International Journal of Food Microbiology 137 (2010) 40–48

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Farm-to-fork characterization of *Escherichia coli* associated with feedlot cattle with a known history of antimicrobial use

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ARTICLE INFO

Article history:
Received 2 July 2009
Received in revised form 22 September 2009
Accepted 11 November 2009

Keywords:
Escherichia coli
Antimicrobial resistance
Growth promoter
Abattoir
Cattle

ABSTRACT

This study investigated antimicrobial-resistant (AR) *Escherichia coli* isolated from "farm-to-fork" production of cattle fed diets containing the antimicrobial growth promoter (AGP) chlortetracycline plus sulfamethazine (44 ppm each, AS700) or no AGP (control). For each treatment, samples included: feces just prior to euthanization; hides after euthanization; intestinal digesta from the lower digestive tract; carcasses immediately after evisceration and after 24 h in the chiller; and ground beef stored at 5 °C for 1 and 8 days. Samples were also collected from the abattoir environment and from air during hide removal. Total, ampicillin (Amp^r)-, and tetracycline (Tet^r)-resistant *E. coli* were isolated on MacConkey agar or MacConkey agar containing ampicillin or tetracycline, respectively. Amp^r and Tet^r *E. coli* were isolated from the feces and hides of all cattle. Compared to the control, the prevalence of Amp^r (26.5% *vs.* 7.9%) and Tet^r (50.9% *vs.* 12.6%) *E. coli* was greater in feces from AS700 treated animals ($P<0.05$), but was similar between treatments for hide samples ($P>0.05$). The prevalence of carcass or ground beef contamination with AR *E. coli* was not different between treatments. Resistant *E. coli* were isolated from the abattoir environment after processing of both groups of cattle. Susceptibilities to 11 antimicrobials and pulsed-field gel electrophoresis (PFGE) analyses were conducted on 360 Amp^r and Tet^r *E. coli* isolates. Twenty-five antibiogram profiles were detected, with isolates exhibiting resistance to up to 9 antimicrobials. Most (28.2%) Amp^r *E. coli* were also resistant to streptomycin and tetracycline, whereas Tet^r *E. coli* (53.5%) were mainly resistant to only tetracycline. Thirty one genotypes were detected by PFGE with most isolates from meat and environmental samples having similar genetic profiles to isolates from hides or digesta. These data demonstrate that antimicrobial-resistant *E. coli* can contaminate meat products during slaughter and enter the food chain regardless of whether or not cattle are administered AGP. The abundance of AR *E. coli* on the hides of animals is likely a key element for controlling end-product contamination.

1. Introduction

Antimicrobial growth promoters (AGP) are commonly used in the North American feedlot industry. The use of AGP has the potential to increase the development of, or select for, pathogenic and commensal antimicrobial-resistant (AR) bacteria (Salyers et al., 2004). There is evidence that the AR bacteria can be transferred from livestock to humans (Barton, 2000; van den Bogaard and Stobberingh, 2000), and consequently, concern for human health, as well as consumer and political pressure, prompted the European Union to ban AGP in 1999 (Casewell et al., 2003).

The presence of AR bacteria in food may pose a direct or indirect threat to human health. The detection of AR bacteria in an abattoir (Aslam et al., 2009) and commercial beef products (Schroeder et al., 2003) has been reported. Although proper cooking would eliminate most contaminating bacteria, undercooked meat may act as a vector for exposure of humans to AR bacteria. In one survey, 10% of respondents in the United States consumed undercooked hamburger (Shiferaw et al., 2000). Additionally, improper handling of contaminated raw meats could lead to cross-contamination and subsequent ingestion of AR bacteria. Options for antimicrobial therapy may be limited if AR virulent strains of bacteria are transferred through contaminated meat to humans. Secondary health threats include the transfer of resistance genes from bacteria in food to pathogens or resident bacteria of the human digestive tract (Hammerum and Heuer, 2009).

Despite reports of AR bacteria in beef products, few studies have attempted to determine a direct link between AR bacteria harboured by livestock and those entering the food supply. Studies conducted to date have investigated point contamination in slaughter houses and have focused mainly on pathogenic bacteria or did not investigate the AR profiles of the isolates obtained (Aslam et al., 2003; Gu et al., 2003).

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doi:10.1016/j.ijfoodmicro.2009.11.008

Isolation of AR *Campylobacter* spp., *Salmonella* spp., and *Escherichia coli* from beef carcasses has been reported (Larkin et al., 2006; Fluckey et al., 2007). One study genetically profiled AR *E. coli* in a beef packing plant and found multiple sources of resistant *E. coli*, with few genotypes being shared across contaminating sources (Aslam and Service, 2006). However, none of these studies reported the specifics of AGP use in the cattle being processed, or were able to determine if the use of AGP increased the contamination of beef products with AR bacteria.

E. coli has been shown to exchange genetic material with other bacterial species (Davison, 1999; Blake et al., 2003) and it is therefore possible that this organism may pass antibiotic resistance genes to transient bacterial pathogens that cause disease in humans (Hummel et al., 1986). Previous studies have detected increased levels of AR *E. coli* in feces shed from cattle fed AGP (Alexander et al., 2008; Sharma et al., 2008). We hypothesized that contamination with AR *E. coli* would be higher in carcasses and ground beef from steers fed AGP. The objective of the present study was to investigate if administering AGP to feedlot cattle affected the prevalence of AR *E. coli* contamination of carcass, abattoir environment, or meat products at a commercial abattoir. Antimicrobial susceptibilities and genotyping were used to investigate the degree of relatedness among isolates.

