BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 50, n. 2, apr./jun., 2014
http://dx.doi.org/10.1590/S1984-82502014000200013

Article

Genetic heterogeneity of *Escherichia coli* isolated from pasteurized milk in State of Paraná, Brazil

Karine Oltramari[1], Rosilene Fressati Cardoso[2], Eliana Valéria Patussi[2], Adolfo Carlos Barreto Santos[3], Jane Martha Graton Mikcha[1,2,*]

[1]Postgraduate Program in Health Sciences, State University of Maringá, Maringá, PR, Brazil, [2]Department of Clinical Analyses and Biomedicine, State University of Maringá, Maringá, PR, Brazil, [3]Department of Biological Sciences, School of Phamaceutical Sciences, University of State of São Paulo "Júlio de Mesquita Filho", Araraquara, SP, Brazil

Food contamination caused by enteric pathogens is a major cause of diarrheal disease worldwide, resulting in high morbidity and mortality and significant economic losses. Bacteria are important agents of foodborne diseases, particularly diarrheagenic *Escherichia coli*. The present study assessed the genetic diversity and antimicrobial resistance of *E. coli* isolates from pasteurized milk processed in 21 dairies in northwestern State of Parana, Brazil. The 95 *E. coli* isolates were subjected to antimicrobial susceptibility testing according to the recommendations of the Clinical and Laboratory Standards Institute and assessed genotypically by Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR). The highest rate of resistance was observed for cephalothin (55.78%). ERIC-PCR revealed high genetic diversity, clustering the 95 bacterial isolates into 90 different genotypic patterns. These results showed a heterogeneous population of *E. coli* in milk samples produced in the northwestern region of Paraná and the need for good manufacturing practices throughout the processing of pasteurized milk to reduce the risk of foodborne illnesses.

Uniterms: Pasteurized milk/processing. Pasteurized milk/bacterial typing. Pasteurized milk/contamination. *Escherichia coli*/genetic diversity. *Escherichia coli*/antimicrobial resistance. Food/microbiological analysis.

A contaminação de alimentos por patógenos entéricos é uma das principais causas de doenças diarréicas em todo o mundo, resultando em altas taxas de morbidade e mortalidade e perdas econômicas significativas. As bactérias são importantes agentes de doenças de origem alimentar, particularmente *Escherichia coli* diarreiogênicas. O presente estudo teve como objetivo avaliar a diversidade genética e a resistência a antimicrobianos de *E. coli* isoladas de leite pasteurizado, processados em 21 laticínios na região noroeste do Paraná - Brasil. Os 95 isolados de *E. coli* foram submetidos a testes de suscetibilidade aos antimicrobianos de acordo com as recomendações do *Clinical and Laboratory Standards Institute* e avaliados genotipicamente por ERIC-PCR *(Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction*). O principal perfil de resistência encontrado entre os isolados foi resistência à cefalotina (55,78%). ERIC-PCR revelou alta diversidade genética, agrupando os 95 isolados bacterianos em 90 diferentes perfis genotípicos. Estes resultados mostraram uma população heterogênea de *E. coli* em amostras de leite produzido na região noroeste do Paraná e a necessidade de boas práticas na manipulação de todo o processamento de leite pasteurizado, a fim de reduzir o risco de doenças transmitidas por alimentos.

Unitermos: Leite pasteurizado/processamento. Leite pasteurizado/tipagem bacteriana. Leite pasteurizado/contaminação. *Escherichia coli*/diversidade genética. *Escherichia coli*/resistência a antimicrobianos. Alimentos/análise microbiológica.

***Correspondence:** Laboratório de Microbiologia de Alimentos. Departamento de Análises Clínicas e Biomedicina, Universidade Estadual de Maringá. Av. Colombo, 5790, Bloco T-20, sala 205, 87020-900 – Maringá - PR, Brasil. Tel: +55 44 3011-4813. E-mail: jmgmikcha@uem.br

INTRODUCTION

Food contamination caused by enteric pathogens is a major cause of diarrheal disease worldwide, resulting in high morbidity and mortality and significant economic losses (Centers for Disease Control and Prevention, 2013). Bacteria are important agents of foodborne diseases (Centers for Disease Control and Prevention, 2013), particularly *Escherichia coli*, which is widely distributed in nature. Despite being commonly found in the gastrointestinal tract of humans and other animals, it can cause a variety of diseases, some of which are life threatening in more vulnerable groups, such as the elderly, children, and immunocompromised individuals (FDA, 2012).

