Shiga Toxin–Producing Escherichia coli in Mastitis: An International Perspective

Shelton E. Murinda, A. Mark Ibekwe, Nora G. Rodriguez, Karina L. Quiroz, Alexander P. Mujica, and Kayla Osmon

Abstract

The pathogen profile of Escherichia coli mastitis reveals a complex etiology involving commensal, environmental, and other distinct E. coli pathotypes such as enteropathogenic E. coli and of recent, Shiga toxin–producing E. coli (STEC) have been associated with bovine intramammary infections (IMI). Many researchers have not been testing for STEC and focused on E. coli detection without further subtyping, and as such, the prevalence of STEC in mastitis remains underdiagnosed and underreported. Owing to the dearth of information on STEC involvement in IMI, this review provides an international perspective on the prevalence of STEC in mastitis. In addition, predominant serotypes, ancillary virulence factors, and antimicrobial resistance profiles of STEC isolated from mastitis cases were summarized. This information is important for public health policy since STEC impact both animal health and human welfare. Importantly, the low infectious doses of STEC are a major concern to public health. The review highlights the need for further surveillance to ascertain the potential for environmental contamination and food chain security by STEC from bovine mastitis, and emphasizes appropriate, science-based mitigation approaches for prevention or control.

Keywords: bovine mastitis, milk, prevalence, Shiga toxin–producing E. coli

Introduction

Mastitis is an intramammary infection (IMI) that affects a high proportion of dairy cows worldwide. Mastitis differs from most other animal diseases in that several diverse bacteria are capable of invading and infecting the udder, multiplying there, and producing harmful substances that result in inflammation, reduced milk production, and altered milk quality (Oliver and Calvinho, 1995; NMC, 1996). Mastitis has economic, veterinary, and public health significance. The production of maximum quantities of high-quality milk is an important goal for every dairy producer. High-quality milk tastes better, is more nutritious, and has a longer shelf-life (Oliver and Murinda, 2011). On the other hand, poor- or reduced-quality milk affects all sectors of the dairy industry, ultimately resulting in milk with decreased manufacturing properties and dairy products with reduced shelf-life. Mastitis is the most important factor associated with reduced milk quality (Oliver and Murinda, 2011).

Bovine mastitis is a very complex disease that is generally categorized into two main forms, that is, subclinical mastitis, which is asymptomatic, and clinical mastitis, both having significant impacts on dairy herds. The National Mastitis Council (NMC) estimates that mastitis costs dairy producers in the United States more than $2 billion annually (NMC, 1996; Hogan et al., 2011). The average cost of clinical mastitis per cow per year in the United States was estimated at $71–$179 (Bar et al., 2008). Thus, mastitis continues to be one of the most significant limiting factors, if not the most significant, to profitable dairy production in the United States and worldwide (Oliver and Murinda, 2011).

Raw milk is one of the products promoted as a “health food.” This results in milk consumption by individuals who may have lowered immunity such as the very young, very old, or immunocompromised people, or those with specific dietary needs (reviewed by Zastempowska et al., 2016). There are inherent dangers associated with consumption of raw milk, since raw milk can be contaminated by a plethora of pathogens originating from within the cow’s udder, surface of the udder, milk handling and storage equipment, and the cow’s environment (Oliver et al., 2005, 2009; Oliver and Murinda, 2011). Shiga toxin–producing Escherichia coli (STEC; also called verotoxin-producing E. coli, VTEC), particularly E. coli O157:H7, have very low infectious doses,

Risk assessment conducted in New Zealand indicated that a typical serving of 250 mL of STEC-contaminated milk, with only 0.4 colony-forming unit/mL STEC, generates a dose of 100 cells, resulting in a 50% risk of infection (King et al., 2014). Due to these low infectious doses, there are specific concerns especially among those with lowered immunities. In a review that highlighted the threats to human health posed by consumption of milk and dairy products, Oliver and Murinda (2011) indicated that the role of STEC in mastitis remained undetermined. Figure 1 depicts a theoretical model for transmission of STEC between dairy cows and their environment.

The main virulence factors of STEC are production of Shiga toxins (Stx1 and Stx2) (Paton and Paton, 1998; Gyles, 2007). Other virulence factors that contribute to STEC pathogenesis are intimin, encoded by the eae gene and responsible for the intimate attachment of STEC to intestinal epithelial cells, an enterohemolysin (EhxA), an STEC autoagglutinating adhesin (Saa), and a novel STEC autotransporter (Sab) (Nataro and Kaper, 1998; Paton and Paton, 1998; Herold et al., 2009). The ehxA, saa, and sab genes are located on a megaplasmid (Paton and Paton, 1998; Paton et al., 2001; Herold et al., 2009).

Shiga toxins (Stxs) are associated with a variety of human illnesses such as diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP), with possible fatal consequences (Nataro and Kaper, 1998; Paton and Paton, 1998; Herold et al., 2009). The ehxA, saa, and sab genes are located on a megaplasmid (Paton and Paton, 1998; Paton et al., 2001; Herold et al., 2009).

Kaipainen et al. (2002) have indicated that bovine mastitis resembles a urinary tract infection in that it is caused by environmental bacteria and ascends the teat canal. To enable the bacteria to colonize, multiply, and survive in the udder, bacterial virulence factors are required to fend off the host’s selection pressure (Kaipainen et al., 2002).

Although STEC can have a reservoir in other domestic bovidae species, camelids, equids, and ovids (reviewed by Ivbade et al., 2014), ruminants, especially cattle, are their main reservoir (Hussein and Sakuma, 2005b; Farrokh et al., 2013). Outbreaks associated with the consumption of ground beef, pasteurized milk, and dairy products (yoghurt and cheese) have been reported worldwide (reviewed by Hussein and Sakuma, 2005a; Farrokh et al., 2013; Zastempowska et al., 2016). Direct contact with cattle and the dairy farm environment has been reported as a possible vehicle for STEC transmission to humans (Oliver et al., 2005).

While there are innumerable international publications on the prevalence of E. coli in mastitis (reviewed by Oliver and Murinda, 2011; PuMed Central, www.ncbi.nlm.nih.gov/pubmed) and its presence in dairy products worldwide (Hussein and Sakuma, 2005a; Farrokh et al., 2013), there have not been many studies that focused on the specific involvement of STEC in mastitis (Oliver and Murinda, 2011; Farrokh et al., 2013).

The goal of this review was to collate worldwide literature (predominantly in English) that addresses the prevalence of STEC in mastitis. The low infectious doses of STEC underscore a heightened concern on their presence in milk and milk products. The pertinent information on STEC prevalence was gathered from a variety of sources. An effort was made to gather information on serology and virulence factors/characteristics, including antimicrobial resistance (AMR) profiles of the STEC isolates. Recommendations for risk mitigation with respect to STEC-associated mastitis are provided.

How Prevalent Are STEC in Mastitis Internationally?

