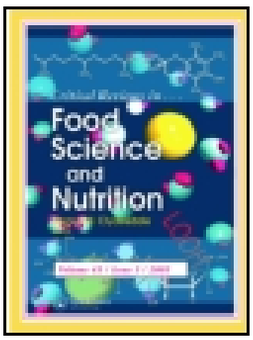

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# Effect of Antimicrobial Use in Agricultural Animals on Drug-resistant Foodborne Campylobacteriosis in Humans: A Systematic Literature Review

M.A. McCrackin[1,2], Kristi L. Helke[2], Ashley M. Galloway[3], Ann Z. Poole[3], Cassandra D. Salgado,[4] Bernadette P. Marriott[3,*]

[1]Ralph H. Johnson VA Medical Center Research Service, Charleston, SC, USA 29401

[2]Department of Comparative Medicine, Professor, College of Medicine, Medical University of South Carolina, Charleston, SC, USA 29425

[3]Nutrition Section, Division of Gastroenterology and Hepatology, Department of Medicine, and Military Division, Department of Psychiatry, College of Medicine, Medical University of South Carolina, Charleston, SC, USA 29425

[4]Division of Infectious Diseases, Department of Medicine, College of Medicine, Medical University of South Carolina, Charleston, SC, USA 29425

[*]**Corresponding author's contact information:** Bernadette P. Marriott, Ph.D.**,** Professor and Director, Nutrition Section**,** Division of Gastroenterology and Hepatology**,** Department of Medicine and**,** Military Division, Department of Psychiatry and Behavioral Sciences**,** 114 Doughty Street STB 630D, MSC 7740, Charleston, SC, 29425-7740

**Last names listed as they should be indexed in PubMed:** McCrackin; Helke; Galloway; Poole; Salgado; Marriott



**ABSTRACT**

Controversy continues concerning antimicrobial use in food animals and its relationship to drug-resistant infections in humans. We systematically reviewed published literature for evidence of a relationship between antimicrobial use in agricultural animals and drug-resistant foodborne

campylobacteriosis in humans. Based on publications from the United States (U.S.), Canada and Denmark from 2010 to July 2014, 195 articles were retained for abstract review, 50 met study criteria for full article review with 36 retained for which data are presented. Two publications reported increase in macrolide resistance of *Campylobacter coli* isolated from feces of swine receiving macrolides in feed, and one of these described similar findings for tetracyclines and fluoroquinolones. A study in growing turkeys demonstrated increased macrolide resistance associated with therapeutic dosing with Tylan® in drinking water. One publication linked tetracycline-resistant *C. jejuni* clone SA in raw cow's milk to a foodborne outbreak in humans. No studies that identified farm antimicrobial use also traced antimicrobial-resistant *Campylobacter* from farm to fork. Recent literature confirms that on farm antibiotic selection pressure can increase colonization of animals with drug-resistant *Campylobacter* spp. but is inadequately detailed to establish a causal relationship between use of antimicrobials in agricultural animals and prevalence of drug-resistant foodborne campylobacteriosis in humans.

**Keywords:**

food safety, tetracycline, macrolides, quinolones, farm-to-fork, meat

***INTRODUCTION***

Antimicrobial resistance is a global threat that has received targeted national attention (CDC, 2013) and government action in the United States (U.S.)(PCAST, 2014). The Centers for Disease Control and Prevention (CDC) estimates that two million illnesses and 23,000 deaths are caused each year by antimicrobial-resistant bacteria and fungi domestically (CDC, 2013). Of the antimicrobial-resistant bacteria, the CDC has identified *Campylobacter* as a pathogen of concern associated with foodborne illness in the United States (CDC, 2013). Domestically acquired, foodborne campylobacteriosis is estimated to account for 845,024 illnesses, 8,463 hospitalizations, and 76 deaths annually, with approximately 49% of laboratory-confirmed *Campylobacter* isolates exhibiting resistance to tetracyclines, 22% to quinolones and 2% to macrolides (CDC, 2015). These drug classes have been in past or current use in food animal production for one or more of the following purposes: growth promotion, disease prevention, treatment or control. Quinolones and macrolides are categorized by the World Health Organization as critically important antimicrobials for use in human medicine (CDC, 2015), so there is concern about resistance of *Campylobacter* to these drug classes and whether their use in food animal production may contribute to resistance.

