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Original Article

Antimicrobial Resistance and Virulence Factors of *Escherichia coli* in Cheese Made from Unpasteurized Milk in Three Cities in Brazil

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Abstract

The production of cheeses from unpasteurized milk is still widespread in Brazil, even with a legal ban imposed on its marketing. The manufacture of this cheese is a public health problem, due to the use of raw milk and the poor hygienic conditions throughout the supply chain process. Contamination may occur from several sources and involve several different pathogenic microorganisms, such as *Escherichia coli*. The latter can cause different clinical manifestations depending on the pathotype involved. Furthermore, some isolates manifest antimicrobial resistance and may be a risk for public health. The purpose of the current study was to investigate the presence of potentially pathogenic *E. coli* in raw-milk cheese in Brazil and their possible risk to public health. A total of 83 cheeses were collected from three different cities and 169 *E. coli* isolates were characterized for the presence of enteropathogenic *E. coli*, Shigatoxigenic *E. coli*, enterotoxigenic *E. coli*, extraintestinal pathogenic *E. coli* (ExPEC) virulence genes, phylogenetic type, antimicrobial resistance, O serogroup, and pulsed-field gel electrophoresis. The number of samples positive for *E. coli* was highest in Aracaju (90.32%, 28/31). The prevalence of samples positive for potential ExPEC genes was similar for Uberaba and Aracaju (23.07%); the most prevalent ExPEC virulence genes were *tsh*, *iucD*, and *papC*. Isolates from Uberaba had a higher prevalence of resistance to tetracycline (38.46%), amoxicillin/clavulanic acid (58.85%), and ampicillin (61.54%) than the other cities. Overall, antimicrobial resistance genes *tetB*, bla_{TEM}, and $bla_{CMY\text{-}2}$ were the most prevalent genes (26.32%, 15.79%, and 28.95%, respectively) and the most prevalent serotypes were O4 (8%), 018 (12%), and O23 (8%). Clones originating from the same regions and from different regions were observed. These results emphasize the presence of a potential danger for humans in the consumption of raw-milk cheeses in three cities in Brazil due to the presence of antimicrobial resistance, which should be monitored.

Introduction

Cheeses made with raw milk are among the most consumed dairy products in Brazil, frequently consumed on the national market. These are soft, white slightly salted cheeses, with a slight lactic acid taste, and are produced by the enzymatic coagulation in unpasteurized milk (Cunha *et al.*, 2006). Contamination and spoilage of these cheeses may occur as a result of poor hygiene, long periods of transportation, and lack of appropriate storage facilities throughout the production chain (Temelli *et al.*, 2006).

Various pathogenic bacteria may be transmitted by dairy products. Carvalho *et al.* (2007) identified *Listeria monocytogenes*, coagulase-positive staphylococci, and fecal coliforms in 93 raw-milk cheeses. Lima *et al.* (2013) found *Staphylococcus aureus* in cheeses in the State of Rio Grande

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do Sul, Southern Brazil. In addition, *Escherichia coli* infection outbreaks have been associated with the consumption of cheese. For example, in 2002, hemorrhagic colitis due to *E. coli* O157:H7 was associated with consumption of unpasteurized Gouda cheese (Honish *et al.*, 2005). Uncooked meat or unpasteurized milk products may frequently be common sources of serious foodborne outbreaks due to enterohemorrhagic serogroup O157 or other non-O157 serogroups like O26, O111, O103, and O145 (European Food Safety Authority [EFSA], 2013; European Food Safety Authority [EFSA]; and European Centre for Disease Prevention, Control [ECDC], 2014).

E. coli usually reside harmlessly in the intestinal lumen of humans and animals. However, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, opportunistic strains can cause infection. Infection with inherently pathogenic *E. coli* strains may result in urinary tract infection, sepsis/meningitis, or enteric/diarrheal disease. *E. coli* strains can cause extraintestinal pathogenic (ExPEC) or enteric/diarrhogenic infections in humans. Enteric *E. coli* infections are classically divided into six pathotypes, which are based on their pathogenicity profiles (virulence factors, clinical disease, and phylogenetic background). The pathotypes are as follows: enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and Shigatoxigenic *E. coli* (STEC). A subset of STEC is enterohemorrhagic *E. coli* (EHEC), including strain serotype O157:H7 (Kaper *et al.* 2004).