The isolate or sample prevalence of mastitis-associated STEC in various geographic regions is summarized in Tables 1–6. Many researchers have not been testing for

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**FIG. 1.** Conceptual framework for sources of Shiga toxin–producing Escherichia coli associated with intramammary infection.
<table>
<thead>
<tr>
<th>Country</th>
<th>Duration of study</th>
<th>No. of farms, animals, samples, and E. coli isolates</th>
<th>No. of STEC/No. of E. coli (%)</th>
<th>Serotypes</th>
<th>Virulence factor profiles (stx1, stx2, eae, ehly, etc.)</th>
<th>Antibiotic resistance profiles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>CM (n = 4 samples); SC (n = 2 samples); 6 E. coli+</td>
<td>4/6 (66.67)</td>
<td>O111, O55 (2), O26</td>
<td>stx2+; stx1/stx2, eaeA, hylA; stx1, stx2, eaeA</td>
<td>n/a</td>
<td>Galal et al. (2013)</td>
</tr>
<tr>
<td>Egypt</td>
<td>6 Months (October 2008 to March 2009)</td>
<td>80 QM samples; CM (n = 48)/SC (n = 32); 20 E. coli (12 CM vs. 8 SC)</td>
<td>5/20 (25)</td>
<td>O157:H7 (1), O111 (4)</td>
<td>eae, stx1 (stx2 not tested)</td>
<td>n/a</td>
<td>Moussa et al. (2010)</td>
</tr>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>101 CM cases; 18 E. coli</td>
<td>8/9 (88.9) (9 of 18 tested; 1 per serotype)</td>
<td>O111:H4, O127:H6, O26, O126, O119:H6, O114:H21, O55:H7, O44:H18, O124</td>
<td>stx1 and stx2; eight of nine were stx1+; stx2 (eight); stx2 (four); stx2/stx1 (three)</td>
<td>n/a</td>
<td>Sayed (2014)</td>
</tr>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>One farm; 40 cows CM; 20 E. coli</td>
<td>2/20 (10)</td>
<td>O125 (Isolated from “different sources” not specifically indicated if any isolated from mastitis)</td>
<td>n/a</td>
<td>n/a</td>
<td>Radwan and Abo-Zaid (2017)</td>
</tr>
<tr>
<td>Egypt</td>
<td>5 Months (May 2014 to September 2014)</td>
<td>5 Small-scale dairy farms: 125 cows; paired milk samples; 10 mastitic cows; 5 E. coli</td>
<td>n/a</td>
<td>O26 (n = 2), O111:H4 (n = 1) buffalo</td>
<td>Virulence tests n/a; appears all six defined as EHEC only from serological tests stx1, stx2, hylA, fliCH7</td>
<td>n/a</td>
<td>Awadallah et al. (2016)</td>
</tr>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>150 SC; buffalos and cows; 100 cows; 15 E. coli, 50 buffalo; 1 E. coli</td>
<td>4/15 (26.67) cows; 1/1 (100) buffalo</td>
<td>O26 (n = 2), O111:H4 (n = 1) buffalo</td>
<td>Virulence tests n/a; appears all six defined as EHEC only from serological tests stx1, stx2, hylA, fliCH7</td>
<td>n/a</td>
<td>El-Bagory and Zayda (2015)</td>
</tr>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>40 Cows mastitis; CM; 8 E. coli</td>
<td>0 n/a</td>
<td>n/a</td>
<td>Virulence tests n/a; appears all six defined as EHEC only from serological tests stx1, stx2, hylA, fliCH7</td>
<td>n/a</td>
<td>Osman et al. (2012)</td>
</tr>
<tr>
<td>Egypt</td>
<td>n/a</td>
<td>840 QM samples, 220 buffalos; 541 CM and SC tested; 63 E. coli+</td>
<td>n/a; only five isolates representing five different serogroups tested</td>
<td>O114, O18, O78, O157, and O55 (other serotypes, O1, 153, O78, O26, and O126, and untypable strains not tested)</td>
<td>eae, stx1; stx2; all five were stx1+; three were eaeA+; stx1 not tested</td>
<td>n/a</td>
<td>Lamuye et al. (2013)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>n/a</td>
<td>5 Cattle herds; suburban Ogun state area; 50 fresh milk samples; E. coli+</td>
<td>1/50 (2)</td>
<td>STEC O157:H7 focus</td>
<td>stx2+; negative for stx2, eaeA, and ehlyA</td>
<td>AMC, AMP, TET, CHL, STR, NAL, SXT, NOR, CIP, NEO; Resistant to AMP, and TET; sensitive to CIP and NEO (sensitivity data on other antibiotics was not provided)</td>
<td>Ivbade et al. (2014)</td>
</tr>
<tr>
<td>South Africa</td>
<td>n/a</td>
<td>2 Dairy farms; 400 samples; 188 E. coli+; CM/SC (200 samples each); 87 E. coli (uidA+)</td>
<td>0 No O-serotyping done; focused on fliCH7 detection</td>
<td>Test only fliCH7 and eae; 21% were eae+; all were stx1- and stx2-</td>
<td>No O-serotyping done; focused on fliCH7 detection</td>
<td>Tested only fliCH7 and eae; 21% were eae+; all were stx1- and stx2-</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Virulence factor-encoding and other genes: eaeA/eae, intimin gene; fliCH7, H7 flagella gene; hlyA, hemolysin A gene; stx1, Shiga toxin 1 gene; stx2, Shiga toxin 2 gene.

Antibiotics: AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; NAL, nalidixic acid; NEO, neomycin; NOR, norfloxacin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

CM, clinical mastitis (= acute mastitis); EHEC, enterohemorrhagic E. coli; n/a, not available or not applicable; SC, subclinical mastitis; STEC, Shiga toxin–producing E. coli.
### Table 2. Shiga Toxin–Producing Escherichia coli Isolated from Mastitic Milk in Asia

<table>
<thead>
<tr>
<th>Country</th>
<th>Duration of study</th>
<th>No. of farms, animals, samples, and E. coli isolates</th>
<th>No. of STEC/No. of E. coli (%)</th>
<th>Serotypes &amp; Virulence factor profiles (stx1, stx2, eae, ehly, etc.)</th>
<th>Antibiotic resistance profiles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>May to August 2012</td>
<td>CM/SC; 8 E. coli+</td>
<td>1/8 (12.5)</td>
<td>stx2 and stx1; only stx2 detected</td>
<td>AMI, AMC, AMP, AT, CTZ;</td>
<td>Ghatak et al. (2013)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>CPM, CFS, C, CFX, CTZ, CHL, CIP, COL, GEN, IMI, LEV, MER, PIP, PIT, TET, SXT (n=21); STEC isolate was positive for ESBL, AMP, PIP, CAR, AMC, CPM, C, CR, CTZ, CFP, CXM, IMI, MEM, GEN, AMK, TGC, CIP, NOR, SXT (n=18). STEC O14:K were susceptible; nontypable STEC was resistant to AMP, CAR, and SXT</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>n/a</td>
<td>17 Dairy farms; 38 samples; 69 E. coli isolates</td>
<td>3/69 (4.35)</td>
<td>stx1, stx2, eaeA; Isolates were stx1+, negative for stx2 and eaeA</td>
<td>n/a</td>
<td>Hinthong et al. (2017)</td>
</tr>
<tr>
<td>China</td>
<td>14 Months (September 2012 to October 2013)</td>
<td>6 Major dairies; 663 milk samples; CM/SC; 70 E. coli</td>
<td>0</td>
<td>stx1, stx2, saa, eaeA, and ehaA, were not detected</td>
<td>n/a</td>
<td>Liu et al. (2014)</td>
</tr>
<tr>
<td>India</td>
<td>n/a</td>
<td>7 Farms; 101 milk samples CM &amp; SC; 37 E. coli+</td>
<td>2/37 (5.4)</td>
<td>stx1/stx2 co-detection</td>
<td>Tested for ESBL production versus CPD; CTZ, AT; C, CR (n=5); both isolates negative</td>
<td>Munir (2015)</td>
</tr>
<tr>
<td>India</td>
<td>July 2001 to March 2002</td>
<td>Samples collected from udder, but mastitis status not indicated; 111 samples</td>
<td>2/111 (1.8% culturable); 4.5% (including nonculturable)</td>
<td>eae, hlyA, etpD, stx1, and stx2; stx1 (n=1); stx1 and hlyA (n=1)</td>
<td>n/a</td>
<td>Das et al. (2005)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>13 Months (July 2006 to January 2007)</td>
<td>20 Dairy cows; 20 households; (random samples)</td>
<td>0</td>
<td>Targeted STEC O157; none could be isolated</td>
<td>stx2, eae, katP, etpD, and ehly</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Vinulence factor-encoding and other genes: *eaeA/eae*, intimin gene; *ehxA*, enterohemolysin A gene; *ehly*, enterohemolysin gene; *etpD*, type II secretion pathway protein D gene; *fimH*, type I fimbriae gene; *fliC*, type VII flagella gene; *hlyA*, hemolysin A gene; *katP*, catalase-peroxidase gene; *rfbE* (O157), O157 antigen-encoding gene; *saa*, STEC agglutinating adhesion gene; *stb*, heat-stable enterotoxin b gene; *stx1*, Shiga toxin 1 gene; *stx2*, Shiga toxin 2 gene. Serotypes: K, rough; NT, nontypable; O, somatic serotype; H, flagella serotype.

