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Frequency of extended-spectrum β-lactamase (ESBL)– and AmpC β-lactamase–producing *Enterobacteriaceae* in a cheese production process

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ABSTRACT

The aim of this study was to investigate the extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase activities of *Enterobacteriaceae* isolated from raw milk and cheese production line and to determine the probability of transmitting these bacteria to consumers. One hundred seventy-three samples from raw milk and cheese production lines were analyzed; 64 isolates were confirmed as *Enterobacteriaceae*. Sixteen of 64 isolates (25%) were resistant to at least one cephalosporin according to European Committee on Antimicrobial Susceptibility Testing Standards (EUCAST) and Clinical and Laboratory Standards Institute criteria. Seven of the 16 resistant isolates (43.75%) had confirmed ESBL activity. Additionally, phenotypic AmpC β-lactamase activity was observed in 31 (48.44%) of 64 *Enterobacteriaceae* isolates and confirmed in 27 of the 31 strains (89.1%). Overall, 3 isolates showed both ESBL activity and AmpC resistance, 28 isolates were only AmpC resistant, and 5 isolates had ESBL activity alone. Of the 173 samples, the proportions of samples that contained ESBL- and AmpC-producing isolates were 4.64 and 15.6%, respectively. Five of the ESBL-positive isolates (62.5%) and 11 of the AmpC-positive isolates (40.7%) were obtained from bulk milk tanks; therefore, the bulk tank plays a very important role in the spread of antibiotic-resistant bacteria. Periodic cleaning and maintenance of bulk tanks should be performed and recorded. Effective food safety and hygiene practices should significantly reduce cross-contamination in dairy plants.

Key words: antibiotic resistance, *Enterobacteriaceae*, cheese, raw milk

INTRODUCTION

Bacterial antimicrobial resistance is a major public threat to public health worldwide (EFSA, 2011; WHO, 2014). Antibiotics of the β-lactam group are the most commonly used antibiotics globally for treating infections caused by gram-negative bacteria because of their limited side effects and strong bactericidal properties (de Oca et al., 2015). Penicillins, cephalosporins, monobactams, and carbapenems are the major β-lactam antibiotics for treating many infectious diseases (Bush and Jacoby, 2010). Gram-negative bacteria can develop resistance to β-lactam-group antibiotics by several mechanisms (Thomson, 2010). β-Lactamase enzyme synthesis is the most important resistance mechanism (Bush and Jacoby, 2010; Schmid et al., 2013). The production of β-lactamase enzymes is encoded by genes on the chromosomes, plasmids, or transposons of microorganisms (EFSA, 2011), and these plasmids and mobile elements are easily transferred to other bacteria. Extended-spectrum β-lactamases (**ESBL**) are enzymes that can hydrolyze third-generation cephalosporins (oxyimino-cephalosporins), such as ceftriaxone, cefpirome, and cefepime. They are also partially susceptible to cephamycin and carbapenem. The ESBL are usually inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam (Thomson, 2010; Schmid et al., 2013; Stefani et al., 2014). The AmpC β-lactamases confer resistance to cephalosporins, including β-lactam inhibitors, monobactams, cephamycins, and some carbapenems, depending on the type of β-lactamases. They are inhibited by cloxacillin and aztreonam (Jacoby, 2009). Although researchers have reported the existence of AmpC β-lactamases less frequently than ESBL, AmpC β-lactamases exhibit resistance to a broader spectrum of β-lactam antibiotics than ESBL (Jacoby, 2009; Ibrahim et al., 2016). Community- and hospital-acquired infections with AmpC β-lactamase-producing bacteria are increasing worldwide (Liebana et al., 2013). In Turkey, the unnecessary use of antibiotics in clinical practice is restricted by government regulations; however, reports from the