E. coli has been isolated from various foodstuffs, such as meat, vegetables, milk, and milk derivatives, which can serve as a vehicle for the transmission of diseases by food (Lee *et al.*, 2009; Solomakos *et al.*, 2009; Brooks *et al.*, 2012; Maffei *et al.*, 2013).

In Brazil, some studies have reported a high prevalence of *E. coli* in raw and pasteurized milk. *E. coli* was isolated from 41.1% of pasteurized milk samples evaluated by Silva *et al.* (2001), while in raw milk, this bacteria was found in 79,2% (Campos *et al.*, 2006) and in 76% (Barreto *et al.*, 2012) of milk samples analyzed. An important subject is the emergence of resistant foodborne pathogens that may be transmitted to humans as food contaminants. Outbreaks of foodborne diseases that involve resistant bacteria associated with food animal sources have been reported (Safe Food, 2010).

Milk may get contaminated with *E. coli* from various sources during different stages of production and processing, which could explain the genetic diversity of these bacteria in milk. The presence of *E. coli* in pasteurized milk may be due to inadequate pasteurization or contamination of the product after pasteurization process. Studies of the epidemiology of foodborne microorganisms, especially the source of contamination, have been conducted using various molecular techniques, such as ribotyping, multilocus sequence typing, randomly amplified polymorphic DNA, enterobacterial repetitive intergenic consensus-polymerase chain reaction, repetitive extragenic palindromic, and pulsed-field gel electrophoresis (Foley, Lynne, Nayak, 2009).

Several studies have used ERIC-PCR to assess the genetic similarity of *E. coli* isolates from different sources. Rantsiou, Alessandria and Cocolin (2012) assessed the genetic diversity of Shiga toxin-producing *E. coli* from meat and dairy products using ERIC-PCR. Wenz *et al.* (2006) described genetic variability among *E. coli* isolates from dairy cattle with mastitis using ERIC-PCR. Ibenyassine *et al.* (2006) used the same technique and evaluated the similarity of isolates from plants infected by irrigation water. Other authors have used ERIC-PCR to compare *E. coli* isolates from humans and animals (Leung *et al.*, 2004; Sabate *et al.*, 2008; Tramuta *et al.*, 2011; De la Fé Rodriguez *et al.*, 2012). Moreover, Casarez, Pillai, Di Giovanni, (2007), Duan *et al.* (2009), and Wan *et al.* (2011) showed that ERIC-PCR could discriminate *E. coli* from water and environmental samples. Knowledge about the epidemiology of diarrheagenic *E. coli* in food is important for understanding its distribution and transmission, besides developing and implementing control measures in food production.

To the best of our knowledge, no studies have used ERIC-PCR to assess the diversity of *E. coli* in pasteurized, ready-to-consume cow milk. Thus, the aim of the present study was to evaluate the genetic diversity and antimicrobial resistance of *E. coli* isolates from cow milk pasteurized in the northwestern region of Paraná, Brazil, using ERIC-PCR.

MATERIAL AND METHODS

Bacterial isolates

Ninety-five *E. coli* isolates from pasteurized, ready-to-consume cow milk samples were analyzed. The isolates were stored in Tryptic Soy Broth (TSB; Difco, Le Pont de Claix, France) with glycerol at -20 ºC and kept at the Laboratory of Food Microbiology, Department of Clinical Analysis and Biomedicine, Maringá State University. The milk samples were obtained from 21 dairies in Paraná, Brazil, from March 2006 to November 2008 in a previous study conducted in our laboratory that evaluated the presence of coliforms in pasteurized milk (Zanella *et al.*, 2010). The different dairies were identified by the abbreviations AA, APRO, CAIUÁ, CC, CIA, COC, COOP, GM, L, LAC, LOU, MOU, MP, NE, P, PN, S, SN, SO, TB, and U.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion technique according to the method described by the Clinical and Laboratory Standards Institute (2009). The antimicrobial agents (Oxoid, Hampshire, England) were the following: amikacin (30μg), amoxicillin-clavulanic acid (10-20 μg), ampicillin (10 μg), aztreonam (30 μg), cephalothin (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefoxitin

(30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamycin (10 µg), imipenem (10 µg), sulphamethoxazole-trimethoprim (1.25-23.75 µg), tetracycline (30 µg), and tobramycin (10 µg). The *E. coli* ATCC 25922 and *E. coli* ATCC 35218 isolates were used as controls.

ERIC-PCR

Genomic DNA of *E. coli* isolates was extracted from overnight bacterial growth on Nutrient Agar (Difco, Le Pont de Claix, France; Swanenburg *et al.*, 1998). DNA quantification was performed using NanoDrop 2000.

The amplification reaction was performed by adding 100 ng of bacterial DNA to a PCR reagent mixture that contained 1 µM of the primers ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'; (Versalovic, Koeuth, Lupski, 1991), PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 200 µM of each nucleotide (dATP, dCTP, dGTP, and dTTP), 1 U of DNA polymerase (Taq DNA polymerase; Invitrogen -Life Technologies, Brazil), and sterile deionized water to a final volume of 25 µl. The amplification was performed in a thermocycler (Gene Amp PCR System 2400, Perkin Elmer, Roche, Branchburg, NJ, USA) with denaturing at 94 ºC for 7 min, followed by 35 cycles at 94 ºC for 30 s, 52 ºC for 1min, and 72 ºC for 8 min and a final extension at 72 ºC for 16min. The PCR product underwent 1.5% agarose (Amersham Pharmacia Biotech AB, Uppsala, Sweden) gel electrophoresis at 7-10 V/cm^2 for 2 h and visualized under UV light. The spectral band analysis was performed using BioNumerics software (version 4.45, Applied Maths, Sint-Martens-Latem, Belgium). The Dendrogram was constructed using the Dice coefficient, and the phylogenetic distance was determined using the Unweighted Pair Group Method with the Arithmetic Mean algorithm. Isolates with ≥95% similarity were considered closely related. The discriminatory power of ERIC-PCR was calculated based on Simpson's diversity index as indicated by Hunter and Gaston (1988).

RESULTS

All 95 *E. coli* isolates were found to be susceptible to amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamycin, imipenem, and tobramycin. Sixty-two isolates (65.26%) were resistant to at least one of the antimicrobial agents tested. Among the resistant isolates, five were simultaneously resistant to three antimicrobial agents. The highest rates of resistance were observed for cephalothin (55.78%) and ampicillin (26.31%). Resistance to cefoxitin (5.26%), tetracycline (3.15%), amikacin (2.10%), chloramphenicol (2.10%), and trimethoprim-sulphamethoxazole (1.05%) was also observed. The *E. coli* isolates were grouped into 11 resistance profiles. The most common resistance profiles were to cephalothin (30 isolates) and cephalothin/ampicillin (14 isolates; Figure 1).

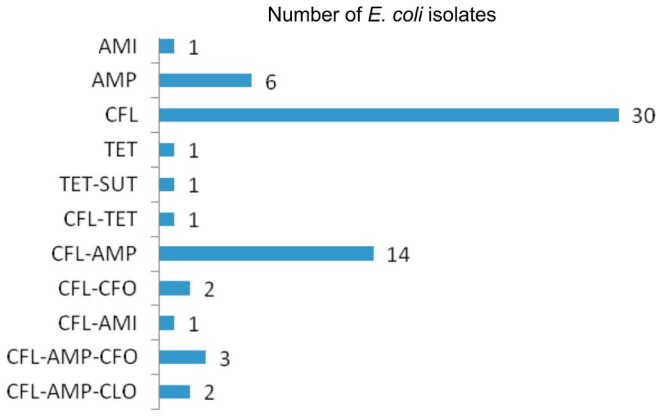

FIGURE 1 – Antimicrobial resistance profiles of *E. coli* isolated from pasteurized milk in State of Paraná, Brazil. AMI: amikacin, AMP: ampicillin CFL: cephalothin, TET: tetracycline, SUT: trimethoprim-sulphamethoxazole CFO: cefoxitin, CLO: chloramphenicol.