Antibiotics: AMC, amoxicillin/clavulanic acid; AMI, amikacin; AMO, amoxicillin; AMP, ampicillin; AT, aztreonam; C, cefotaxime; CAR, carbencillin; CEP, cefalothin; CF, cefoxitin; CF, cefoxitin; CFZ, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; COT, cotrimoxazole; CPD, cepodoxime; CPA, cephalaxin; CPM, cefepime; CR, ceftiraxone; CTZ, ceftazidime; CXM, cefuroxime; DOX, doxycycline; ENF, enrofloxacin; ERY, erythromycin; FLO, florfenicol; GEN, gentamicin; IMI, imipenem; KAN, kanamycin; LEV, levofloxacin; LIN, lincomycin; MCG, ceftizoxime; MEM, meropenem; MER, meropenem; NAL, nalidixic acid; NEO, neomycin; NET, netilmicin; NT, nitrofurantoin; NOR, norfloxacin; NOV, novobiocin; OTE, oxytetracycline; PIP, piperacillin; PIT, pipacillin/tazobactam; STR, streptomycin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline; CM, clinical mastitis (=acute mastitis); ESBLS, extended-spectrum beta lactamases; n/a, not available or not applicable; QM, quarter milk; SC, subclinical mastitis; STEC, Shiga toxin–producing *E. coli.*
STE.C; therefore, the true prevalence of STEC in mastitis remains underdiagnosed and underreported. Some who have tested for STEC have only focused on detection of serotype O157:H7 (Bean et al., 2004; Daood, 2007; Rangel and Murin, 2009; Islam et al., 2010; Momtaz, 2010; Caine et al., 2014; Ivbadi et al., 2014; Iweriebor et al., 2015; Turkyilmaz et al., 2017), disregarding the possible involvement of non-O157 STEC serotypes in mastitis.

Other researchers have also targeted testing for a single stx gene, for example, stx2 (Moussa et al., 2010; Lamey et al., 2013) or stx1 (Naser, 2016), whereas Murin (2015) co-detected stx1 in E. coli mastitis isolates without distinguishing stx1 from stx2. Very few have attempted to test for stx variants as Keane (2016) and Bean et al. (2004) who, respectively, detected stx2 and stx3 in their studies. An earlier study by Barrow and Hill (1989) used Shiga toxin (verotoxin toxin) assays to detect for presence of Stx production by E. coli from mastitis.

Several Egypt studies (Table 1) conducted with E. coli isolates from clinical and subclinical mastitis have reported a prevalence rate of STEC ranging from 0% to 88.9%. Galal et al. (2013) isolated STEC serotypes O111, O26, and O55 (66.67% of E. coli). In a study by Moussa et al. (2010), the STEC serotypes causing bovine mastitis were similar to those causing diarrhea or being shed in the feces of apparently healthy calves. The results confirmed conclusions by previous researchers who reported that E. coli serovars that cause bovine mastitis were similar to those from fecal isolates (reviewed by Moussa et al. 2010). The stx2 gene was detected in 5 of 20 (25%) E. coli isolates of serotype O157:H7 (n = 1) and O111 (n = 4) from mastitis.

Sayed (2014) obtained 18 E. coli of various serotypes O111:H4 (n = 3), O127:H6 (n = 3), O26 (n = 2), O126 (n = 2), O44:H18 (n = 1), O55:H7 (n = 1), O114:H21 (n = 1), O119:H6 (n = 1), and O124 (n = 1), including some (n = 3) that were serologically untypeable. In this study, the true prevalence rate of STEC could not be determined due to selective testing of stx in representative isolates from the different serotypes. Of nine E. coli (out of 18) that were tested, one representing each of nine serotypes, eight (~89%) were STEC (Sayed, 2014). In another study (reviewed by Sayed, 2014), most STEC serotypes (39.9% of E. coli) were isolated from mastitic cows.

Radwan and Abo-Zaid (2017) identified STEC of serotype O44 and O146 as causes of 10% of E. coli mastitis. Awa-dallah et al. (2016) reported that milk collected from cows with mastitis was four times more likely to be contaminated with E. coli than milk collected from normal cows (odds ratio, 4; 95% confidence interval, 1.07–14.99; p < 0.05). The stx1 gene was identified in 7 of 12 E. coli O125 from mastitis and other sources. However, the study does not specifically indicate the number of stx-positive isolates from mastitis.

El-Bagory and Zayda (2015) isolated serotypes O111:H4 (n = 2) and O26 (n = 2) from bovine clinical mastitis (26.67%...
of *E. coli* isolates and serotype O111:H4 (*n* = 1) from buffalo’s milk. These isolates were defined as enterohemorrhagic *E. coli* (EHEC); however, the method for determination of virulence markers was not indicated. Lamey et al. (2013), who studied buffalos, identified five *E. coli* isolates of serotype O114, O18, O78, O157, and O55 that had *stx*2 genes; 3 had the *eaeA* gene. However, the presence of *stx*1 genes was not tested in any of the 63 *E. coli* isolates. Ibade et al. (2014) isolated one STEC strain in Nigeria (Table 1), whereas none of the *E. coli* isolates from mastitis studied by Osman et al. (2012) in Egypt or Caine et al. (2014) in South Africa was identified as STEC.

STEC have been isolated from several mastitis cases in Asia (Table 2). In a study conducted in India, only one of eight *E. coli* isolates (12.5%) from mastitis was positive for *stx*2, whereas none had the *stx*1 gene (Ghatak et al., 2013). Two of three STEC isolates from milk samples in Thailand possessed the O114:K serotype (the K-type was not reported), whereas one isolate was serologically untypeable (Hinthong et al., 2017). The STEC prevalence rate was 3.5%.