E. coli may be used as an indicator to assess inadequate pasteurization, poor hygienic conditions during processing (especially when carried out by hand), or postprocessing contamination (Kornacki and Johnson, 2001). In addition, the level of antimicrobial resistance in *E. coli* is considered to be a good indicator of the selection pressure exerted by the use of antimicrobials (Lei *et al.*, 2010) and of potential antimicrobial resistance problems in bacterial infectious diseases (Álvarez-Fernándes *et al.*, 2013). The importance of these aspects in raw-milk cheeses in Brazil is not well known. The purpose of the current study was to investigate the presence of potentially pathogenic *E. coli* strains in raw-milk cheeses and document potential risk to public health in Brazil.

Materials and Methods

Sampling and initial procedures

Cheeses were collected from cities in three different provinces in Brazil where raw-milk cheeses are commonly produced (Fig. 1). These were selected for ease of transport of samples to the laboratory. In addition, Minas Gerais was selected as, at the time of sampling, only this province permitted production of raw-milk cheeses. At each site, cheeses were randomly selected to represent the cheeses available to the public, comprising $\sim$20% of the total number of cheeses offered at each store. All selected cheeses were purchased. In Uberaba, 30 cheeses were collected on the same day, at one market. In Ribeirao Preto, 22 cheeses were collected on the same day. They were collected from 11 different stores at the municipal market. In Aracaju, 31 cheeses were collected on the same day from seven different markets or street sellers. All cheeses originated from various farms, although the precise farm of origin could not be verified. Sampling was performed during February 2010.

For *E. coli* isolation, 25 g of each cheese was enriched in Lauryl Tryptose Broth (Difco), streaked onto eosin methylene blue agar (EMB; Difco), and incubated at 37°C for 24 h (Apha, 2001). Thereafter, in each cheese analyzed, 5–10 typical *E. coli* colonies were randomly selected and identified biochemically by the IMViC tests (indole production, methyl red, Voges–Proskauer, and citrate) (Koneman *et al.*, 2001). A

FIG. 1. Map of Brazil indicating all states and showing the three regions where cheeses were collected. For the sampling, 30 cheeses were collected in Uberaba from one market and a total of 51 *Escherichia coli* isolates selected. In Ribeirao Preto, 22 cheeses were collected from 22 different stores in the municipal market and a total of 25 *E. coli* isolates selected. In Aracaju, 31 cheeses were collected from eight different markets or street sellers and a total of 93 *E. coli* isolates selected.

total of 169 *E. coli* isolates, 51, 25, and 93 from Uberaba, Ribeirao Preto, and Aracaju, respectively, that is, one to two representative isolates from each positive sample, were retained and sent to the Reference Laboratory for *E. coli*, Faculté de médecine vétérinaire, Université de Montréal.

Polymerase chain reaction for determination of the pathotype of isolates

Colonies were plated onto MacConkey agar (MA; Oxoid). A loop from the confluent growth or individual colonies on MA plates was inoculated into 5 mL Luria Bertani (Luria-Bertani–LB; Difco) broth and enriched overnight at 37°C. DNA templates were prepared from the processed samples by boiled cell lysis for examination by polymerase chain reaction (PCR) for the presence of the virulence genes, which define the *E. coli* pathotypes commonly found in animals, as described previously by Maluta *et al.* (2014) and in the animal pathogenic zoonotic *E. coli* website (http://apzec.ca/en/Protocols).

PCR for determination of the phylogenetic group

Phylogenetic grouping was carried out for the 169 selected *E. coli* isolates using a multiplex PCR-based assay as described by Clermont *et al.* (2000). Based on the presence or absence of two genes (*chuA* and *yjaA*) and a noncoding DNA fragment (TSPE4.C2), isolates were classified into four main *E. coli* phylogenetic groups (A, B1, B2, or D).

Antimicrobial resistance testing

A total of 95 isolates, including those positive for virulence genes and some randomly selected isolates, thus being representative of possible pathogenic and commensal isolates, were examined for resistance to the 15 antimicrobials used for testing generic *E. coli* in the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (Government of Canada), by the disk-diffusion (Kirby–Bauer) method. The following disks (BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs) were used: amoxicillin + clavulanic acid (20 + 10 μg), ceftiofur (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), amikacin (30 μg), ampicillin (10 μg), cefoxitin (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), sulfisoxazole (0.25 mg), and trimethoprim + sulfamethoxazole (1.25 + 23.75 μg). Breakpoints were those recommended by the Clinical Laboratory Standards Institute (CLSI, 2008). Isolates nonsusceptible to three or more classes of antimicrobial agents were considered to be multidrug resistant (Magiorakos *et al.*, 2012).

