Food Control 71 (2017) 353–357

Contents lists available at ScienceDirect

Food Control

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

Short communication

Microbial efficacy and impact on the population of *Escherichia coli* of a routine sanitation process for the fabrication facility of a beef packing plant

Xianqin Yang*, Hui Wang, Annie He, Frances Tran

Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, Alberta, T4L 1W1, Canada

ARTICLE INFO

Article history:
Received 7 May 2016
Received in revised form
13 July 2016
Accepted 15 July 2016
Available online 16 July 2016

Keywords:
Sanitation process
Escherichia coli
MLVA
Contacting surface
Non-contacting surface
Fabrication equipment

ABSTRACT

The aim of this study was to assess the microbiological effect of a sanitation process used at a beef fabrication facility. On each of three fabrication days and the following mornings, samples were collected from meat contacting surface (CS) and non-contacting surface of two conveyor belts and from surfaces of cuttings tables before cleaning and before work, respectively, for recovery of total aerobes, coliforms and *Escherichia coli*. Selected presumptive *E. coli* isolates from belt 2 were purified and the confirmed isolates were genotyped using multiple-locus variable-number tandem-repeat analysis (MLVA). The numbers of aerobes before cleaning were mostly 6 log cfu/1000 cm^2 and were not significantly ($p > 0.05$) different from those before work. The log total numbers of coliforms and *E. coli* before cleaning and before work were largely similar. However, the numbers of samples from which no coliforms or *E. coli* were recovered were fewer before cleaning than before work. Of the presumptive *E. coli* isolates from CS and NCS before cleaning and before work, 88 (95%) and 1 (5%), and 134 (84%) and 78 (65%), respectively, were confirmed. MLVA of 89 (CS) and 212 (NCS) *E. coli* isolates revealed 18 and 16 distinct genotypes, respectively. Of the *E. coli* from CS, 98% were found at one sampling time. Of the *E. coli* from NCS, however, >90% were found more than once, and both before cleaning and before work. The findings show that the sanitation process did not have significant impact on the numbers of aerobes or coliforms, but was effective for removing *E. coli* from CS and to a lesser degree from NCS of conveyor belt.

1. Introduction

Contamination of beef with enteric pathogens such as *Escherichia coli* O157:H7 has long been linked to outbreaks of foodborne illnesses (CDC, 1993, 2014). To control the contamination of beef with *E. coli* O157:H7, all federally inspected beef packing plants in North America are required to implement Hazard Analysis Critical Control Points (HACCP) programs in which many large beef packing plants also include multiple antimicrobial interventions, particularly in the carcass dressing process, including spraying carcasses with solutions of lactic, acetic or peroxyacetic acid, and pasteurizing carcasses with hot water or steam (Gill, 2009). Consequently, the microbiological condition of beef carcasses has greatly improved in recent years. For instances, some plants at least can produce most dressed carcasses essentially free of *E. coli* (Arthur et al., 2004; Ruby & Ingham, 2009; Yang, Badoni, Youssef, & Gill, 2012), in contrast to the organism being recoverable from most dressed carcasses at 1 log CFU/cm^2 in the 90s (Gill, McGinnis, & Badoni, 1996; Sofos, Kochevar, Reagan, & Smith, 1999). Despite that, outbreaks associated with consumption of domestic beef contaminated with *E. coli* O157:H7 still occur (CDC, 2014). This is likely because the improvement of the microbiological condition of dressed carcasses is not equally reflected on that of meat products, i.e. cuts and trimmings. Chilled beef carcasses are broken into large portions which are subsequently deboned and fabricated into primal or subprimal cuts (cuts), with small portions of beef trimmed away in the process (trimmings). A number of studies have demonstrated that the numbers of *E. coli* on cuts and trimmings were higher than those on chilled carcasses, indicating recontamination during the meat fabrication process (Yang et al., 2012; Youssef, Badoni, Yang, & Gill, 2013). Thus, to prevent recontamination of meat during the fabrication process, the hygienic condition of surfaces of equipment involved in the process is imperative.

Federally inspected beef packing plants in Canada must

* Corresponding author.
E-mail address: xianqin.yang@agr.gc.ca (X. Yang).

http://dx.doi.org/10.1016/j.foodcont.2016.07.016
0956-7135/

implement a documented sanitation program for cleaning the facility as part of the pre-requisite program for HACCP (CFIA, 2014). However, a pre-operational visual inspection before work, rather than microbiological testing, is required to determine the effectiveness of cleaning. As with all sanitation procedures for food processing facilities, the sanitation programs commonly used at beef packing plant aim to reduce meat residue (soil) from and reduce undesirable bacteria on equipment through cleaning and sanitization, respectively (Gibson, Taylor, Hall, & Holah, 1999; Heinz & Hautzinger, 2007; Keener, 2005). By definition, sanitization should reduce the contamination level on clean surface by 99.999% (5 logs) in 30 s (Keener, 2005; Marriot, 2006). However, the effectiveness of sanitizers for target organisms can be affected by various environmental factors (Li, Kuda, & Yano, 2014) and by variations in strain/species response to sanitizers (Kastbjerg & Gram, 2009; Møretrø, Langsrud, & Heir, 2013). Thus, the overall effect of sanitation procedures in commercial setting may differ significantly from that for clean surface tested under laboratory conditions. Published accounts on the effectiveness of commonly used sanitation programs in commercial beef plants are largely lacking. The objectives of this study were: 1. to assess the microbiological effect of the sanitation program used for fabrication equipment in a commercial beef packing facility, and 2. to infer mechanisms by which *E. coli* survived the sanitation process, by comparing the populations of *E. coli* before and after sanitation.

