Food Microbiology 36 (2013) 260–266

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Lactic acid resistance of Shiga toxin-producing *Escherichia coli* and multidrug-resistant and susceptible *Salmonella* Typhimurium and *Salmonella* Newport in meat homogenate

Aliyar Fouladkhah, Ifigenia Geornaras, Hua Yang, John N. Sofos*

Center for Meat Safety & Quality, Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171, USA

ARTICLE INFO

Article history:
Received 17 November 2012
Received in revised form
3 June 2013
Accepted 11 June 2013
Available online 19 June 2013

Keywords:
Shiga toxin-producing *Escherichia coli*
Multidrug-resistant *Salmonella*
Lactic acid

ABSTRACT

This study compared lactic acid resistance of individual strains of wild-type and rifampicin-resistant non-O157 Shiga toxin-producing *Escherichia coli* (STEC) and of susceptible and multidrug-resistant (MDR) and/or MDR with acquired *ampC* gene (MDR-AmpC) *Salmonella* against *E. coli* O157:H7. After inoculation of sterile 10% beef homogenate, lactic acid was added to a target concentration of 5%. Before acid addition (control), after acid addition (within 2 s, i.e. time-0), and 2, 4, 6 and 8 min after addition of acid, aliquots were removed, neutralized, and analyzed for survivors. Of wild-type and of rifampicin-resistant non-O157 STEC strains, irrespective of serogroup, 85.7% (30 out of 35 strains) and 82.9% (29 out of 35 strains), respectively, reached the detection limit within 0–6 min. Of *Salmonella* strains, 87.9% (29 out of 33 isolates) reached the detection limit within 0–4 min, irrespective of antibiotic resistance phenotype. Analysis of non-log-linear microbial survivor curves indicated that non-O157 STEC serogroups and MDR and susceptible *Salmonella* strains required less time for 4D-reduction compared to *E. coli* O157:H7. Overall, for nearly all strains and time intervals, individual strains of wild-type and rifampicin-resistant non-O157 STEC and *Salmonella* were less ($P < 0.05$) acid tolerant than *E. coli* O157:H7.

1. Introduction

Escherichia coli O157:H7 and other Shiga toxin-producing *E. coli* (STEC) serotypes are major concerns in primary and further processing of muscle foods as they have been involved in several foodborne illness episodes in recent years (Bosilevac et al., 2009). Pathogenic *E. coli* serotypes, and especially enterohemorrhagic *E. coli* (EHEC) strains, are capable of producing Shiga toxins that may lead to hemolytic uremic syndrome (HUS) a potentially life-threatening kidney complication in infected people. HUS is characterized by hemolytic anemia and acute renal failure and is primarily a concern for children, the elderly, and individuals with suppressed immune systems (Ahn et al., 2009; Johannes and Römer, 2010).

After an outbreak of gastrointestinal illness in 1983, *E. coli* O157:H7 was first recognized as a pathogenic agent capable of causing HUS (Feng, 1995). Soon after a multistate Pacific Northwest outbreak of *E. coli* O157:H7 in 1992–1993 (Oliver et al., 2009), followed by recalls of ground beef and beef trimmings contaminated with *E. coli* O157:H7, this pathogen became the first microbial agent considered as an "adulterant" in ground beef in the United States (Grant et al., 2011). Emergence of this pathogen has led to much advancement in food regulation and production systems including mandatory implementation of Hazard Analysis Critical Control Point (HACCP) based food safety management systems for muscle foods (Oliver et al., 2009; Sofos, 2009). Most recent epidemiological investigations estimate that every year in the United States *E. coli* O157:H7 is responsible for 3268 illness episodes with 46.2% and 0.5% hospitalization and death rates, respectively (Scallan et al., 2011). Along with *E. coli* O157:H7, in recent years other serogroups of STEC have been involved in foodborne episodes, including a recall of ground beef associated with *E. coli* O26 in the United States (USDA-FSIS, 2010), a recall of ground beef contaminated with *E. coli* O111 in Japan (USDA-FSIS, 2011a), and an outbreak through consumption of beef sausage contaminated with *E. coli* O26 in Denmark (Ethelberg et al., 2009). *E. coli* serogroups O26, O45, O103, O111, O121, and O145 are responsible for the majority of episodes of foodborne illness and HUS, associated with non-O157 STEC in the United States (Bettelheim, 2007; Mathusa et al., 2010; Grant et al., 2011). It is estimated that 1579 illness episodes, with a 12.8% hospitalization rate and a 0.3% death rate, are related to non-O157 STEC every year (Scallan et al., 2011). Recently

* Corresponding author. Tel.: +1 970 491 7703; fax: +1 970 491 5326.
E-mail address: john.sofos@colostate.edu (J.N. Sofos).

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.06.005

(USDA-FSIS, 2011b) the U.S. Department of Agriculture-Food Safety and Inspection Service declared these six non-O157 STEC serogroups (i.e. O26, O45, O103, O111, O121 and O145) as adulterants in raw non-intact beef products and components of such products (e.g. beef manufacturing trimmings), and in June, 2012 initiated a sampling and testing program for these particular serogroups in beef manufacturing trimmings.

Recent investigations show that 0.3–19.7% of feedlot cattle, 0.7–27.3% of cattle on irrigated pasture, and 0.9–6.9% of cattle in rangeland forage carry STEC serotypes in their gastrointestinal system, while at slaughtering facilities, prevalence of STEC serotypes ranges from 0.2 to 27.8% (Hussein and Bollinger, 2005a). In a one-year study of beef processing plants (Barkocy-Gallagher et al., 2003) it was shown that 60.6% and 56.6% of cattle hides, 5.9% and 19.4% of cattle manure samples, and 26.7% and 58.0% of carcasses were contaminated with O157 and non-O157 STEC, respectively.

