1% of isolates were resistant to streptomycin, and those from group B1 were more resistant to this antimicrobial than isolates from group A. Isolates belonging to phylogroup B1 were also reported to have a higher frequency of virulence genes than isolates of group A, which could be related to potential differences in antimicrobial resistance among phylogroups.

Despite these promising results, we recognized that a lower sampling size than expected was used for evaluation of descriptors at the cow or herd level and phylogenetic groups, especially for less frequent phylogroups. In addition, further studies evaluating AMS and the presence of resistance genes for these less frequently

identified phylogroups are needed to better understand their epidemiology and potential role in the etiology of mastitis. Despite these limitations, this is the first study evaluating the phylogeny and susceptibility of *E. coli* strains isolated from CM in dairy herds in Brazil. The results observed in this report may be used as a reference for further studies evaluating the pharmacoepidemiology and pathogenicity of *E. coli* causing CM in dairy cows.

In conclusion, *E. coli* isolates associated with CM mainly belonged to phylogenetic groups A and B1, although isolates were also assigned at lower frequency to phylogenetic groups B2, C, D, and E. None of the cow- and herd-level descriptors were associated with the *E. coli* phylogenetic groups. Resistance was observed for all evaluated antimicrobials. However, *E. coli* isolates were more susceptible to antimicrobials pertaining to the cephalosporin class. Finally, strains belonging to group A1 were inhibited at lower cephalothin concentration than strains assigned to phylogroup B1, which indicates that specific genetic properties among certain strains may be involved with cephalothin susceptibility. Based on our results, management strategies to reduce exposure of cows' teat ends to commensal *E. coli* (regardless of phylogenetic group) are still the best practice for CM control in dairy herds. In addition, prudent antimicrobial use should be performed to treat CM caused by *E. coli*, as phylogenetic properties may affect treatment outcomes.

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