ERIC-PCR applied to 95 *E. coli* isolates generated patterns of four to 20 bands, with sizes that ranged from 100 to 2,000 bp. Considering the similarity level ≥95%, 90 ERIC-PCR patterns were observed. Of these, 85 (89.47%) showed unique profiles, and the remaining 10 (10.53%) were included in five clusters, comprising two isolates each (isolates 14 GM and 16 GM, 14 CC and 15 CC, 6 MP and 10 MP, 10 COC and 11 COC, and 12 L and 14 L; Figure 2).

The clusters 1, 2, 4, and 5, that contained two isolates each, clustered isolates from four milk samples processed in four distinct dairies. Distinct antimicrobial resistance profiles between each of two clustered isolates were observed. Cluster 3 contained two isolates (6 MP and 10 MP) that were resistant to cephalothin and were obtained from different pasteurized milk samples in the same dairy (Figure 2). None of the clusters was obtained from milk samples processed in different dairies. The discriminatory index of ERIC-PCR was 0.999.

DISCUSSION

Pathogenic microorganisms have been isolated from milk and dairy products (D'Amico, Donnelly, 2010;

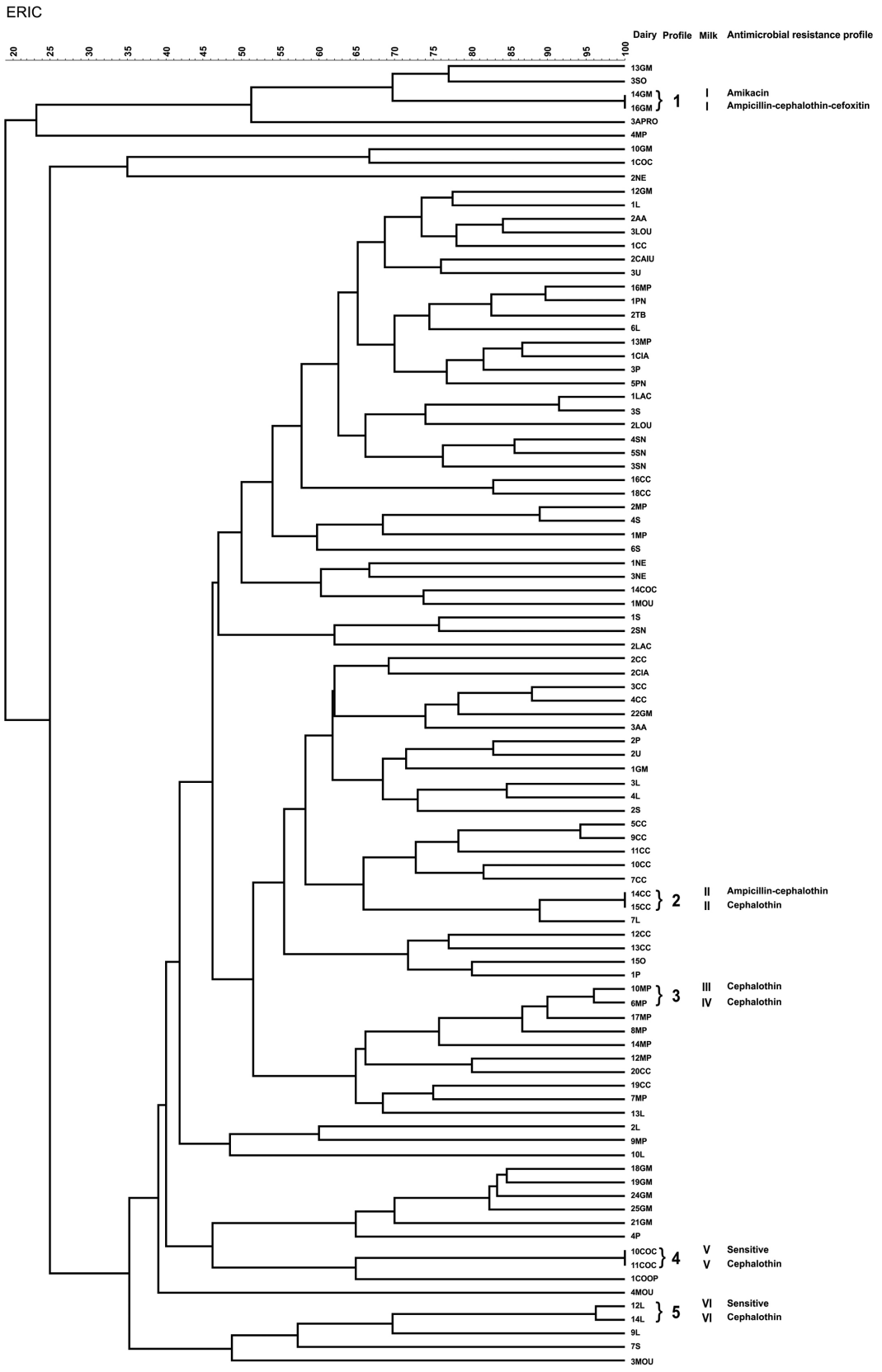

FIGURE 2 - Dendrogram that represent the genetic relationship of ERIC-PCR, milk samples and antimicrobial resistance of 95 *E. coli* isolated from pasteurized cow milk in State of Paraná, Brazil.

Costa Sobrinho *et al.*, 2012). Among them, diarrheagenic *E. coli* is notable and can cause diseases with serious consequences, especially in children and the elderly (FDA, 2012). The analysis and comparison of *E. coli* isolates from different sources can generate important information about the origin and transmission of these bacteria (Foley, Lynne, Nayak, 2009).

Importantly, antimicrobial-resistant bacteria can act as reservoirs of resistance genes. Several studies of bacteria isolated from food have shown resistance to one or more antimicrobials, which is worrying because these microorganisms can be spread in the population as food contaminants (Lutgen *et al.*, 2009; Solomakos *et al.*, 2009; Dutil *et al.*, 2010; Rahimi, Chaleshtori, Parsaei, 2010).