2. Materials and methods

2.1. Animals

Beef cattle were housed in the Lethbridge Research Centre research feedlot (Sharma et al., 2008). Calves originated from a common location and had not received antimicrobial agents subtherapeutically before the initiation of the experiment. Upon arrival at the feedlot, calves were assigned arbitrarily to one of two treatments ($n=50$ each): (1) no antimicrobial agents (i.e. control); and (2) 44 ppm of a mixture of both chlortetracycline and sulfamethazine (Aureo S-700 G, Alpharma Inc., NJ; treatment denoted AS700). AS700 is commonly used in the Canadian beef industry, and was fed at the concentration recommended by the manufacturer. Each treatment was replicated in five pens arranged as a randomized complete block design; each block consisted of a separate pen containing ten steers. Water troughs were shared between adjacent pens, but treatments were arranged in a manner that only cattle in the same treatment group (control or AS700) could drink from the same water troughs.

All of the animals involved in this study were cared for according to the guidelines of the Canadian Council on Animal Care (1993). For a 179 day finishing period, cattle were fed a diet that consisted, on a dry matter basis, of 85% barley grain, 10% barley silage and 5% supplement. Cattle were fed once per day at a level that ensured that all feed allotted to each pen was consumed. AS700 was fed continuously in the diet until 28 days prior to slaughter. At this point, AS700 was removed from the diet in order to ensure compliance with the regulatory withdrawal period (Canadian Food Inspection Agency, 1994). To avoid cross-contamination, the antimicrobial agent was mixed with 5 kg of a supplement containing minerals and vitamins, and the mixture was spread manually over the surface of feed within each of the appropriate pens. All animals in the pen were able to access the feed trough at the same time. Cattle assigned to the control treatment were fed supplement that contained no antimicrobial agents.

Fifteen Angus-cross steers from the control and 15 from the AS700 treatments were randomly selected for sampling in the abattoir. In total, three steers were processed from each of the five replicate pens per treatment. Steers were transported to the abattoir, a distance of 30 km, on the evening prior to euthanization. Control animals were transported on July 17th and slaughtered on July 18th, and AS700 animals were transported on July 18th and slaughtered on July 19th. Following transport of the control animals, the stock trailer was thoroughly cleaned using a high pressure washer with water heated to 60 °C. At the abattoir, steers were housed in clean pens with a cement floor and maintained on a barley silage diet. Steers were kept separate from animals outside the respective treatment groups and were euthanized humanely according to the Canadian Council on Animal Care (1993).

2.2. Abattoir

The abattoir was a provincially inspected facility of moderate capacity located in the province of Alberta. Standard sanitation procedures for the "slaughter floor" at the end of the day included: (1) removal of all edible offal and trimmings; (2) pre-rinsing all equipment, knock box, walls, leg bench, viscera tub, inspection tray, cradle, scale, splitting saw, brisket saw, gutting stand, doors, door handles and head rack with warm water (i.e. >32 °C); (3) removal and rinsing of floor gates; (4) pumping of blood pit; (5) scraping and discarding of excess material from the floor; (6) treatment of all equipment and surfaces with HydroChem foam (HydroChem Industrial Services, Inc., Deer Park, TX) for a minimum of 15 min; (7) further hand washing of all foamed equipment, inspection tray, and brisket saw; (8) rinsing all foamed equipment and surfaces with hot water (starting at the top and working down, and washing the floors in a direction toward the centre floor drain); (9) application of a 12% bleach solution (0.6% sodium hypochlorite) to all equipment and surfaces with a minimum exposure time of 5 min, followed by final rinsing with hot water; and (10) application of the quaternary ammonium product, Quatromycide (Dustbane Products Ltd., Ottawa, ON, Canada), at a dilution of 500:1 to all surfaces and equipment. This procedure was fully implemented before the commencement of the study and between the euthanizations of the control steers on July 18 and the AS700 steers July 19.

2.3. Sampling

2.3.1. Feces

On July 15, 2005 (i.e. just before transport to the abattoir), fecal samples were obtained from each of the 30 animals by rectal sampling.

2.3.2. Abattoir environment

Five environmental samples were obtained from the abattoir prior to the commencement of and immediately after slaughter of the fifteen animals processed on each day by swabbing with sterile 2 cm × 4 cm cellulose acetate sponges (Nasco Canada, Newmarket, ON) moistened in Columbia broth (BD, Franklin Lakes, NJ). Samples were obtained from the: (1) hydraulic gate of the knock box; (2) blade of the splitting saw; (3) wall immediately behind the inspection area; (4) viscera tub; and (5) wall adjacent to the rendering room entrance.

2.3.3. Hides and carcasses

Within 5 min of euthanasia, swab samples were obtained from the surface of the hide and carcasses of all steers. A 900 cm^2 area of the brisket and rump was swabbed with a cellulose acetate sponge moistened in Columbia broth. The sampling area (i.e. 30 × 30 cm) was delineated using a sanitized wire frame. Carcasses were sampled immediately after evisceration and breaking, and after having hung for 24 h in a chiller held at 6 °C. After evisceration and breaking, the carcass was sprayed with warm water (38 to 43 °C) according to the standard operating procedures of the abattoir. Following washing, the brisket and rump of the right side of the carcass were sampled before placement of the carcass in the chiller. After 24 h in the chiller, the brisket and rump of the left side of the carcass were swabbed as described above.

2.3.4. Air

Air samples were obtained during hide removal from each steer. An inertial air sampler (MAS-100; EMD Chemicals Inc., Gibbstown, NJ)