*Campylobacter* is a motile, gram negative, microaerophilic and thermophilic bacterium that typically causes self-limiting gastroenteritis in humans linked to the consumption of undercooked contaminated chicken (CDC, 2013) or from cross-contamination of other foods prepared in the same area as the chicken. In recent years, raw milk and foods prepared with cheese made from unpasteurized milk, such as queso fresco, have been increasing as sources of foodborne campylobacteriosis (IFSAC, 2015; Mungai, *et al.*, 2015). We focused on three species

of *Campylobacter: jejuni*, *coli* and *lari*. In high income economies, most human infections are caused by *C. jejuni*. *C. jejuni* is most frequently recovered from poultry and cattle while *C. coli* is commonly identified in swine (USDA, 2014). *C. lari* is uncommonly found in humans (CDC, 2015) and animals (USDA, 2014). In agricultural animals, colonization of the intestinal tract with *C. jejuni*, *C. coli* or *C. lari* is usually asymptomatic, and the animals serve as healthy reservoirs. In recent years, a highly pathogenic, abortifacient and tetracycline-resistant *C. jejuni* clone, called clone SA for *s*heep *a*bortion, was identified (Sahin, *et al.*, 2008) and has been responsible for sheep and cattle abortions in the U.S. and human campylobacteriosis linked to raw cow's milk (Sahin, *et al.*, 2012).

Campylobacteriosis is a worldwide zoonosis known to affect humans of all ages; however, during illness, the organism is isolated from children more often than adults. Symptoms of campylobacteriosis can range from mild diarrhea to a more systemic illness of bloody diarrhea, malaise, fever and abdominal cramps. Illness typically begins two to five days after exposure to the organism and lasts for about one week. Definitive diagnosis is made by culturing the organism from the diarrheal stool. Most patients will recover, but sometimes serious post-infectious complications occur such as arthritis or temporary paralysis known as Guillain-Barré Syndrome (GBS). The CDC estimates that one of every 1,058 cases of *C. jejuni* infection is followed by GBS and that up to 40% of all GBS cases in the U.S. are associated with campylobacteriosis (Nachamkin, *et al.*, 1998).

In most cases of human infection due to *Campylobacter*, recovery occurs with symptomatic treatment (rest and hydration), but for patients with severe disease or for those who may be at high risk for serious complications, such as those with compromised immune systems,

antimicrobials are recommended. Macrolides and fluoroquinolones have been the mainstays of therapy, but resistance to these antibiotics, particularly fluoroquinolones such as ciprofloxacin, is now common, limiting treatment options (Luangtongkum, *et al.*, 2009). Macrolides and quinolones have been used in the production and veterinary care of agricultural animals, and there is concern that continued use of these drug classes will increase the prevalence of antibiotic-resistant *Campylobacter* in animal-derived food products and, therefore, antibiotic-resistant human campylobacteriosis. Bacterial contamination may occur at multiple points throughout the food production chain, including on farm, during slaughter, processing, packaging, distribution, preparation and consumption, making foodborne illness difficult to control. Although the CDC has several surveillance systems in place to detect and prevent foodborne illness and outbreaks, the incidence of foodborne illness overall has remained unchanged since 2010 (CDC, 2014).

This review aimed to systematically examine what is and is not known about the relationship between the use of antimicrobials in food animal production and the emergence and spread of domestically acquired, foodborne antimicrobial-resistant *Campylobacter* from 2010 through July, 2014.