In the present study, 65.26% of the isolates showed resistance to at least one of the antimicrobial agents tested. Similar results were reported by Rahimi, Chaleshtori and Parsaei (2010), who found that 88.9% of the isolates were resistant to one or more of the tested antimicrobials. The highest rates of resistance were observed for cephalothin (55.78%) and ampicillin (26.31%). Paneto *et al.* (2007) evaluated *E. coli* isolated from cheese and provided indices of resistance to ampicillin and cephalothin that were similar to those observed in the present study. Rahimi, Chaleshtori and Parsaei, (2010) found that 44.4% of the *E. coli* isolates from dairy products showed ampicillin resistance. Solomakos *et al.* (2009) found that all of the *E. coli* isolates from milk were resistant to ampicillin, whereas Campos *et al.* (2006) found increased resistance to ampicillin and cephalothin and resistance to tetracycline in *E. coli* isolated from milk and cheese.

With regard to resistance profiles, the *E. coli* isolates were differentiated into 11 profiles. Heterogeneous profiles of antimicrobial resistance were also described by Rangel and Marin (2009) and Solomakos *et al.* (2009) in *E. coli* isolated from milk.

In the present study, ERIC-PCR showed high genetic diversity among the 95 *E. coli* isolates, which were clustered into 90 profiles. Among the clusters generated by ERIC-PCR, eight clustered *E. coli* isolates from four milk samples processed in four different dairies, suggesting a single clone contaminant per milk sample (Figure 2). Nevertheless, one cluster that comprised two *E. coli* isolates from different milk samples processed in the same dairy could suggest a common source of contamination. These results indicate failures in pasteurization process or contamination in post-pasteurization processing and show the need for control measures to minimize *E. coli* contamination. The present results are consistent with Wenz *et al.* (2006), who studied *E. coli* isolates from dairy cattle with mastitis, and Rantsiou, Alessandria and Cocolin (2012), who used ERIC-PCR to assess the genetic similarity of *E. coli* isolated from meat products and unpasteurized dairy products. *E. coli* is widely distributed in nature, and the sources of milk contamination can be diverse, explaining the high genotypic diversity observed in the present study.