Serotyping and PCR for determination of O type

Fifty-two (30.8%) of the 169 isolates, including 11 isolates (6.50%) possessing tested virulence genes and 32 isolates (18.9%) resistant to one or more antimicrobials (one isolate per antimicrobial resistance pattern was selected for each cheese), and eight randomly selected isolates were examined for determination of the O serogroups described at www.ecllab.com/en/products/serotyping.asp using standard agglutination methods (Orskov *et al.*, 1977).

PCR for O4, O18, and O141 was performed with the standard protocol. All PCRs were carried out using negative control strain ECL3463 and the appropriate positive control strains (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/fpd).

PCR for determination of presence of antimicrobial resistance genes

The 52 selected isolates were examined for the presence of five β-lactamase resistance genes (bla_{SHV}, bla_{TEM}, bla_{CMY}, bla_{OXA}, $bla_{CTX\text{-}M}$) and tetracycline genes (*tetA* and *tetB*) by multiplex PCR. The protocol was provided by the National Microbiology Laboratory of the Public Health Agency of Canada and used with some adjustments. The primers, annealing temperatures, and controls are described in Supplementary Table S1. The cycling conditions consisted of an initial step at 95°C for 5 min, followed by 30 cycles, each consisting of denaturation at 94°C for 30 s, annealing 63°C for 90 s, and extension at 72°C for 90 s. The final extension was at 72°C for 7 min (Mataseje *et al.*, 2012).

Pulsed-field gel electrophoresis

The 52 selected isolates were subtyped by the standardized rapid pulsed-field gel electrophoresis (PFGE) protocol used by laboratories in PulseNet, as described previously (RIBOT *et al.*, 2006). Chromosomal DNA was digested with *Xba*I. Electrophoresis conditions comprised an initial time of 2.2 s, final time of 54.2 s at a gradient of 6 $V \cdot cm^{-1}$, and an included angle of 120°. The gels were electrophoresed for 18 h. The similarities of fragments were compared using a Dice coefficient at 1% tolerance and 0.5% optimization, and a dendrogram was constructed with the UPGMA clustering method using the software BioNumerics (Applied Maths). Clusters were established by the cutoff value by BioNumerics. Subclusters were determined empirically, and the isolates were considered similar with at least 60% of similarity.

Results

Prevalence of E. coli *in raw-milk cheese samples*

The prevalence of samples positive for *E. coli* (90.32%) and the prevalence of samples with *E. coli* possessing at least one ExPEC gene (32.14%) were highest in Aracaju (Table 1). In contrast, among samples positive for *E. coli*, Uberaba had a higher frequency of isolates (92.30%) resistant to one or more antimicrobials compared to other districts and the highest prevalence of tested O serotypes was in Ribeirao Preto (45.45%).

E. coli *isolates in raw-milk cheese were mostly of phylogenetic groups A and B1 and some were potential ExPEC*

Overall, 17 of 169 *E. coli* isolates (10.6%) were potential ExPEC: being of virotypes *tsh, iucD, papC, tsh:iucD, or tsh:papC*. These potential ExPEC genes were from Uberaba (4/7.84%) and Aracaju (13/13.97%) (Table 2). We did not find genes for STEC (*stx1* and *stx2*), EPEC (*eae*), and ETEC (STa, STb, LT, and F4).

Overall, isolates most commonly belonged to phylogenetic group A; most of the potential ExPEC isolates belonging to phylogenetic group B1 (Table 2). Interestingly, isolates most commonly belonged to phylogenetic group B1 in Uberaba

Table 1. Presence of *Escherichia coli* in Raw-Milk Cheese from Three Different Regions of Brazil

	City			
	Uberaba/MG (%)	*Ribeirao Preto/SP (%)*	*Aracaju/SE (%)*	*All samples (%)*
No. of samples positive for *E. coli*	13/30 (43.33)	11/22 (50)	28/31 (90.32)	52/83 (62.65)
No. of samples with antimicrobial-resistant *E. coli*[a]	12/13 (92.30)	6/11 (54.54)	10/28 (35.71)	28/52 (53.84)
No. of samples with *E. coli* possessing at least one ExPEC gene[b]	3/13 (23.07)	0/11 (0)	9/28 (32.14)	12/52 (23.07)
No. of samples with *E. coli* belonging to one of the tested O serogroups	5/13 (38.46)	5/11 (45.45)	5/28 (17.85)	15/52 (28.84)