## *METHODS*

### *Research Question and Approach*

Specifically, this review addressed the question: "Is there evidence from the literature that antimicrobial use in food animals is directly or indirectly involved in the emergence and spread of foodborne antibiotic-resistant *Campylobacter*?" The review included all published research studies conducted in North America [the U.S. and Canada] and Denmark; Denmark was included

due to its long standing practice of restricting use of antibiotics in food animal production. The project was initiated in August 2014, so the search time frame ended July 31, 2014. Other search parameters were limited to English, antibiotic resistance, *Campylobacter* and food animal production. The search was narrowly focused on domestically acquired, foodborne illnesses associated with products from agricultural animals, so travel-associated diarrhea, direct animal contact, pet, plant and environmental contributors to human campylobacteriosis cases were excluded. The literature review process consisted of three phases: Phase 1: Title and Abstract Review; Phase 2: Full Article Review; and Phase 3: Final Library Creation.

An evidence-based approach was employed for collecting, reviewing and synthesizing the literature that was modeled after the processes used by the Agency for Health Care Research and Quality Evidence-based Practice Centers (EPC) (AHRQ, 2014). As such, a stepwise, systematic approach was used for literature inclusion, exclusion, data management, and evidence table presentation. The standard EPC approach was tailored for the task by including a mix of multidisciplinary, highly experienced scientific professionals who were knowledgeable in the specific areas of the review and by employing the evidence grading approach recommended by the Grades of Recommendation, Development and Evaluation (GRADE) Working Group (Guyatt, *et al.*, 2011) (**Table 1**). This structured review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidance (Liberati, *et al.*, 2009).

The team defined search parameters, inclusion and exclusion criteria and engaged in a bias discussion at the beginning of the project. All team members voiced that they could review and evaluate the literature on this topic fairly, disclosed no conflicts of interest, and signed a statement to this effect. No team members expressed opposition to the use of animals for food.

***Search and Review Process***

The SCOPUS[1] database, which incorporates PubMed citations and includes international publications, was selected for this review. A preliminary search was conducted to identify the depth and breadth of information available that limited the search parameters to English language, publication years 2005-2014, and document types: journal article, review, conference paper, article in press or conference review. Based on the number of relevant articles identified in the preliminary review, the final document types were limited to published articles and articles in press from January 2010 through July 2014, review articles and position statements were excluded, and searches in the AGRICOLA database were added to identify more agricultural studies. A secondary search was conducted in the SCOPUS and AGRICOLA databases using the search string "human AND health AND agriculture* AND resistance*" to identify any pertinent publications on antibiotic resistance not specific to *Campylobacter*. The preliminary and final search terms are shown in **Table 2**.

For the title/abstract screening step of the review, the predetermined exclusion criteria, which resulted in articles being excluded from further review, included if the research article focus was: 1) not on antibiotic resistance, 2) on domesticated pets or their environment, 3) on plants and not on agriculture, 4) on experimental treatment of animals only, 5) not based in the U.S., Canada or Denmark, 6) a review article rather than primary literature or there was no abstract present, or 7) other. If the "other" category was selected, the reviewer was required to specify the reason for rejection.

[1] SCOPUS©, Elsevier, B.V., http://www.elsevier.com/online-tools/scopus

 

For all citations for which full article review was conducted, information was systematically documented on the type of article (case study, observational/longitudinal, prevalence/incidence of disease, basic science, government report, other); animal species focus (n/a, beef, dairy, swine, poultry-all, fish/seafood, wild game, human, other); food processing (n/a, abattoir, processing/packaging, preparation); product (n/a, meat, milk, cheese, yogurt, other dairy, eggs, other) and antibiotic use (not mentioned, no, human, animal, laboratory/treatment, control, prevention, growth, resistance measured, other). The reviewers also summarized the article by recording the study duration, sample type, sample size, methods, results, and conclusion. The reviewer gave each publication a grade, based on the criteria in **Table 1**, and provided specific comments related to the rationale for the grade. One team member conducted the searches and compiled the results, four performed title/abstract screening and two conducted full article reviews. To manage the literature, provide transparency of the review process, and rapid access to the data, all literature search results and reviews were captured in custom forms created using EndNote® x7 reference manager software[2]. The EndNote® reviewer form for each article included the review elements for both title/abstract screening (inclusion; if exclusion, reasons) and full article review (study purpose, type of article, species focus, food processing, product, antibiotic use, study duration, study sample type and size, method summary, results, conclusion and grade).