Foodborne *Salmonella* serovars are also a significant public health concern; more than 30 of salmonellosis outbreaks in the United States and around the world have been associated with fresh meat as well as processed low-moisture food products in recent years (FDA, 2009). Of particular concern is the emergence of *Salmonella* strains that are multidrug-resistant (MDR) or MDR with an acquired *ampC* gene (MDR-AmpC) (Arthur et al., 2008; Bosilevac et al., 2009; Zhao et al., 2009). *Salmonella* strains with a MDR phenotype are resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline, and strains with a MDR-AmpC phenotype are resistant to at least the above-mentioned drugs as well as amoxicillin-clavulanic acid and ceftiofur (Greene et al., 2008; CDC, 2009). Recent investigations indicate that 0.6% of ground meat samples harbored drug-resistant *Salmonella* (Bosilevac et al., 2009) with approximately 7% of them displaying the MDR-AmpC phenotype (Zhao et al., 2009). Ground beef contaminated with *S.* Typhimurium strains with the MDR phenotype or *S.* Newport strains with the MDR-AmpC phenotype have been associated with foodborne illness outbreaks in the United States (Talbot et al., 2006).

Due to involvement of *E. coli* O157:H7 in numerous national and international foodborne illness episodes in the last few decades, this pathogen has been the primary target for control in beef processing (Oliver et al., 2009; Sofos, 2009). Lactic acid is one of the most common antimicrobial interventions in primary processing of fresh beef in the United States, and its efficacy has been reported by numerous investigators (Cutter and Rivera-Betancourt, 2000; Castillo et al., 2001; Arthur et al., 2004; Harris et al., 2006; Arthur et al., 2008).

The purpose of this study was to compare the lactic acid resistance of individual strains of the six non-O157 STEC serogroups (i.e. O26, O45, O103, O111, O121 and O145), and antibiotic susceptible and multidrug-resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium, to that of a 5-strain mixture of *E. coli* O157:H7. Additionally, the lactic acid resistance of spontaneous rifampicin-resistant variants of the STEC strains was compared to that of their parental (wild-type) counterparts.

2. Materials and methods

2.1. E. coli strains

Four to seven individual strains from each of six non-O157 STEC serogroups (i.e. O26, O45, O103, O111, O121, and O145) were used in this study. They were kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA), and Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE). A list of these strains, their identification codes, and isolation sources are reported by Geornaras et al. (2011). In addition to these wild-type strains, spontaneous rifampicin-resistant (100 μg/ml) variants of the non-O157 STEC strains were selected, based on the method described by Kaspar and Tamplin (1993), and included in the study. Wild-type and rifampicin-resistant variants of *E. coli* O157:H7 strains used in this study were ATCC 43888, ATCC 43895, C1-057, C1-072, and C1-109 (Carlson et al., 2009) and were available in the Pathogen Reduction Laboratory of the Center for Meat Safety & Quality of Colorado State University. Lactic acid resistance was tested on both, wild-type (parental) and rifampicin-resistant strains of *E. coli* O157:H7 and non-O157 STEC.

2.2. Salmonella strains

Individual strains of each of MDR and/or MDR-AmpC and susceptible *S.* Newport and *S.* Typhimurium were used in this study. The strains were kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. FDA, Laurel, MD). A list of the strains, their identification codes, and isolation sources are reported by Geornaras et al. (2011). The antibiotic resistance profiles of the *Salmonella* strains were confirmed using the Sensititre® antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH); specifically panel CMV2AGNF which was designed for the National Antimicrobial Resistance Monitoring System (NARMS). With this panel, minimum inhibitory concentrations (MIC) were determined, in accordance with the manufacturer's instructions, for ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. It should be noted that azithromycin was also included in the panel but no breakpoints were found for this antimicrobial (Geornaras et al., 2011). *Salmonella* strains with a MDR phenotype were resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT), and strains with a MDR-AmpC phenotype were resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and had a decreased susceptibility to ceftriaxone (MIC $\geq$2 μg/ml) (Greene et al., 2008; CDC, 2009).

2.3. Preparation of strains and mixtures

Strains were individually prepared and subcultured at 35 °C for 20–24 h in 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) (for *Salmonella*, and wild-type STEC strains) or TSB supplemented with rifampicin (100 μg/ml, Sigma–Aldrich, St. Louis, MO; TSB + rif) (for rifampicin-resistant STEC strains). Broth cultures were then streak-plated onto tryptic soy agar (TSA; Acumedia, Lansing, MI) (for wild-type STEC strains), TSA supplemented with 100 μg/ml rifampicin (TSA + rif) (for rifampicin-resistant STEC strains), or xylose lysine deoxycholate (XLD) agar (Acumedia) (for *Salmonella* strains); plates were incubated at 35 °C for 24 h. Inocula of each strain were prepared by suspending single colonies from the above-mentioned cultured plates into 5 ml phosphate-buffered saline (PBS, pH 7.4; 0.2 g/l KH_2PO_4, 1.5 g/l $Na_2HPO_4 \cdot 7H_2O$, 8.0 g/l NaCl, and 0.2 g/l KCl). The bacterial suspensions were standardized to a 0.5 McFarland standard (cell concentration of approximately 1.5×10^8 CFU/ml) using a spectrophotometer (600 nm) and nephelometer (Sensititre, Trek Diagnostics). For the inoculum comprised of a composite of five *E. coli* O157:H7 strains, bacterial suspensions to a 0.5 McFarland standard were initially prepared for each of the five strains separately, before combining the strains. All bacterial suspensions were diluted tenfold in PBS before use.