ERIC-PCR is effective in typing *E. coli* isolates from animals (Mohapatra, Broersma, Mazumder, 2007; Prabu *et al.*, 2010; Wan *et al.*, 2011; De la Fé Rodriguez *et al.*, 2012) and water (Casarez, Pillai, Di Giovanni, 2007). However, some reports indicated that ERIC-PCR is not effective in typing *E. coli* isolated from humans, animals, and food (Giammanco *et al.*, 2002; Leung *et al.*, 2004; Costa *et al.*, 2006).

The present study showed heterogeneous profiles of antimicrobial resistance and a high genotypic diversity using ERIC-PCR in *E. coli* isolated from dairy milk processed in northwestern Paraná. The high genetic diversity observed in the present study can be justified by several sources of contamination of cow milk by *E. coli* during the entire production process.

ACKNOWLEDGEMENTS

This research received financial support from Fundação Araucária/Apoio ao Desenvolvimento Científico e Tecnológico do Paraná.

REFERENCES

BARRETO, N.S.; SANTOS, G.C.F.; CREPALDI, A.L.; SANTOS, R.A. Microbial quality and antimicrobial susceptibility of informally traded milk in Cruz das Almas county, Bahia. *Semina: Ciências Agrárias*, v.33, p.2315-2326, 2012.

BROOKS, J.C.; MARTINEZ, B.; STRATTON, J.; BIANCHINI, A.; KROKSTROMB, R.; HUTKINS, R. Survey of raw milk cheese for microbiological quality and prevalence of foodborne pathogens. *Food Microbiol.*, v.31, p.154-158, 2012.

CAMPOS, M.R.H.; KIPNIS, A.; ANDRÉ, M.C.D.P.B.; BORGES, L.J.; VIEIRA, C.A.S.; BORGES, L.J.; SANTOS, P.P.; SERAFINI, A.B. Caracterização fenotípica pelo antibiograma de cepas de *Escherichia coli* isoladas de manipuladores, de leite cru e de queijo "Minas Frescal" em um laticínio de Goiás, Brasil. *Ciên. Rural*, v.36, p.1221-1227, 2006.

CASAREZ, E.A.; PILLAI, S.D.; DI GIOVANNI, G.D. Genotype diversity of *Escherichia coli* isolates in natural waters determined by PFGE and ERIC-PCR. *Water Res.*, v.41, p.3643-3648, 2007.

CENTERS FOR DISEASE CONTROL AND PREVENTION. Incidence and trends of infection with pathogens transmitted commonly through food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996-2012. *Morb. Mortal. Wkly Rep.*, v.62, p.283-287, 2013.

CLINICAL AND LABORATORY STANDARDS INSTITUTE. CLSI. Performance standards for antimicrobial susceptibility testing: Wayne, 2009. 149 p. (M100-S16 Nineteenth Informational Supplement).

COSTA, M.M.; SILVA, M.S.; SPRICIGO, D.A.; WITT, N.M.; MARCHIORO, S.B.; KOLLING, L.; VARGAS, A.P.C. Epidemiology, molecular characterization and resistance to antimicrobials of *Escherichia coli* isolated from South-Brazilian pig herds. *Pesq. Vet. Bras.*, v.26, p.5-8. 2006.

COSTA SOBRINHO, P.S.; FARIA, C.A.M.; PINHEIRO, J.S.; ALMEIDA, H.G.; PIRES, C.V.; SANTOS, A.S. Bacteriological quality of raw milk used for production of a Brazilian farmstead raw milk cheese. *Foodborne Pathog. Dis*., v.9, p.138-144, 2012.