***Applying the GRADE System***

Each article receiving full article review was graded on the basis of quality, directness and consistency based on the GRADE approach for grading quality of evidence developed by the

[2] EndNote® x7, Thomson Reuters, http://endnote.com/product-details/X7

GRADE Working Group (Guyatt, *et al.*, 2011). The GRADE approach has been adopted by the Cochrane Collaboration for evaluating quality of evidence reported in systematic reviews in human research. We could not identify a similar grading system that had been used for animal research; therefore, we adapted GRADE for this review. In our review, two scores were given for quality, one based on evidence of statistical analysis and one based on the probability of bias or study design limitations. The directness score reflected whether the methods and results presented were clear and straightforward. The consistency score reflected whether the results and conclusions presented appeared to be consistent with the methods. Combining the three scoring categories, each article could receive a maximum grade of (+) four and a minimum grade of (-) six (**Table 1**).

***RESULTS***

***Search Results, Screening and Review***

The primary SCOPUS and AGRICOLA searches yielded 621 records and the secondary search and other sources 46. After removing 118 duplicates, 549 publications remained. The scope of the project was narrowed to literature from 2010 through July 2014, resulting in 195 articles for title/abstract screening. Of these, 50 met the study criteria and received full article review. An additional 14 journal articles were excluded during full review, leaving 36 for which data are presented in **Figure 1, Tables 3, 4 and 5**. Of these, 11 earned a GRADE quality score of three or four, 13 a score of zero to two, and 12 a score below 0 (**Tables 4 and 5**).

***Article Characteristics***

***Type of article***

Articles were assigned to one or more of the following categories: observational or longitudinal (25), prevalence or incidence of disease (18), basic science (7), case studies (5), and other unique publications, including one mathematical model risk analysis and one survey-based study.

***Species Focus***

Most articles focused on a single animal species, including poultry (15), swine (6), cattle (beef or dairy) (5), human (2) and seafood (1). Of the seven remaining articles, six included more than one animal species and one focused on animal feed. No publications about farmed fish were identified that matched the search criteria.

***Food Processing***

Articles were categorized based on whether samples were collected along the slaughter to retail product chain, including slaughterhouse (abattoir), food processing or packaging, and preparation (retail products). Of the 36 articles retained after full review, eight included samples from slaughterhouses, five described processing or packaging, and four sampled retail products ready for preparation by consumers. Four of these 17 articles addressed two categories, three including both abattoir and food processing or packaging (Logue, *et al.*, 2010; Quintana-Hayashi, *et al.*, 2012; Tadesse, *et al.*, 2011) while one collected samples from both abattoir and retail products (Sahin, *et al.*, 2012). None of the publications receiving full review included all three steps of the meat processing journey from slaughterhouse to retail stores.

***Product***

Food products analyzed in each article were recorded. Sixteen articles did not study food products, 17 sampled meat or meat products, two organ meats and one each milk, packaged seafood and animal feed.

***Antibiotic Use***

Specific details of antibiotic usage in animals or humans were not recounted in 21 of the 36 articles that received full review. Of the remaining 15, ten publications mentioned treatment of animals with antibiotics, four did not use antibiotics and one article detailed antibiotic use in humans. Of the ten articles that cited antibiotic use in animals, seven indicated use for treatment purposes, five for prevention, three for growth promotion and two for disease control. Five of these papers included two or more antibiotic use categories. Fifteen of 36 articles contained analyses of antibiotic resistance.