2. Material and methods

2.1. Fabrication process at the packing plant

Beef is fabricated at the plant on Monday and Tuesday of each week. Carcass sides hung on a rail are cut by an air saw into quarters and subsequently into large portions. The large portions are dropped onto conveyor belts (Belt 1 and 2), from which they are taken to cutting tables alongside the belts for the removal of bones. Bone removal and subsequent fabrication of primal cuts are performed by multiple workers. The cutting tables are flipped once at lunch break. The side that is used in the morning is designated as S1 and the other side S2. The ambient temperature in the fabrication facility is maintained at 6–7 °C during production and it may go up to 15 °C during the sanitation process.

2.2. Sanitation procedures for fabrication equipment

The fabrication facility including fabrication equipment is cleaned daily at the end of each fabrication day, with the equipment being sanitized at the end of the cleaning process. The sanitation process is commonly used for beef processing equipment and facilities (Heinz & Hautzinger, 2007; Youssef, Klassen, & Gill, 2014). Briefly, visible meat debris is first removed and then the equipment is pre-rinsed with pressurized water at temperatures between 40 and 50 °C. After rinsing, the equipment is sprayed with a solution of 2–5% (w/v) Powerfoam Plus, a chlorinated foaming alkaline cleaner (Epsilon, Edmonton, Alberta, Canada) which is subsequently rinsed off after a contacting time of 15 min. The equipment is then sprayed with a solution of 200 ppm E-San, a quaternary ammonium based sanitizer (Epsilon). The sanitation process usually starts at 3–4 p.m. and ends at 2 a.m. Work starts at 5:30 a.m.

2.3. Collecting and processing of samples

Samples were collected after fabrication, but before cleaning on each of three Mondays and before work on each of the following Tuesdays, from both sides of the conveyor belts (meat contacting and non-contacting surface) and of the cutting tables. At each sampling time, five areas, each approximately 1000 cm^2, of each surface type of the conveyor belts and cutting tables were swabbed using a synthetic sponge (Whirl-Pak™ Speci-Sponge™ Bags; Nasco, WI). The sponges were premoistened with 7 ml of 0.1% peptone water (w/v). After swabbing, the sponge was replaced back in the stomacher bag which was kept on ice. Upon collection of sample before work the next day, 7 ml of double strength neutralizing buffer (Difco, BD, Sparks, MD) was immediately added to each sponge that had been used for swabbing the surfaces. Then samples were then processed, for recovery of total aerobes, coliforms and *E. coli* using Petrifilm™ Aerobic Count Plates (3M Corp., St. Paul, MN, USA), Lactose Monensin Glucuronate agar (LMG; Acumedia, Lansing, MI, USA) and Buffered MUG agar (BMA; Acumedia, Lansing, MI, USA), respectively (Liu, Youssef, & Yang, 2016). Briefly, 1 ml portions of appropriate decimal dilutions prepared in 0.1% peptone water from each swab sample were used to inoculate Petrifilm Aerobic plates. After incubation at 25 °C for 72 h, colonies on Petrifilm plates were counted following manufacturer's instructions. The remaining sponge fluid and 10^{-1} dilution of the sponge fluid were each filtered through a hydrophobic grid membrane filter (HGMF; Oxoid). The filters were incubated successively on LMG and BMA at 35 °C for 24 and 2 h, respectively. Blue-white fluorescent colonies on BMA were regarded as presumptive *E. coli*.

2.4. Analysis of microbiological data

All bacterial counts were transformed to log values. Counts of the same type obtained from samples collected from the same equipment surface type before cleaning or before work were regarded as a set. For aerobic and coliform counts, values for the mean log ($\bar{x}$), and standard deviation (SD) were calculated. For all three groups of bacteria, a value for the log of the total number of bacteria recovered (n) was calculated for each set of counts by summing the counts in each set and obtaining the log of the sum. All calculations were carried out using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA). A Ryan-Joiner test for normal distribution was applied to each set of aerobic counts and coliform counts using Minitab version 16 (Minitab Inc., State College, PA). Mean values for groups of sets that were all or mostly normally distributed ($P > 0.05$) were separated using the Tukey option of the one-way analysis of variance function in Minitab.

2.5. Collection and genotyping of E. coli isolates

Blue-white fluorescent colonies were picked, as presumptive *E. coli*, from HGMF filters used for enumeration of *E. coli* in samples from CS and NCS of Belt 2. When more than 15 presumptive *E. coli* colonies were obtained from each sample, 15 colonies were picked at random. Otherwise, all presumptive *E. coli* colonies were picked. The selected presumptive *E. coli* isolates were each streaked on plates of MacConkey agar which were incubated overnight at 35 °C. The streaking and incubation were repeated until homogenous colonies of typical *E. coli* morphology were obtained. The identities of purified isolates were confirmed by real-time PCR and the confirmed *E. coli* isolates were genotyped using multiple-locus variable-number tandem-repeat analysis (MLVA), as described previously (Yang, Badoni, Tran, & Gill, 2015). Each *E. coli* was identified by a string of 7 allele numbers in the order CVN001-CVN002-CVN003-CVN004-CVN007-CVN014-CVN015. The strings of allele numbers were imported as non-categorical data into BioNumerics 7.6 (Applied Maths, Austin, TX) and a genotype number was arbitrarily assigned to each unique MLVA type.