[a]Antimicrobial-resistant *E. coli*: *E. coli* isolates resistant to one or more of the tested antimicrobials.
[b]Presence of ExPEC genes (*cnf*1/2, *tsh*, *papC*, and *iucD*) as determined by PCR.
ExPEC, extraintestinal pathogenic *E. coli*; PCR, polymerase chain reaction.

and Ribeirao Preto and to group A in Aracaju. Group B2 and D isolates were only found in the latter region.

The most common serogroups observed were O4, O18, and O23. O4 and O18 isolates belonged to phylogenetic group B1 and mostly found in Uberaba. Two isolates of O4 and two isolates of O18 possessed *tsh* gene. In Ribeirao Preto, one O18 and three O23 isolates were found, none of these isolates possessing virulence genes. Each of the O4, O18, and O23 isolates was from a different cheese.

Antimicrobial resistance was high in Uberaba and bla_{CMY-2} *was the most commonly found β-lactamase resistance gene*

In Uberaba, *E. coli* samples were more frequently resistant to ampicillin, amoxicillin/clavulanic acid, and tetracycline (Table 3). In contrast, the frequency of antimicrobial resistance was low in Aracaju, although one isolate was resistant to ciprofloxacin. Multidrug resistance (nonsusceptibility to three or more classes of antimicrobial agents as defined by Magiorakos *et al.*, 2012) was more frequently observed in isolates from Uberaba, resistance being up to six classes (Fig. 2). In contrast, most isolates from Aracaju were non-MDR. All 95 tested isolates were susceptible to ceftriaxone, amikacin, gentamicin, and chloramphenicol.

The presence of gene encoding resistance to ampicillin, tetracycline, and the third-generation cephalosporin ceftiofur was examined in the 52 selected isolates. In Uberaba, 9 (36%) isolates possessed one or more resistance genes (1 bla_{TEM} and *tetB*; 1 *tetA* and 4 bla_{CMY-2}; 1 *aadA*, *tetB*, and bla_{CMY-2}; 1 *tetB*, 1 *tetB*, and bla_{CMY-2}). This city had the highest prevalence of β-lactamase gene bla_{CMY-2}, but low for bla_{TEM}. Although Aracaju demonstrated the lowest overall antimicrobial resistance, 57.93% isolates possessed one or more resistance genes (2 *tetB*, 2 bla_{TEM}, and bla_{CMY-2}, 1 *tetA*, 1 bla_{TEM}, and *tetB*; 3 bla_{CMY-2}; 2 bla_{TEM}, bla_{CMY-2}, and *tetB*). In contrast, only 4 (50%) isolates from Ribeirao Preto were resistance gene positive (3 *tetB* and 1 *tetA*), no β-lactamase resistance genes being detected.

Most of the isolates possessing the bla_{CMY-2} gene were resistant to amoxicillin/clavulanic acid and most bla_{TEM}-positive isolates were resistant to ampicillin (Table 4). In contrast, 6 (11.54%) isolates possessing bla_{CMY-2} or bla_{CMY-2}:bla_{TEM} did not demonstrate β-lactamase antimicrobial resistance.

Table 2. Phylogenetic Group and Virulence Factors in *E. coli* Isolates from Raw-Milk Cheese in Three Different Cities in Brazil

City	*Total no. of isolates*	*No. of isolates of phylogenetic group*			
		A	*B1*	*B2*	*D*
Uberaba	51	10	41[a]	0	0
Ribeirao Preto	25	9	16	0	0
Aracaju	93	73[b]	18[c]	1	1
Total	169	92	75	1	1

[a]1 *iucD, 4 tsh.*
[b]*1 iucD and tsh, 1 papC, 7 tsh.*
[c]3 *tsh.*

Clones were found in the isolates from different cities, different cheeses, and from the same cheeses

Certain isolates from the same city demonstrated a high level of similarity. Clones (isolates with the same PFGE profile) were observed in the same cheese (isolates 71 and 72, 18 and 21), in different cheeses (isolates 29 and 77, 53 and 54A, 18 and 19B) from the same city, and unexpectedly, two identical isolates from different cities were observed (Fig. 3).