***Laboratory Methods***

***Culture and Isolation Techniques***

Bacterial culture and isolation techniques employed varying enrichment methods and sample collection strategies to improve success in growing fastidious *Campylobacter*. Some fecal samples were collected from the ground, others from the rectum of the animals and still others directly from the intestines at slaughter. Several studies mentioned using swabs for undefined sampling areas when collecting environmental or hide samples while others defined surface areas and locations and used larger surface area sponges or similar to increase the likelihood of success.



 

Archived frozen stocks of *Campylobacter* can be difficult to resuscitate from the frozen state, so the number of isolates in manuscripts sometimes changed between initial prevalence calculations and antimicrobial susceptibility or molecular testing.

***Antimicrobial Susceptibility Testing***

Antimicrobial susceptibility testing methods were variable, in part because available technology changed over time. The USDA animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) used a commercially available testing method for measuring minimum inhibitory concentrations MIC called Etest®[3] from 1998-2004. In 2005, the Sensititre® broth microdilution system[4] was established as the standard for NARMS testing (USDA, 2014). Different researchers used different panels of antibiotics ranging from four (Rao, *et al.*, 2010) to 16 (Noormohamed, *et al.*, 2012, 2013), and some antibiotics used for antimicrobial susceptibility testing were antibiotics either used in the selection media or to which *Campylobacter* exhibits intrinsic resistance (Deckert, *et al.*, 2010).

Most authors followed standardized guidance for antibiotic resistance break points and interpretation, when available, but guidance evolved for *Campylobacter* over the past 15 years. Resistance break points changed over this period, with the most recent change in NARMS methodology being the use of epidemiological cut-off values (ECOFFs) rather than break points beginning with the 2012 retail meat and human isolates annual reports (CDC, 2015; FDA, 2015b). This resulted in an interpretation of susceptible or resistant with no intermediate category.

[3] AB Biodisk; Solna, Sweden
[4] Trek Diagnostic Systems, Inc., Thermo Fisher Scientific; Oakwood Village, OH

***Definition of Multidrug Resistance***

A standardized definition for multidrug resistance (MDR) does not appear to exist in the *Campylobacter* literature, and we found that some authors defined MDR as resistance to ≥ 2 (Zhao, *et al.*, 2010), and some ≥ 3 (Noormohamed, *et al.*, 2013; Quintana-Hayashi, *et al.*, 2012), drugs. Despite differing definitions, there was a lower prevalence of MDR in *C. jejuni* than *C. coli*, consistent with recent NARMS reports for animal and meat reports (FDA, 2015b; USDA, 2014).

***Prevalence of Campylobacter***

***Poultry - Chicken***

*C. jejuni* was the only strain (100%) of *Campylobacter* isolated from feces of organic chickens in Quebec, Canada (Thibodeau, *et al.*, 2011) and commercial broilers from large-scale production units in North Carolina, U.S. (Thakur, *et al.*, 2013), with overall prevalences of 50% and 30%, respectively. In Denmark, the yearly mean prevalence of *Campylobacter* on post-chill broiler meat was determined to be 54.2% for organic and 19.7% for conventional production methods, resulting in 1.7 times higher risk from the organic than the conventional products (Rosenquist, *et al.*, 2013). Prevalence of *Campylobacter* contamination of broiler meat post-chill was also studied in chickens from small-scale pasture-raised production settings and processed using one of three methods: on-farm, small USDA-inspected facilities (USDA-IF), or mobile processing unit (MPU) (Trimble, *et al.*, 2013). Prevalence ranged from 70% on-farm to 82% USDA-IF to 100% MPU; a statistically significant difference was not found among processing methods.