DE LA FÉ RODRIGUEZ, P.Y.; KIIRU, J.N.; MARTIN, L.O.M.; MUÑOZ, E.C.; BUTAYE, P.; COX, E.; GODDEERIS, B.M. Characterization and clonal grouping of pathogenic *Esherichia coli* isolated from intestinal contents of diarrheic piglets in Villa Clara province, Cuba, according to their antibiotic resistance and ERIC-PCR profiles. *Vet. Microbiol.*, v.154, p.425-428, 2012.

D'AMICO, D.J.; DONNELLY, C.W. Microbiological quality of raw milk used for small-scale artisan cheese production in Vermont: effect of farm characteristics and practices. *J. Dairy Sci.*, v.93, n.1, p.134-147, 2010.

DUAN, H.; CHAI, T.; LIU, J.; ZHANG, X.; QI, C.; GAO, J.; WANG, Y.; CAI, Y.; MIAO, Z.; YAO, M.; SCHLENKER, G. Source identification of airborne *Escherichia coli* of swine house surroundings using ERIC-PCR and REP-PCR. *Environ. Res*., v.109, p.511-517, 2009.

DUTIL, L.; IRWIN, R.; FINLEY, R.; NG, L.K.; AVERY, B.; BOERLIN, P.; BOURGAULT, A.M.; COLE, L.; DAIGNAULT, D.; DESRUISSEAU, A.; DEMCZUK, W.; HOANG, L.; HORSMAN, G.B.; ISMAIL, J.; JAMIESON, F.; MAKI, A.; PACAGNELLA, A.; PILLAI, D.R. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerg. Infect. Dis.*, v.16, p.48-54, 2010.

FOOD AND DRUG ADMINISTRATION. FDA. The bad bug book: foodborne pathogenic microorganisms and natural toxins. 2.ed. Washington: Center for Food Safety and Applied Nutrition, 2012. p.67-80.

FOLEY, S.L.; LYNNE, A.M.; NAYAK, R. Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. *Infect. Genet. Evol*., v.9, p.430-440, 2009.

GIAMMANCO, G.M.; PIGNATO, S.; GRIMONT, F.; GRIMONT, P.A.D.; CAPRIOLI, A.; MORABITO, S.; GIAMMANCO, G. Characterization of Shiga toxin-producing *Escherichia coli* O157:H7 isolated in Italy and in France. *J. Clin. Microbiol.*, v.40, p.4619-4624, 2002.

HUNTER, P.R.; GASTON, M.A. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol*., v.26, p.2465-2466, 1988.

IBENYASSINE, K.; AITMHAND, R.; KARAMOKO, Y.; COHEN, N.; ENNAJI, M.M. Use of repetitive DNA sequences to determine the persistence of enteropathogenic *Escherichia coli* in vegetables and in soil grown in fields treated with irrigation water. *Lett. Appl. Microbiol.*, v.43, p.528-533, 2006.

LEE, G.Y.; JANG, H.I.; HWANG, I.G.; RHEE, M.S. Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. *Int. J. Food Microbiol.*, v.134, p.196-200, 2009.

LEUNG, K.T.; MACKERETH, R.; TIEN, Y.C.; TOPP, E. A comparison of AFLP and ERIC-PCR analyses for discriminating *Escherichia coli* from cattle, pig and human sources. *FEMS Microbiol. Ecol*., v.47, p.111-119, 2004.

LUTGEN, E.M.; MCEVOY, J.M.; SHERWOOD, J.S.; LOGUE, C.M. Antimicrobial resistance profiling and molecular subtyping of *Campylobacter* spp. from processed turkey. *BMC Microbiol.*, v.9, p.203, 2009.

MAFFEI, D.F.; SILVEIRA, N.F.A.; CATANOZI, M.P.L.M. Microbiological quality of organic and conventional vegetables sold in Brazil. *Food Control*, v.29, p.226-230, 2013.

MOHAPATRA, B.R.; BROERSMA, K.; MAZUMDER, A. Comparison of five rep-PCR genomic fingerprinting methods for differentiation of fecal *Escherichia coli* from humans, poultry and wild birds. *FEMS Microbiol. Lett.*, v.277, p.98-106, 2007.