One predominant clone (18, 19B, and 21 in subcluster VIIb of Fig. 3) was found in five different cheeses. All of these cheeses were from the same market and probably from the same farm, but it is not known if they are from the same animal. There were two clones from the same city, but from different markets, one of them being resistant to sulfisoxazole and the other possessing a resistance gene for tetracycline (*tetB*). In addition, the likelihood that these originated from the same farm is low. No predominant clone possessing the bla_{CMY-2} gene was observed. Surprisingly, two isolates of the same clone originated from different cities although they demonstrated differences in antimicrobial resistance and presence of resistance genes. Nevertheless, many isolates from the same cheese demonstrated a high level of variability on PFGE, having less than 60% of similarity. This can be

TABLE 3. RESISTANCE TO ANTIMICROBIALS OF HIGH IMPORTANCE IN HUMAN MEDICINE IN UBERABA, RIBEIRAO PRETO, AND ARACAJU OF *E. COLI* FROM CHEESE MADE OF UNPASTEURIZED MILK

		Number of isolates (percentage) of samples resistant per category,[a] *antimicrobial class,*[b] *and antimicrobial*[c]														
		Category I					*Category II*							*Category III*		
		FLQ		*PEN/I*	*CPS*		*PEN*	*CPM*	*AMG*			*FOL*			*PHE*	*TET*
City	*No. of samples (No. of isolates)*	*NAL*	*CIP*	*AMC*	*TIO*	*CRO*	*AMP*	*FOX*	*GEN*	*KAN*	*STR*	*SXT*	*FIS*		*CHL*	*TET*
Uberaba	13 (30)	2 (15.4)	0	8 (61.5)	0	0	8 (61.5)	2 (15.4)	0	2 (15.4)	3 (23.1)	1 (7.7)	1 (7.7)		0	5 (38.5)
Ribeirao Preto	11 (16)	0	0	2 (18.2)	1 (9.1)	0	3 (27.3)	0	0	0	1 (9.1)	0	1 (9.1)		0	5 (45.5)
Aracaju	28 (49)	1 (3.6)	1 (3.6)	1 (3.6)	0	0	4 (14.3)	1 (3.6)	0	0	0	1 (3.6)	0		1 (3.6)	8 (28.6)

[a]Category of human antimicrobial importance: (I) very high importance, (II) high importance, and (III) moderate importance.
[b]Antimicrobial classes: FLQ, fluoroquinolones; PEN/I, Penicillin+ β-lactamases inhibitors; CPS, cephalosporins; PEN, Penicillin; CPM, cephamycin; AMG, aminoglycosides; FOL, folate; PHE, phenicols; TET, tetracycline.
[c]Antimicrobials: NAL, nalidixic acid; CIP, ciprofloxacin; AMC, amoxicillin/clavulanic acid; TIO, ceftiofur; CRO, ceftriaxone; AMP, ampicillin; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; FIS, sulfisoxazole; CHL, chloramphenicol; TET, tetracycline.

FIG. 2. Multidrug resistance in *E. coli* isolates from raw-milk cheese in three areas of Brazil. According to definition of Magiorakos *et al.* (2012), multidrug resistance (MDR): nonsusceptible to at least one antimicrobial for three or more antimicrobial classes.

seen in isolates 121 and 122 or in isolates 118 and 119, which originated from the same cheeses in Aracaju.

Discussion

Raw-milk cheese is a product consumed in Brazil, for which there is as yet little regulatory control, and thus, the potential to transmit zoonotic disease has become a focus of current research. The aim of this study was to evaluate the possible risk of raw-milk cheese for the human population, with respect to *E. coli*. The overall prevalence of cheeses positive for *E. coli* was 62.65% in the three examined regions, being highest in Uberaba (92.30%). Similarly, in comparison, in a previous study at Campinas, Sao Paulo, Brazil, of 31 cheeses produced in a similar way to that described in the present study, only 64.5% were contaminated with *E. coli* (Carvalho *et al.*, 2007).

Although most *E. coli* are commensal and, thus, not harmful, some *E. coli* possessing virulence factors are potentially pathogenic and, hence, a public health risk. In the present study, only a low proportion of isolates were potentially pathogenic, possessing the ExPEC virulence-associated genes *tsh*, *iucD*, and/or *papC*, although not in combinations usually associated with severe disease.