Of *Campylobacter* cultured from retail packaged chicken meat, *C. jejuni* accounted for the following percentages of total isolates recovered: 90% from fresh chicken legs and thighs in Ontario, Canada (Deckert, *et al.*, 2010), 72% from fresh skin-on chicken breasts from CDC Foodborne Diseases Active Surveillance Network (FoodNet) sites in the U.S. (CA, CO, CT, GA, MD, MN, NM, NY, OR, TN) (Zhao, *et al.*, 2010), and 63% from chicken breasts in Iowa, U.S. (Thakur, *et al.*, 2010). *C. coli* comprised 9%, 28% and 37% of the total *Campylobacter* isolates from retail chicken meat, with *C. lari* identified 1%, 0.1% and 0%, respectively. The overall prevalences of *Campylobacter* in these studies of retail chicken meat were 60% (Deckert, *et al.*, 2010), 50% (Zhao, *et al.*, 2010) and 84% (Thakur, *et al.*, 2010). A survey of retail chicken livers and gizzards in Oklahoma, U.S. reported an overall prevalence of 67% for *Campylobacter*, with 51% of isolates identified as *C. jejuni* and 49% *C. coli* (Noormohamed, *et al.*, 2012).

***Poultry -- Turkey***

Two studies conducted in the U.S. examined retail ground turkey for the presence of *Campylobacter*. An overall prevalence of 8% was reported from Iowa, with 27% of the isolates identified as *C. jejuni* and 73% as *C. coli* (Thakur, *et al.*, 2010). Data from samples collected from FoodNet sites revealed an overall prevalence of 1.6%, with 56% of isolates speciated as *C. jejuni* and 41% as *C. coli* (Zhao, *et al.*, 2010).

***Cattle***

In cattle, the most common strain of *Campylobacter* recovered varied among reports. *C. jejuni* was predominant in feedlot cattle with a prevalence of 55% (Rao, *et al.*, 2010). *Campylobacter* in dairy cattle demonstrated an overall prevalence of 37%, with 92% of these isolates identified as *C. jejuni* (Sanad, *et al.*, 2013). Cattle slaughtered for meat consumption in

various regions of the U.S. had an overall prevalence of *Campylobacter* of 19% in 181 collected fecal samples (Sanad, *et al.*, 2011). Since more than one isolate was recovered from some fecal samples, the distribution of strains among 213 isolates was 14% *C. coli* and 8% *C. jejuni*.

Among retail beef samples, prevalence of *Campylobacter* varied as well, ranging from 0% in beef cuts (steak, stew, shoulder, bone in) (Noormohamed, *et al.*, 2013), < 0.5% in ground beef (Zhao, *et al.*, 2010), 1% in grain-fed veal (*C. jejuni*) (Cook, *et al.*, 2011) to 78% in beef liver (68% *C. coli*, 32% C. *jejuni*) (Noormohamed, *et al.*, 2013).

***Swine***

*C. coli* was the predominant strain of *Campylobacter* (95-99%) isolated from fresh feces of swine in two articles comparing conventional to antimicrobial-free (ABF) production systems in North Carolina, U.S. (Quintana-Hayashi, *et al.*, 2012) and in two Midwestern regions of the U.S. (Ohio and Michigan, Wisconsin and Iowa) (Tadesse, *et al.*, 2011). Tadesse et. al. found a *Campylobacter* prevalence range of 2.9-100% for conventional and 0-72% for ABF farms with an overall prevalence of 56%. Ninety-five percent of these isolates were *C. coli*. Quintana-Hayashi et. al. found 71% of conventional and 51% of ABF sows positive for *Campylobacter*. Interestingly, the prevalence of *Campylobacter* in pigs reared from these sows was 67% for conventional and 73% for ABF farms (Quintana-Hayashi, *et al.*, 2012). There was no statistically significant difference detected in prevalence between production methods.