### Table 4. Shiga Toxin–Producing Escherichia coli Isolated from Mastitic Milk in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>Duration of study</th>
<th>No. of farms, samples, and <em>E. coli</em> isolates</th>
<th>No. of STEC/No. of <em>E. coli</em> (%)</th>
<th>Serotypes</th>
<th>Virulence factor profiles (<em>stx</em>1, <em>stx</em>2, eae, ehly, etc.)</th>
<th>Antibiotic resistance profiles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>England and Wales</td>
<td>1 Year</td>
<td>237 <em>E. coli</em> isolates; severity n/a</td>
<td>0.5% (1/237)</td>
<td>n/a</td>
<td>Conducted verotoxin assays</td>
<td>n/a</td>
<td>Barrow and Hill (1989)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>10 Months</td>
<td>2416 QM samples; 145 samples different animals <em>E. coli</em>+</td>
<td>2.76% (4/145)</td>
<td>n/a</td>
<td>(1) O105:K+H18; (2) O105:K+H18; (3) OX3:K?:H2; (4) O8:K4+:H25; all four were eae-negative</td>
<td>(1) Sensitive; (2) sensitive; (3) AMP, NEO, TET, SU; (4) sensitive: AMP, CFP, CEP, GEN, NEO, TET, CHL, POL, COL, and SMX (n = 10)</td>
<td>Stephan and Kuhn (1999)</td>
</tr>
<tr>
<td>Austria</td>
<td>Not indicated</td>
<td>CM; 200 <em>E. coli</em> isolates</td>
<td>1.5% (3/200)</td>
<td>(1) OX; (2) 026:K71;OX</td>
<td><em>stx</em>1, <em>stx</em>2, <em>traT</em>, <em>fimH</em>, <em>trat</em>, <em>fyua</em>, <em>hlyD</em>, <em>fimH</em>, <em>fyuA</em>, <em>hlyD</em>, <em>fimH</em>; None was eaeA+</td>
<td>n/a</td>
<td>Sabry et al. (2006)</td>
</tr>
<tr>
<td>Southern Finland</td>
<td>Days 0 and 21</td>
<td>65 Dairy herds; 144 cows; 144 CM samples and 10 follow-up samples; 37 <em>E. coli</em> isolates</td>
<td>0%</td>
<td>n/a</td>
<td><em>stx</em>1 and <em>stx</em>2 not found</td>
<td>n/a</td>
<td>Suojala et al. (2011)</td>
</tr>
<tr>
<td>Ireland (Republic of)</td>
<td>1 Year</td>
<td>154 <em>E. coli</em> isolates</td>
<td>2.7% (1/37)</td>
<td>n/a</td>
<td><em>stx</em>2a (variant), eaeA–</td>
<td>n/a</td>
<td>Keane (2016)</td>
</tr>
<tr>
<td>Turkey</td>
<td>n/a</td>
<td>35 Farms; 484 mastitic samples; 51 <em>E. coli</em>+</td>
<td>7/51 (13.72%)</td>
<td>O157:H7</td>
<td>7 isolates carried <em>stx</em>2, eae, ehlyA, and tir genes</td>
<td>n/a</td>
<td>Turkyilmaz, et al. (2017)</td>
</tr>
<tr>
<td>Turkey</td>
<td>3 Years</td>
<td>100 Cows: CM</td>
<td>0%</td>
<td>0</td>
<td><em>stx</em> not found</td>
<td>n/a</td>
<td>Guler and Gunduz (2007)</td>
</tr>
</tbody>
</table>

*Many other virulence genes were tested. Virulence factor-encoding and other genes: eaeA/eae, intimin, attaching and effacing factor; ehly, hemolysin gene; ehlyA, enterohemolysin A gene; fimH, mannose-specific adhesion type 1 fimbiae gene; fyua, yersiniabactin gene; hlyD, alpha hemolysin toxin gene; *stx*1, Shiga toxin 1 gene; stx2, Shiga toxin 2 gene; *stx*2a, Shiga toxin 2d variant gene; hlyA, hemolysin A gene; tir, translocated intimin receptor gene; retA, serum survival/resistance gene.

Antibiotics: AMC, amoxicillin/clavulanic acid; AMP, ampicillin; C, cefotaxime; CEP, cefalotin; CFP, cefoperazone; CFT, cefotiofur; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; CPL, ceftriaxone, CRO, cefotaxime; ENF, enrofloxacin; FFN, florfenicol; GEN, gentamicin; KAN, kanamycin; MCG, cefalexin; NAL, nalidixic acid; NEO, neomycin; POL, polymyxin B; SMX, sulfamethoxazole; SPE, spectinomycin; STR, streptomycin; SU, sulfonamide; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.

CM, clinical mastitis (acute mastitis); n/a, not available or not applicable; QM, quarter milk; SC, subclinical mastitis; STEC, Shiga toxin–producing *E. coli* (=VTEC, verotoxin-producing *E. coli*; verotoxin = Shiga toxin).
Table 5. Shiga Toxin–Producing E. coli Isolated from Mastitic Milk in New Zealand

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of forms, samples, and E. coli isolates</th>
<th>No. of STEC (%)</th>
<th>No. of E. coli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand</td>
<td>30880 (37.5)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Duration of study 17 Months

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of STEC</th>
<th>No. of E. coli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand</td>
<td>84 cows</td>
<td>100</td>
</tr>
</tbody>
</table>

Shiga Toxin–Producing E. coli

The isolates were stx1 positive, and stx2 and eaeA negative. A total of 70 E. coli isolates recovered from clinical and subclinical mastitis samples in China were stx negative (Liu et al., 2014). Two of 37 (5.4%) E. coli isolated in India were positive for stx in an stx1/stx2 co-detection assay (Munir, 2015) that did not distinguish the two toxin genes. In a study that represented the first survey of STEC that targeted serotype O157 in Bangladesh, the researchers were not able to isolate STEC (Islam et al., 2010).

Studies conducted in the Middle East (Table 3), particularly Iran, appear to have generated some of the highest prevalence of STEC associated with mastitis. Out of 400 samples, 42 were positive for E. coli and 10 (23.8%) of these were stx positive (Montaz, 2010). Tavakoli and Pourtaghi (2017) reported an STEC prevalence rate of 21.6% (13/60; corrected to 15%; 9/60) in bovine E. coli mastitis, whereas Naser (2016) reported a prevalence of 13.3% in sheep. Notably, Tavakoli and Pourtaghi (2017) miscounted/misclassified four enteropathogenic E. coli (EPEC) strains as STEC; these did not have stx genes. Studies in Iraq reported a 10.34% (9/87) prevalence rate of STEC in mastitis (Zafarana et al., 2017).

At least six countries in Europe have reported STEC associated with E. coli mastitis (Table 4). In earlier studies of E. coli mastitis, in England and Wales, Barrow and Hill (1989) found that 0.5% (1/237) of E. coli produced Shiga toxins, whereas Stephan and Kuhn (1999) identified STEC in 2.8% (4/145) of cows in Switzerland. The latter study identified serogroups O105, O8, and OX3 (Stephan and Kuhn, 1999). In Austria, Sabry et al. (2006) identified stx genes in 1.5% (3/200) of isolates of serotype O126:K71 (n = 1) and OX (n = 2) from E. coli mastitis. Two isolates had stx1, stx2, traT, and fimH genes (serotypes O126:K71 and OX), and one isolate (serotype OX) had stx2, traT, fyuA, hlyD, and fimH genes. Studies conducted in Southern Finland with 65 dairy herds failed to yield STEC from E. coli mastitis cases (Suojala et al., 2011). The researchers targeted samples collected on day 0 and a follow-up sample on day 21.