Furthermore, only one isolate belonged to the phylogenetic group B2, the group most frequently associated with ExPEC isolates causing disease in humans. This group is important because Johnson *et al.* (2001) found that isolates from phylogroups B2 and D possessed more ExPEC virulence factors than isolates from the phylogroups A and B1. In contrast, the present study showed that the isolates were mostly classified as A (prevalent in cheeses from Aracaju city) and B1 (prevalent in cheeses from Uberaba and Ribeirao Preto city). This difference could be explained by the presence of rearing systems involving other animal species in each city or different sources of contamination although these data were not available to us. Indeed, Carlos *et al.* (2010) showed that group A isolates were most commonly

TABLE 4. FREQUENCY OF β-LACTAMASE GENOTYPES AMONG β-LACTAM ANTIMICROBIAL-RESISTANT *E. COLI* ISOLATES FROM CHEESE MADE OF UNPASTEURIZED MILK FROM UBERABA, RIBEIRAO PRETO, AND ARACAJU

		No. of isolates positive for resistance genes			
β-lactam resistance profile	*Total no. of isolates*	bla_{TEM}	bla_{CMY}	bla_{TEM}:bla_{CMY}	*None*
AMP, AMC	6	1	0	0	5
AMP, AMC, FOX	3	0	2	0	1
AMC	3	0	2	0	1
AMP	6	1	0	1	4
AMC, FOX	2	0	0	2	0
XNL	1	0	0	0	1
None	31	0	4	2	25
Total	52	2	8	5	37