Despite a statistically significant higher prevalence of *Campylobacter* in ABF carcasses (73%) compared to conventional (28%) at postevisceration (Quintana-Hayashi, *et al.*, 2012), post chill prevalence of *Campylobacter* was equally low for conventional and ABF pork, 1.6 percent

or less in one study (Quintana-Hayashi, *et al.*, 2012) and undetectable in another (Tadesse, *et al.*, 2011).

Among retail pork samples, prevalence of *Campylobacter* was extremely low, varying from < 0.5% (Zhao, *et al.*, 2010) to 2% (Noormohamed, *et al.*, 2013).

***Seafood***

*C. jejuni* was reported once in a survey of retail seafood available in grocery stores in Louisiana (Y. Wang, *et al.*, 2014). Retail samples collected in this study were imported yet reviewed here since imported seafood represents about 80% of retail seafood sold in the U.S. each year (Y. Wang, *et al.*, 2014).

***Antibiotic Resistance***

***Poultry***

*Campylobacter* isolated from broiler chickens in North Carolina, U.S. (Thakur, *et al.*, 2013) and organically raised chickens in Quebec, Canada (Thibodeau, *et al.*, 2011) demonstrated resistance to tetracyclines, with prevalences of 56% and 44%, respectively. The organic chicken showed low prevalences of resistance to erythromycin (6%), azithromycin (6%) and clindamycin (2%) (Thibodeau, *et al.*, 2011).

In retail poultry, resistance rates were similar to those reported for live birds. The most common antibiotic resistance detected in *Campylobacter* isolated from chicken breasts was against tetracyclines, with reported rates of 37-57% (Deckert, *et al.*, 2010; Thakur, *et al.*, 2010; Zhao, *et al.*, 2010). Resistance to fluoroquinolones was also common in the U.S., with rates of 19% (Thakur, *et al.*, 2010) and 17% for ciprofloxacin (Zhao, *et al.*, 2010) and 19% for nalidixic

acid (Zhao, *et al.*, 2010). This contrasted with a report from Canada showing *Campylobacter* resistance to ciprofloxacin and nalidixic acid to be 1.9% (Deckert, *et al.*, 2010).

Despite *C. coli* causing human disease much less frequently than *C. jejuni*, it consistently more often displayed multi-drug resistance. *C. coli* exhibited higher rates of resistance compared to *C. jejuni*, with the exception of doxycycline/tetracycline (Zhao, *et al.*, 2010). The proportion of *C. coli* isolates resistant to clindamycin and erythromycin was significantly higher compared to *C. jejuni* in a Canadian study (Deckert, *et al.*, 2010). Multi-drug resistance was seen more commonly in *C. coli* with ciprofloxacin and erythromycin resistance reported as 29% and 16% compared to *C. jejuni* with 19% of isolates resistant to ciprofloxacin (Thakur, *et al.*, 2010). *C. coli* also showed higher rates of multi-drug resistance when isolated from chicken organ meats (Noormohamed, *et al.*, 2012).

One article from Canada described an increasing prevalence of ciprofloxacin resistance in *Campylobacter* isolated from retail chicken, with an overall prevalence of *Campylobacter* in 2009 in British Columbia of 53% with 29% resistance to ciprofloxacin. They also noted that most ciprofloxacin-resistant isolates (64%) were concomitantly resistant to tetracycline (Agunos, *et al.*, 2013).

***Cattle***

Resistance to tetracyclines was the most commonly identified antimicrobial resistance in *Campylobacter* isolated from fresh cattle feces, with resistance rates recorded from 38% in feedlot cattle in Alberta, Canada (Rao, *et al.*, 2010) to 68% in beef (Sanad, *et al.*, 2011) and 73% in dairy (Sanad, *et al.*, 2013) cattle in the U.S. Retail beef liver was shown to have both *C. coli* and *C. jejuni* commonly recovered, with *C. coli* demonstrating higher levels of drug resistance

than *C. jejuni* to tetracycline (97% *C. coli* vs 73% for *C. jejuni*), ciprofloxacin (62% vs 39%) and multi-drug resistance (62% vs 39%) (Noormohamed, *et al.*, 2013).