PANETO, B.R.; SHOCKEN-ITURRINO, R.P.; MACEDO, C.; SANTO, E.; MARIN, J.M. Occurrence of toxigenic *Escherichia coli* in raw milk cheese in Brazil. *Arq. Bras. Med. Vet. Zootec.*, v.59, p.508-512, 2007.

RAHIMI, E.; CHALESHTORI, S.S.; PARSAEI, P. Prevalence and antimicrobial resistance of *Escherichia coli* O157 isolated from traditional cheese, ice cream and yoghurt in Iran. *Afr. J. Microbiology Res.*, v.5, p.3706-3710, 2010.

RANGEL, P.; MARIN, J.M. Analysis of *Escherichia coli* isolated from bovine mastitic milk. *Pesq. Vet. Bras.*, v.29, p.363-368, 2009.

RANTSIOU, K.; ALESSANDRIA, V.; COCOLIN, L. Prevalence of Shiga toxin-producing *Escherichia coli* in food products of animal origin as determined by molecular methods. *Int. J. Food Microbiology*, v.154, p.37-43, 2012.

SABATE, M.; PRATS, G.; MORENO, E.; BALLESTE, E.; BLANCH, A.R.; ANDREU, A. Virulence and antimicrobial resistance profiles among *Escherichia coli* strains isolated from human and animal wastewater. *Res. Microbiol.*, v.159, p.288-293, 2008.

SAFE FOOD. The problem of antimicrobial resistance in the food chain. 2010. Available at: <http://www.safefood.eu/SafeFood/files/8a/8abb9354-4cc2-49a4-b586-bf0008eb8cf.pdf>. Accessed on: 28 May 2013.

SILVA, Z.N.; CUNHA, A.S; LINS, M.C.; CARNEIRO, L.A.M.; ALMEIDA, A.C.F.; QUEIROZ, M.L.P. Isolation and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil. *Rev. Saúde Pública*, v.35, p.375-379, 2001.

SOLOMAKOS, N.; GOVARIS, A.; ANGELIDIS, A.S.; POURNARAS, S.; BURRIEL, A.R.; KRITAS, S.; PAPAGEORGIOU, D.K. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. *Food Microbiol.*, v.26, p.865-871, 2009.

SWANENBURG, M.; URLINGS, H.A.P.; KEUZENKAMP, D.A.; SNIJDERS, J.M.A. Validation of ERIC-PCR as a tool in epidemiology research of *Salmonella* in slaughter pigs. *J. Ind. Microbiol. Biotechnol.*, v.21, p.141-144, 1998.

TRAMUTA, C.; NUCERA, D.; ROBINO, P.; SALVARANI, S.; NEBBIA, P. Virulence factors and genetic variability of uropathogenic *Escherichia coli* isolated from dogs and cats in Italy. *J. Vet. Sci.*, v.12, p.49-55, 2011.

VERSALOVIC, J.; KOEUTH, T.; LUPSKI, J.R. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res.*, v.19, p.6823-6831, 1991.

WAN, L.; WANG, Z.B.; YAN, Q.G.; WANG, X.; LEI, Y.; ZUO, L.; CHENG, Y.; REN, Y.P.; GUO, W.Z. Genetic diversity of *Escherichia coli* isolated from commercial swines farms revealed by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and repetitive extragenic palindrome PCR (REP-PCR). *Afr. J. Biotechnology*, v.10, p.10543-10550, 2011.

WENZ, J.R.; BARRINGTON, G.M; GARRY, F.B.; ELLIS, R.P.; MAGNUSON, R.J. *Escherichia coli* isolates' serotypes, genotypes, and virulence genes and clinical coliforms mastitis severity. *J. Dairy Sci.*, v.89, p.3408-3412, 2006.

ZANELLA, G.N.; MIKCHA, J.M.G.; BANDO, E.; SIQUEIRA, V.L.D.; MACHINSKI, M. JR. Occurrence, antibiotic resistance of coliform bacteria and antimicrobial residues from cow's milk from Brazil. *J. Food Prot.*, v.73, p.1684-1687, 2010.

Received for publication on 19th June 2013
Accepted for publication on 04th October 2013