In a 1-year study involving 18 farms in Ireland, 37.5% (30/80) of E. coli isolates from mastitis were positive for stx1 (n = 25) and stx2 (n = 5), a variant of stx2 (Keane, 2016). The rfbO157 gene was detected in only 3.75% of the isolates (suggesting other STEC serotypes were dominant). In a study carried out in Turkey to determine the prevalence of E. coli O157:H7 in E. coli-positive mastitic bovine milk (n = 51), seven of eight E. coli O157:H7 that were isolated were STEC, whereas one possessed the other STEC virulence factors, except stx (Turkyilmaz et al., 2017). STEC prevalence rate was 13.72%. However, Guler and Gunduz (2007) did not find STEC in their 3-year study of E. coli mastitis in 100 cows.

While no published studies on STEC-related mastitis from Australia was found, in New Zealand, Bean et al. (2004) established an STEC prevalence rate of ~37% (30/80) in E. coli mastitis (Table 5). Two STEC isolates had both cnf2 and stx1 genes, while one had eaeA and stx2 genes. They focused on the detection of rfbO157 and H7 flagellar genes, which were detected at frequencies of 3.75% and 1.25%, respectively.

In South America, the only available reports associating STEC with mastitis were from Brazil (Table 6). Research on prevalence of STEC in E. coli mastitis in Brazil indicated an isolation rate of 12.09% (22/182) from 2144 samples over an
Table 6. Shiga Toxin–Producing *Escherichia coli* Isolated from Mastitic Milk in South America

<table>
<thead>
<tr>
<th>Country</th>
<th>Duration of study</th>
<th>No. of farms, samples, and E. coli isolates</th>
<th>No. of STEC/No. of E. coli (%)</th>
<th>Serotypes</th>
<th>Virulence factor profiles (stx, eae, ehly, etc.)</th>
<th>Antibiotic resistance profiles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>18 Months (March 1997 to August 1998)</td>
<td>2144 Samples of mastitic milk; 182 E. coli isolates</td>
<td>22/182 (12.09)</td>
<td>O26 (7), O111 (5), O119 (5), O55 (4), O157 (1)</td>
<td>stx1, stx2, hly, and eaeA; 11 (50%) of the 22 STEC strains carried the eae gene; 5 (22.7%) of the STEC isolates were positive only for stx1, stx2 gene alone was detected in 10 (45.5%) isolates, stx1 and stx2 gene in combination was present in 7 (31.8%) isolates. 16 (72.7%) STEC isolates showed positive hybridization with the hly gene.</td>
<td>TRI, GEN, C; AMC; NET, TET, CEP; DOX, CFZ, and NAL (n=10); most resistance to CEP 86.3%, TET (63.6%), and DOX (63.6%) and less frequently to NAL (18.1%), TRI (18.1%), and CFZ (9.1%)</td>
<td>Lira et al. (2004)</td>
</tr>
<tr>
<td>Brazil</td>
<td>10 Months (February 2003 to November 2003)</td>
<td>528 Milk samples; 31 E. coli isolates</td>
<td>20/31 (64.5)</td>
<td>O55 (6), O111 (4), O119 (2), O125 (1), O86 (3), O114 (3), O142 (1)</td>
<td>stx1, stx2, eae. Diverse virulence factor profiles even among same serotypes; genotypes: stx1 only (8); stx2 only (1); stx1/stx2 only (1); stx1/eae (5); stx2/stx2 (1); stx1/stx2/eae (3)</td>
<td>NAL, AMP, CEP, CHL; ERY, STR, GEN, KAN, LI, PEN, NOV, NEO, TET, NIT, and TMP (n=15). Most resistance to NOV (100%), LI (96.8%), PEN (96.8%), and ERY (90.3%). All were sensitive to GEN and KAN. (All E. coli data—did not segregate STEC.)</td>
<td>Kobori et al. (2004)</td>
</tr>
<tr>
<td>Brazil</td>
<td>10 Months (February to November 2004)</td>
<td>2 States; 37 dairy farms; 670 mastitic cows; 51 E. coli; 231 E. coli isolates</td>
<td>20/231 (8.6)</td>
<td>Targeted O157:H7 (not detected); other serotypes not determined</td>
<td>stx1, stx2, eae; 8 stx1+ only; 12 stx2+ only; no stx/stx+</td>
<td>AMP, AMO, AMC, TET, AMI, CEP, CR, GEN, STR, NAL, SXT, and CIP (n=12). Antibiotic resistance not indicated for STEC. E. coli isolates had high resistance to TET (92.2%), STR 90.4%, and NAL (88.3%). Low resistance to AMC (14.2%) and CR (17.7%).</td>
<td>Rangel and Marin (2009)</td>
</tr>
<tr>
<td>Brazil</td>
<td>n/a</td>
<td>7 Dairy farms; 27 cows and samples; CM; 27 E. coli</td>
<td>2/27 (7.4)</td>
<td>OR:HNT, ONT:H31</td>
<td>stx1, stx2, ehly, saa, fimH, and stb; both isolates were stx2+ and fimH+ and stb+ and saa negative</td>
<td>AMP, GEN, SXT, NEO, CPL, CFT, FLO, and CFP (n=8); OR:HNT sensitive to NEO, AMP, and resistant to SXT. Resistance of STEC to other drugs n/a</td>
<td>Fernandes et al. (2011)</td>
</tr>
</tbody>
</table>

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*Numbers of each serotype.

*Numbers tested many other E. coli virulence genes.

Serotypes: H, flagella serotype; NT, nontypable; S, somatic serotype; R, rough.

Virulence factor-encoding and other genes: *eaeA/eae*, intimin gene; *ehxA*, enterohemolysin A gene; *fimH*, type 1 fimbriae gene; *hlyA*, hemolysin gene; *hlyA*, hemolysin A gene; *saa*, STEC agglutinating adhesion gene; *stb*, heat-stable enterotoxin b gene; *stx1*, Shiga toxin 1 gene (=vt1, verotoxin 1 gene); *stx2*, Shiga toxin 2 gene (=vt2, verotoxin 2 gene).

Antibiotics: AMC, amoxicillin/clavulanic acid; AMI, amikacin; AMO, amoxicillin; AMP, ampicillin; C, cefotaxime; CEP, cephalothin; CFP, cefoperazone; CFT, ceftriaxone; CFT, ceftiofur; CFZ, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin; CPL, cepalexin; CR, ceftriaxone; DOX, doxycycline; ENF, enrofloxacin; ERY, erythromycin; FLO, florenicol; GEN, gentamicin; IML, imipenem; KAN, kanamycin; LI, lincomycin; MCG, ceftizoxime; NA, nalidixic acid; NEO, neomycin; NET, netilmicin; NIT, nitrofurantoin; NOR, norfloxacin; NOV, novobiocin; OTE, oxytetracycline; STR, streptomycin; SXT (=cotrimoxazole), trimethoprim/sulfamethoxazole; TET, tetracycline; TMP, trimethoprim/sulfadiazine; TRI, trimethoprim.