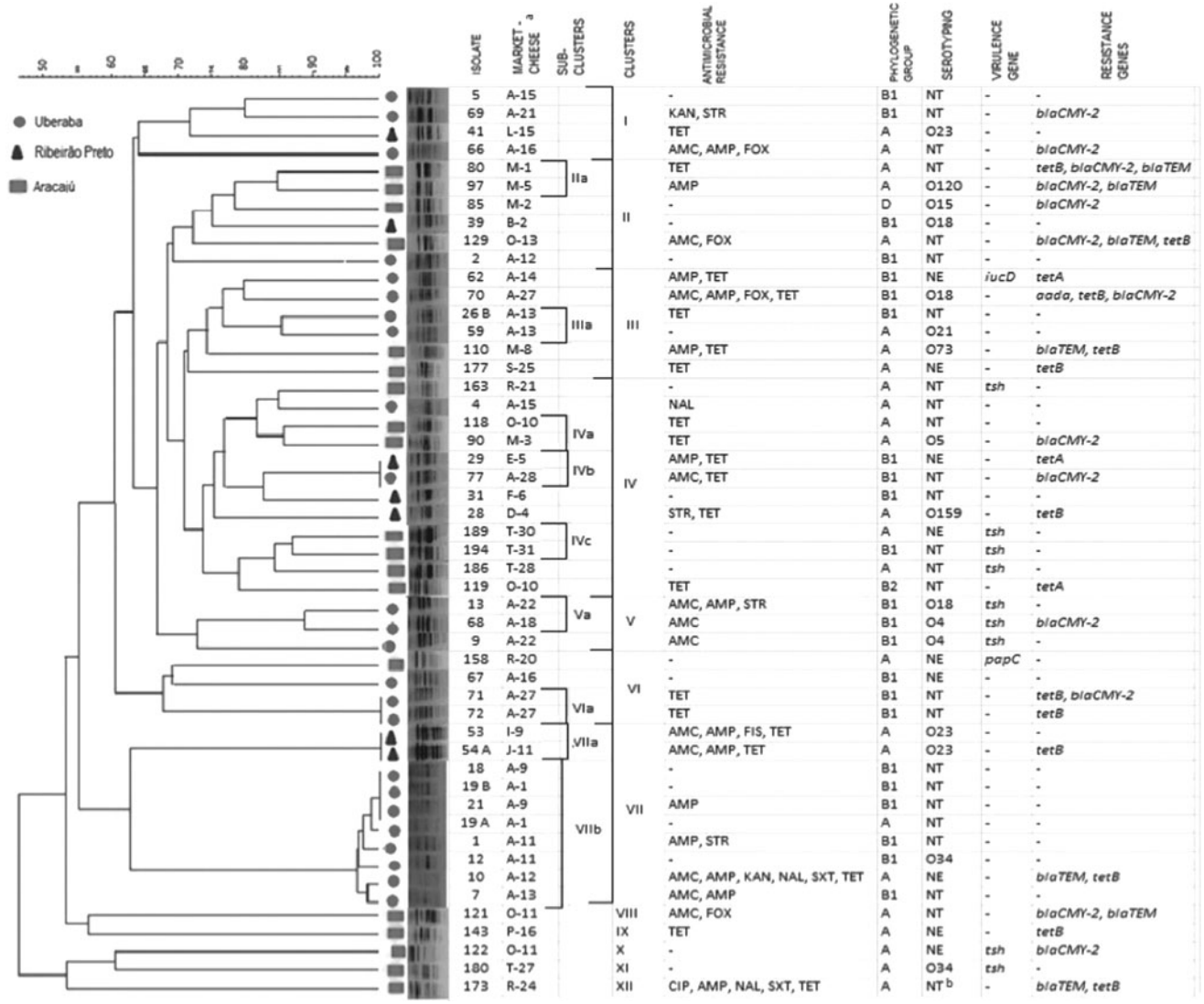

FIG. 3. Results of cluster among some isolates from cheese in three different regions. Clustering was performed to illustrate similarities between the prevalence of the genes examined, antimicrobial resistance, phylogenetic group, resistant gene, and serotype. [a] Market-cheese markets are identified by letter and cheeses by numbers (For example, A-15 is from market A, cheese 15). [b]NT, nontypeable; NE, nonspecific.

found in humans, whereas group B1 isolates were most commonly found in cattle.

Another important factor that represents a public health danger is antimicrobial resistance. The prevalence of antimicrobial resistance has increased worldwide. In this study, we found high levels of resistance to such antimicrobials as ampicillin and amoxicillin/clavulanic acid in Uberaba and Ribeirao Preto and resistance to ciprofloxacin in Aracaju. These results may be explained by the findings of a previous study conducted in Brazil showing that the group of β-lactams is the most commonly used antimicrobials to treat infections in dairy cattle, representing 38.22% of all antimicrobials, followed by aminoglycosides (25.19%) and tetracycline (15.41%) (Netto *et al.*, 2005). A study in carcasses of beef cattle in Brazil (Rigobelo *et al.*, 2006) also found high prevalence of resistance to ampicillin and amoxicillin/clavulanic acid in *E. coli*. This carcass contamination probably originates from feces, as in the present study, probably reflecting the use of antimicrobials in dairy farms and resistance elements in the environment.

In Uberaba and Ribeirao Preto cities, antimicrobial resistance and multidrug resistance were more common, possibly being explained by the antimicrobial usage patterns on farms in these cities. Indeed, Uberaba and Ribeirao Preto are located in the region of Brazil where agriculture is most developed, in other words, larger more progressive farms. Nevertheless, the variation of frequency of resistance to antimicrobials between regions observed in the present study underlines the importance of monitoring antimicrobial resistance in *E. coli* isolates from raw-milk cheese to ascertain the potential danger of eating such products originating from each particular region.

As in the present study, Nagy *et al.* (2015) in a study of *E. coli* isolates from foods of animal origin illegally imported to the EU by airline passengers showed a high frequency of resistance to ampicillin, tetracycline (100%), streptomycin (86%), and to sulfonamide compounds (93%) with less frequent resistance to chloramphenicol, florfenicol, and sulfamethoxazole/trimethoprim (50% each).

The presence of $bla_{CMY\text{-}2}$ in isolates in Uberaba and Aracaju most likely reflects the use of ceftiofur in the cattle and could represent a public health problem. Decreased susceptibility to ceftiofur, ceftriaxone, and other cephalosporins has been previously linked to the presence of plasmid-borne cephamycinase $bla_{CMY\text{-}2}$ genes (Zhao *et al.*, 2001). The presence of this gene in raw-milk cheeses may be due to the circulation of either plasmid-mediated ceftiofur resistance among *E. coli* or of resistant clones, which may occur between farms, markets, cities, and humans. Our results suggest the former, as no predominant $bla_{CMY\text{-}2}$-positive clones were observed.

In conclusion, we have demonstrated in raw-milk cheeses from three cities in Brazil contamination with *E. coli*, which, although not demonstrating a high pathogenic potential, showed antimicrobial resistance and possessed genes conferring resistance to clinically important antimicrobials in humans, which varied depending on the region of origin of the cheeses. As these cheeses are consumed without pasteurization to remove such bacteria, these results thus emphasize the presence of a potential danger for humans, which should be monitored.

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Disclosure Statement

No competing financial interests exist.

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