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National Surveillance Network of Antimicrobial Resistance by the Ministry of Health in Turkey (Şimşek, 2017) show an increasing prevalence of ESBL-producing invasive *Escherichia coli* (33.2% in 2008 and 51% in 2015) and ESBL-producing *Klebsiella pneumoniae* (40% in 2008 and 65% in 2015). These rates are much higher than those in European countries. Antibiotic usage in veterinary medicine and for growth promotion and disease prevention is also an important problem in antibiotic resistance (Capita and Alonso-Calleja, 2013). In recent years, many researchers have shown the emergence of *Enterobacteriaceae* producing ESBL- and AmpC β-lactamases on dairy farms in numerous countries (Stefani et al., 2014; Gonggrijp et al., 2016; Ibrahim et al., 2016; Santman-Berends et al., 2017). Recently, ESBL- and AmpC-producing *Enterobacteriaceae* in food from milk, red meat, and poultry have been identified (Liebana et al., 2013; Kurekci et al., 2016; Tark et al., 2017). Therefore, food-producing animals and foods of animal origin such as meat, milk, and cheese are under suspicion for being transmission vectors for colonization and infection of the humans with ESBL-producing *Enterobacteriaceae* (Verraes et al., 2013; Nguyen et al., 2016). Veterinary medicine is unregulated in Turkey, although several congresses, training sessions, and seminars have been produced to raise awareness.

In Turkey, in studies in farm animals, the prevalence of ESBL-positive *E. coli* is reported to range from 1.5 to 15.53% (Elmacıoğlu, 2013; Pehlivanoğlu et al., 2016). Various researchers have reported the presence of antibiotic-resistant *Enterobacteriaceae* in milk and cheese samples, and the percentage of ESBL-producing *Enterobacteriaceae* strains ranges from 9.9 to 50% (Arslan and Özdemir, 2008; Gundogan and Avcı, 2013; Kurekci et al., 2016; Tekiner and Özpınar, 2016). However, there are limited data about the prevalence of ESBL- and AmpC-producing *Enterobacteriaceae* in various geographical locations.

Here, we aimed to investigate ESBL and AmpC β-lactamase activity in *Enterobacteriaceae* isolated from raw milk collected on farms and in the cheese processing line, including curd and cheese, in a small artisanal dairy factory in order to determine the transmission probability for these bacteria in the final food product.

MATERIALS AND METHODS

Sampling Area

The small dairy factory used for analyzing the cheese production process has been operating since 1987 in Çanakkale, Turkey. They have 75 employees and a closed area of 1,500 m^2. Their capacity averages 25 t of cheese per day. Twelve dairy products are produced, including cheese, yogurt, and buttermilk. Approximately 2 t of white cheese is produced daily and sold to both domestic and overseas markets.

Table 1. Sampling plan

Sample	No. of samples
Raw milk[1]	136
Raw milk from farm bulk tanks	17
Cheese production process[2]	20
Total	173

[1]Raw milk from each farmer who gave milk to the sampled dairy for cheese production.

[2]Cheese production process sampling points: (1) raw milk from reception and weighing unit in dairy, (2) raw milk before pasteurization, (3) pasteurized milk, (4) pasteurized milk on the transfer line, (5) milk from cheese vat, (6) curd in cheese vat (before pressure), (7) curd without salt (after pressure), (8) raw cheese in brine, (9) cheese in tin (first day), (10) cheese ripened for 3 mo.

Sample Collection

One hundred seventy-three samples were obtained under aseptic conditions during the raw milk and cheese production process. Samplings of raw milk were performed in 136 dairy farms and 17 bulk tanks in Çanakkale province, Turkey. A cheese processing line from the small dairy factory was also sampled (Table 1). Milk samples (250 mL each) and cheese and curd samples (approximately 250 g each) were collected aseptically using a sterile ladle into sterile plastic containers. We transported the samples to the laboratory in an insulated container at 4°C within 2 to 4 h after collection. All samples were analyzed the same day. Experiments were done in duplicate. Samples were collected twice between October 2015 and June 2016.

Reference Cultures

References cultures were obtained from the microbiology laboratory culture collection of the Food Engineering Department of Çanakkale Onsekiz Mart University. An ESBL-positive strain (*Klebsiella pneumoniae* ATCC 700603) and an ESBL-negative strain (*Escherichia coli* ATCC 25922) were used as positive and negative controls, respectively.

Isolation and Identification

Milk samples (1 mL) were diluted with 9 mL of 0.1% sterile peptone water (Merck, Darmstadt, Germany), and 0.1-mL aliquots from undiluted milk and its dilutions was spread onto plates containing MacConkey agar (Merck) containing cefotaxime (2 μg/