CM, clinical mastitis (acute mastitis); n/a, not available or not applicable; QM, quarter milk; SC, subclinical mastitis; STEC, Shiga toxin–producing *E. coli*. 
18-month period (Lira et al., 2004). The STEC belonged to five serotypes: O26 (31.8%), O119 (22.7%), O111 (22.7%), O55 (18.2%), and O157 (4.5%). Eleven (50%) of the STEC strains carried both stx and eae genes, while five were positive for stx1 only. The stx2 gene alone was detected in 10 (45.5%) isolates, whereas stx1/stx2 genotypes were found in 7 (31.8%) isolates. Sixteen of the STEC isolates (72.7%) were hly positive (Lira et al., 2004). In the same year, Kobori et al. (2004) isolated STEC of diverse serotypes (O55 [n = 6], O111 [n = 4], O86 [n = 3], O114 [n = 3], O119 [n = 2], O125 [n = 1], and O142 [n = 1]) from 528 milk samples at a relatively high rate, that is, 64.5% (20/31 of E. coli-positive samples). The STEC had diverse virulence factors even among the same serotypes. Eight (40%) of STEC strains carried only the stx1 gene, whereas three (15.0%) carried only the stx2 gene (with or without eae) and four (20.0%) carried both stx1 and stx2 genes. Ten of the STEC were positive for the eae gene.

Rangel and Marin (2009) demonstrated the presence, in a low number (8.6%), of STEC strains among E. coli isolates from bovine mastitic milk in Brazil. Twelve isolates were stx2 positive, whereas eight were stx1 positive, and none of the isolates had both stx1 and stx2 genes. While they could not detect the presence of serotype O157:H7, they did not identify the non-O157 STEC serotypes. In a study by Fernandes et al. (2011), in Brazil, 27 E. coli isolates from mastitis were analyzed, and 2 (7.4%) were shown to produce Stx. All isolates produced biofilms and the fimH gene (encoding type I fimbriae) was found in all isolates and was associated with other virulence markers, including stx2. STEC prevalence data from other major dairy producing countries in South America were not available.

Unlike other regions of the world (summarized in Tables 1–6), an exhaustive investigation of the literature failed to yield any report or systematic study on prevalence of STEC-associated mastitis in dairy cows in the United States and Canada. There are, however, numerous reports on the isolation of STEC from dairy farm environments, animals, animal feces, and milk (bulk tank milk/BTM and fresh, filtered samples) from mastitis were reported (Table 7). The most prevalent serotypes were O26 (20.44%), O157 (14.60%), O55 (10.22%), O111 (9.49%), O119 (6.57%), O114 (5.11%), and O91 (4.38%). Other serotypes were isolated at prevalence rates ranging from 0.73% to 2.92%, predominantly at a 0.73% rate. In the latter case, the serotypes were only isolated once. The Big 7 STEC serotypes are O26, O45, O103, O111, O121, O145, and O157 (Bosilevac and Koolmariae, 2012; ATCC, 2014). Importantly, three of the Big 7 STEC members, serotype O26, O111, and O157, were well represented in STEC mastitis (9.49%–20.44% prevalence), whereas the other four serotypes (i.e., O45, O103, O121, and O145) were isolated at lower prevalence rates, ranging from 1.46% to 2.92% (Table 7). Serotype O26 had the second widest distribution. It was isolated everywhere except Asia and New Zealand. STEC O157 prevalence had the widest distribution; they were isolated in Africa, the Middle East, Europe, Australia, and South America, except Asia. This is obviously, partly, due to the inherent isolation bias as some researchers only targeted detection of serotype O157 in their studies (e.g., Islam et al., 2010; Momtaz, 2010; Caine et al., 2014; Ivbade et al., 2014; Turkyilmaz, et al., 2017). Therefore, there are serious underreporting issues, since absence of O157 does not entail absence of STEC. On the other hand, some isolates that

**SHIGA TOXIN-PRODUCING E. COLI IN MASTITIS**

**Preliminary STEC Serotypes Isolated from Mastitis**

Based on O, H, and K antigens, over 700 serovars of E. coli have been recognized (Kaper et al., 2004). Notably, more than 200 E. coli serovars can produce Stxs (Nataro and Kaper, 1998; WHO, 1998). Mastitis-causing STEC belonged to a broad range of serotypes. Twenty-seven different O-serotypes, ranging from O8–O157, including OX and X3, were reported (Table 7). The most prevalent serotypes were (Table 7) O26 (20.44%), O157 (14.60%), O55 (10.22%), O111 (9.49%), O119 (6.57%), O114 (5.11%), and O91 (4.38%). Other serotypes were isolated at prevalence rates ranging from 0.73% to 2.92%, predominantly at a 0.73% rate. In the latter case, the serotypes were only isolated once. The Big 7 STEC serotypes are O26, O45, O103, O111, O121, O145, and O157 (Bosilevac and Koolmariae, 2012; ATCC, 2014).

Is STEC Infection Associated with Greater Severity of Mastitis?

Previous studies have shown that adherence of E. coli to the epithelial surface of the udder does not play a role in the pathogenesis of bovine mastitis; bacteria multiply in the udder in secreted milk without attachment to the mammary epithelium (Gyles, 1993; Burvenich et al., 2003). Adherence can be important in persistence of the pathogen due to biofilm formation (Herold et al., 2009; Liu et al., 2014). It is accepted that bacterial, environmental, management, and cow factors may affect the occurrence and severity of mastitis. Studies suggest that the severity of E. coli mastitis mainly depends on cow factors rather than the characteristics of E. coli (Burvenich et al., 2003; Lehtolainen, 2004).

Many studies that were performed during the last decade indicate that the severity of E. coli mastitis is mainly determined by host factors rather than by E. coli pathogenicity (reviewed by Burvenich et al., 2003). Out of 279 E. coli isolates investigated by Linton et al. (1979), 217 (77.1%) could be O-serogrouped into 67 different O-serogroups. This indicates that E. coli mastitis is not caused by a limited number of specific pathogenic strains, but seems to be associated with environmental fecal contamination, and is multifactorial (Kobori et al., 2004).
were identified as STEC were not serotyped (Barrow and Hill, 1989; Bean et al., 2004; Keane, 2016). Knowledge of serotypes will enable targeted surveillance and control of STEC. Africa demonstrated the greatest serotype diversity \((n = 14)\), followed by the Middle East \((n = 10)\), South America \((n = 9)\), and Europe \((n = 6)\), whereas New Zealand (O157) and Asia (O114) had one reported serotype each (Table 8). Some isolates in Africa, Europe, and Asia were O-untypable, although they were typable at flagella level.

### STEC Ancillary Virulence Factors

Beyond \(stx\), STEC may be endowed with additional virulence characteristics that augment their pathogenicity (Nataro and Kaper, 1998; Paton and Paton, 1998, Boisen et al., 2015). Adherence to mammary epithelial cells is an important first step for \(E. coli\) invasion of the mammary gland. Adhesin factors, including F17-, P-, and S-fimbriae, afimbrial adhesins, and intimins, all play an important role in bovine \(E. coli\) mastitis (reviewed by Liu et al., 2014). Beyond that, \(E. coli\) can also produce other virulence factors that could improve their iron uptake ability, a feature that would contribute to enhancing bacterial resistance to host immunological defenses. Furthermore, virulence factors may be linked to phylogeny groups and AMR traits (reviewed by Liu et al., 2014). Some STEC were defined as EHEC without further clarification or determination of \(eaeA\) and \(ehly\), in addition to \(stx\) found in these isolates (El-Bagory and Zayda, 2015). Very few researchers attempted detection of \(stx\) variant genotypes, except for Keane (2016) and Bean et al. (2004), who, respectively reported presence of \(stx_{2a}\) and \(stx_{2e}\) in their STEC isolates.

### AMR of STEC Isolated from Mastitis

The development of bacterial resistance to antimicrobial agents poses a serious threat to human health. Many
significant antimicrobial-resistant human pathogens, including *Campylobacter* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), STEC, and extended-spectrum beta lactamase (ESBL) gene carrying bacteria have been isolated from raw milk (reviewed by Zastempowska et al., 2016). In this review, some STEC were ESBL positive (Ghatak et al., 2013), whereas others were ESBL negative (Munir, 2015; Hinthong et al., 2017). The disk diffusion method was the most widely used and standardized method for antibiotic resistance determination. Antimicrobial therapy remains an important component of the decision-making process in determining appropriate antimicrobial therapy against bacterial infection of the mammary gland.

Antibiotic resistance monitoring of mastitis causing *E. coli* can be an important tool in controlling mastitis within dairy herds. IMI involving *E. coli* can result in the release of toxins, and this mainly occurs upon death of the bacterial cells (Wong et al., 2000; Bauwens et al., 2017). These toxins are among the main contributors of clinical signs of mastitis. The use of antibiotics for the treatment of *E. coli* mastitis is therefore not recommended by some veterinarians (Petersson-Wolfe and Currin, 2011). Antibiotic treatment could enhance the expression of Shiga toxin genes; thus, antimicrobial treatment is contraindicated for human *E. coli* O157:H7 infections (Zhang et al., 2009).

The emergence and dissemination of AMR among STEC strains may have potentially negative clinical implications, although the diarrheal phase of illnesses associated with STEC strains is usually self-limiting; thus, the role of early antimicrobial therapy in the prevention of HUS is still unclear (Molbak et al., 2002). Antimicrobial-resistant STEC strains could possibly possess selective advantages over other bacteria colonizing the gastrointestinal tracts of animals that are treated with antibiotics (therapeutically or subtherapeutically). Resistant STEC strains could then become the predominant *E. coli* present under antibiotic selective pressures. This could result in STEC population increases and perhaps greater fecal shedding, which could lead to greater contamination of animal food products with STEC (Zhao et al., 2001).

In one study conducted in Nigeria, all the STEC O157 isolates from various milk and milk products, including one isolate from raw milk (1 of 50 samples; 2% prevalence), were susceptible to ciprofloxacin and neomycin, but were resistant to ampicillin and tetracycline (Ivbade et al., 2014). The presence of virulent multidrug-resistant (MDR; i.e., resistance to two or more classes of antibiotics) *E. coli* O157 strains in milk and milk products, as revealed by Ivbade et al. (2014), unveils a risk of human exposure to these potentially fatal pathogens following consumption of contaminated dairy products.

Twenty-one antibiotics were evaluated against the single STEC isolate (KOEC6) from the study by Ghatak et al. (2013) in India. It was positive for ESBLs and demonstrated resistances to amoxicillin clavulanate, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefazidime, ciprofloxacin, levofloxacin, piperacillin, and trimethoprim/sulfamethoxazole, and sensitivity to amikacin, cefotixin, chloramphenicol, colistin, gentamicin, imipenem, and piperacillin/tazobactam. MDR STEC could be a dangerous precedent to public health and spread of genes to other species (Ghatak et al., 2013). In Thailand (Hinthong et al., 2017), 18 antibiotics were tested (Table 2), and none of the STEC from mastitis possessed the ESBL phenotype. STEC O114::K (n = 2) were susceptible; and one nontypable STEC was resistant to ampicillin, benzylpenicillin, and sulfamethoxazole/trimethoprim. Munir (2015) conducted tests for ESBL production versus cefpodoxime, cefotiofur, aztreonam, cefotaxime, and ceftriaxone (n = 5); the two STEC isolates from mastitis were ESBL negative. Das et al. (2005), in India, conducted antibiotic resistance tests with 15 antibiotics against STEC and non-STEC *E. coli* from mastitis (Table 2). None of the *E. coli* and STEC strains were found to be resistant to gentamicin, ciprofloxacin, cefotaxime, ceftriaxone, nalidixic acid, and norfloxacin; however, no STEC-specific resistance data were provided.

Several studies have reported antibiotic resistance of STEC in the Middle East (Table 3). In tests conducted by Tavakoli and Pourtaghi (2017), multidrug resistance patterns were noted among STEC (Table 3). For example, 5 STEC (38.46%) were multiple resistant to 12 of 14 tested antibiotics; however, all were susceptible to enrofloxacin. In Iraq, antibiotic susceptibility testing revealed that all of the STEC isolates (n = 10) were resistant to ampicillin and tetracycline. High resistance frequency was observed for trimethoprim/sulfamethoxazole (66.6%) followed by chloramphenicol (55.5%), whereas all isolates were susceptible to imipenem.

The significant relationship between those *E. coli* producing only Stx1 and the existence of antibiotic resistances in STEC originating from cattle feces observed by Burnens et al. (1995) was not present in the mastitis *E. coli* studied by Stephan and Kuhn (1999) in Switzerland. The strain producing only Stx2 was multiple resistant to ampicillin, neomycin, tetracycline, and sulfonamide, unlike the other STEC strains that were highly sensitive to all antibiotics (Table 4).

All 8 STEC O157:H7 reported by Turkyilmaz et al. (2017) in Turkey were susceptible to 11 antibiotics (i.e., ampicillin, amoxicillin/clavulanic acid, gentamicin, kanamycin, cefoperazone, cefotaxime, cefoxizome, ceftriaxone, nalidixic acid, chloramphenicol, and trimethoprim/sulfamethoxazole), whereas a few were tetracycline resistant (n = 2), while 1 was streptomycin resistant.

In a study by Lira et al. (2004) in Brazil, all the STEC isolates were tested for sensitivity to 10 antimicrobials. The resistances most commonly observed were to cephalothin (86%), tetracycline (63.6%), and doxycycline (63.6%). When *E. coli* strains (STEC included, but not separated) were tested for resistance to 15 antimicrobial agents (Kobori et al., 2004), they were resistant most commonly to novobiocin (100%), lincomycin (96.8%), penicillin (96.8%), and erythromycin (90.3%). All test *E. coli* strains showed resistance to at least one antimicrobial agent, but none showed resistance to all of them. MDR was very common; 96.8% of the strains showed resistance to three antimicrobial agents, Rangel and Marin (2009) tested *E. coli* isolates from mastitis for resistance to 12 antimicrobial agents. Antibiotic resistance was not specifically indicated for STEC. The predominantly observed resistance was to tetracycline (92.2%), streptomycin (90.4%), nalidixic acid (88.3%), amikacin (86.5%), and cephalothin (84.8%). Lower resistance to amoxicillin/clavulanic acid and ceftriaxone was detected in 14.2% and 17.7% of the isolates (Rangel and Marin, 2009).

It appears that tetracycline resistance in STEC predominates worldwide (Lira et al., 2004; Das et al., 2005; Rangel and Marin, 2009; Ivbade et al., 2014; Turkyilmaz et al., 2017).
There are some indications some STEC could be acquiring ESBL phenotypes (Ghatak et al., 2013). Evidently, despite MDR, STEC are still highly susceptible to some antibiotics (Stephan and Kuhn, 1999; Kobori et al., 2004; Das et al., 2005; Fernandes et al., 2011; Ivbade et al., 2014; Naser, 2016; Hinthong et al., 2017; Tavakoli and Pourtaghi, 2017; Turkylilmaz et al., 2017; Zafarane et al., 2017). It is, however, not clear whether STEC resistance rates are any different from those reported for non-STECE. coli, which were isolated from mastitis in the same studies.

Recommendations for Risk Mitigation to Reduce STEC in Mastitis

Transmission of antimicrobial-resistant mastitis pathogens and/or foodborne pathogens to humans, including STEC, could occur if contaminated unpasteurized milk is consumed, which is another important reason why people should not consume raw milk. AMR among dairy pathogens, particularly those found in milk, is not likely a human health concern as long as the milk is pasteurized (Oliver and Murinda, 2011, 2012). AMR in STEC might impact the outcome of antibiotic therapy—the common treatment for bovine clinical mastitis (Oliver and Murinda, 2011). Widespread use of antimicrobials in farm animals has been incriminated in the rise of antimicrobial-resistant strains of bacteria, which can increase treatment cost and period (Sawant et al., 2007).

Given the severity of the clinical manifestations of the STEC disease in humans and the inability and/or the potential risks of antibiotic administration for treatment, it appears that the most direct and effective measures toward ensuring public health reside in the prevention of STEC infections in humans. Although finding effective preharvest control measures is not easy, preventive approaches should focus on reducing the prevalence of STEC in animal reservoirs in primary production (Hussein and Sakuma, 2005b; Farrokhi et al., 2013), on reducing the pathogens’ prevalence in food and water, and on consumer education.

Establishing a regular monitoring system for identification of cases with clinical or subclinical mastitis and conducting antibiotic sensitivity tests are recommended before antibiotic treatment.

Due to the reservoir or carrier status of dairy cows (Nataro and Kaper, 1998; Paton and Paton 1998; Hussein and Sakuma, 2005b; Farrokhi et al., 2013), reducing the amount of STEC in live cattle will likely lower contamination not only of meat but also of other food and water supplies that come into contact with bovine fecal matter. A variety of strategies have been proposed to reach this goal. These include modification of farm practices (e.g., manure disposal) and bovine diet, vaccination, and administration of lytic phages or probiotic bacteria (Gansheroff and O’Brien, 2000; Cho et al., 2009). On the other hand, according to Suojala et al. (2011), the lack of correlation between virulence factors and the severity of mastitis make mastitis vaccine formulation challenging.

It is unrealistic to expect eradication of STEC on dairy farms. Farm biosecurity, hazard analysis and critical control points, good manufacturing practices, and good farming practices are also important approaches (CDPH, 2015). Lambertini et al. (2015) indicated that due to the low prevalence and low pathogenicity observed in milk samples in this study, hygienic measures to prevent fecal contamination of milk appeared to be effective, although exceptions (from unknown causes) can still result in significant risk. There is evidence that different farm management strategies such as spreading manure on pastures, extent of contact between animals, organic versus conventional approaches, diet, and hygiene may impact the risk of pathogenic E. coli occurrence and transmission (reviewed by Lambertini et al., 2015). However, such evidence is still ambiguous, and climatic and geographical variables are also potential risk factors (APHIS, 2003).

While eradication of pathogenic E. coli on dairy farms still appears a far-fetched goal due to the high prevalence and widespread geographical distribution of the pathogen, as well as its persistent fluctuating occurrence in several farm compartments, a better understanding of the ecology of pathogenic E. coli and the mobile genetic elements associated with its virulence can lead to improved strategies to control E. coli pathogenicity on farms (Lambertini et al., 2015).

In studies conducted by Lambertini et al. (2015), their data suggested that the stress or the negative energy balance associated with lactation may result in increased STEC shedding during the warm summer months and future prevention strategies aimed at reducing stress during lactation or isolating high-risk animals could be implemented to reduce herd-level shedding and avoid transmission of STEC to susceptible animals and people (Venegas-Vargas et al., 2016).

It is impractical to eliminate bacteria on the farm, as they are ubiquitous in the animal’s environment, mouth, coat, animal feces, soil feed, water, manure, and bedding; thus, best strategies entail reduction of pathogens to minimize risk of infection of the udder and transmission to humans.

The practice of feeding waste/mastitic milk to young calves needs to be critically reevaluated in light of biosecurity; it is still a common practice (Kertz, 2002), and should be discouraged both from the animal health perspective and as a preventative measure to reduce both colonization of cattle by these strains and ultimately carriage rates and dissemination to consumers (Bean et al., 2004; Cursons et al., 2005). Although no other side effects were observed, high bacterial load, however, was associated with an increased prevalence rate of diarrhea in calves (Abb-Schwedler et al., 2014). Contrary to these reports, milk from cows treated with antibiotics for mastitis and other disorders has been fed to young calves in fresh or fermented form without adverse health disorders in comparison to those fed control milk (Kesler, 1981).

STEC can contaminate milk and milk products, and cows with IMI could be culled and beef from these animals can pose a risk to consumers by causing serious public health concerns, for example, HC, HUS, TTP, and even death.

Conclusions

STEC is considered a risk factor for human infections. It was notable that there is considerable misinformation regarding STEC prevalence from dairy animals or milk, which is often misrepresented as mastitis prevalence. Even data from “reputable” peer-reviewed journals need to be critically revalidated and interpreted with caution.

There are also inherent difficulties in analyzing mastitis data from the literature as some of the information on prevalence is reported as sample prevalence by some and isolate
prevalence by others. Furthermore, there are also major difficulties in interpreting data from non-native English writers that have published on mastitis. There is no doubt that poor sample collection methods can include extramammary STEC isolates that could be mistaken as if they came from inside the udder. In addition, many different approaches were employed, especially for sample processing and isolation of bacteria, including methods used for detection of virulence factors and AMR of isolates. Many researchers did not provide information on serotypes or AMR profiles of STEC isolates they studied (Tables 1–6). Future studies require more systematic and comprehensive approaches to study STEC prevalence in IMI.

Raw milk presents a potential source of STEC infections. Particular significance should be attributed to fecal contamination of raw milk (Stephan and Kuhn, 1999). Unpasteurized raw milk constitutes a pathway for entry of zoonotic pathogens into the food chain. Data from this review confirm reports from earlier studies that showed the significance of contamination of BTM by zoonotic pathogens, including STEC, which concluded that the consumption of raw milk and raw milk products presents a health risk (Murinda et al., 2002, 2004; Hussein and Sakuma, 2005a; Oliver et al., 2009; Farrokh et al., 2013).

Importantly, STEC are no longer emerging pathogens. They are well established in their animal reservoirs and environment; hence, their continued isolation in milk and IMI is worrisome. There is an urgent need in development of enhanced programs for surveillance and control of STEC, including judicious use of veterinary drugs to mitigate the continued increase in antibiotic resistance.

Disclosure Statement

No competing financial interests exist.

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