***Swine***

On both conventional and ABF swine farms, *C. coli* demonstrated tetracycline resistance most commonly, reported as a combined overall prevalence of 65% in one study covering four U.S. states in two geographic regions (Tadesse, *et al.*, 2011). Another study found tetracycline resistance in 88% of conventional versus 48% of ABF pigs (Quintana-Hayashi, *et al.*, 2012), a statistically significant difference between production management systems.

The overall prevalence of *C. coli* (67-73%) was not statistically different among pigs at farrowing, nursery, and finishing compared to their environment when raised on conventional compared to ABF farms (Quintana-Hayashi, *et al.*, 2012). However, these authors found statistically significant associations between exposure to antibiotics provided in feed on conventional farms and antibiotic resistance characterized in *C. coli* isolated from these pigs, including oxytetracycline use and tetracycline resistance (nursery pigs), tiamulin use and azithromycin and erythromycin resistance (nursery, finishing), and enrofloxacin exposure and resistance to ciprofloxacin and nalidixic acid (farrowing) (Quintana-Hayashi, *et al.*, 2012). In another study, *C. coli* prevalence (54-59%) did not vary significantly between pigs raised on conventional compared to ABF farms, but *Campylobacter* isolates were more likely to show resistance to erythromycin when they originated from conventional farms (Tadesse, *et al.*, 2011). A relationship between production system and fluoroquinolone resistance was not demonstrated in that study.

***Farm Environment***

Broiler house litter (Roberts, *et al.*, 2013) and animal feed ingredients (Ge, *et al.*, 2013) were not implicated as environmental reservoirs for *Campylobacter.* In fact, in both of these studies, *Campylobacter* was not isolated. In contrast, in the ABF swine farm environment (water, feed, soil, drag swabs of floors and structures), the resistance to some antimicrobials (azithromycin, erythromycin, and clindamycin) was higher than that found in the animals (Quintana-Hayashi, *et al.*, 2012).

Two articles attempted to show evidence of pathogenic bacteria or resistance genes in poultry house bioaerosols (Just, *et al.*, 2012) and human nasal colonization or exposure to antimicrobial resistance genes in swine barn bioaerosols (Létourneau, *et al.*, 2010) in Canada. The poultry house study used solely non-culturing molecular techniques, so it was not possible to determine from the data whether viable *Campylobacter* were associated with the findings (Just, *et al.*, 2012). The authors detected *Campylobacter* only in floor-housed poultry operations where antibiotics were commonly used compared to caged housing operations. Prevalence of antibiotic resistance genes was also higher in floor-housed operations. However, no disease in humans nor horizontal transfer among humans was demonstrated, so it was not clear if the presence of either *Campylobacter* or antibiotic resistance genes held practical implications for zoonotic transmission. In the swine barn bioaerosol project, *Campylobacter* was found in the nasal flora of four workers, and genes capable of conferring tetracycline resistance, including *tetA/tetC*, *tetG*, and ribosomal protection protein gene, were detected in all 18 buildings in the study (Létourneau, *et al.*, 2010). Of the organisms studied, *Campylobacter* was identified the least often. As in the poultry study, no disease in humans nor horizontal transfer among humans was demonstrated, and the duration of suspected nasal colonization was not addressed.

***Humans***

A highly virulent strain of *Campylobacter*, *C. jejuni* clone SA, was first reported in 2008 as an agent of sheep abortion in the U.S. and underwent extensive molecular studies that were published in 2012 (Sahin, *et al.*, 2012). *C. jejuni* clone SA was isolated from cattle, sheep, and goat abortions, and in recent years from both humans with campylobacteriosis and raw milk. Exact pulsed-field gel electrophoresis (PFGE) matches were found between tetracycline-resistant *C. jejuni* clone SA from sheep, 67 human isolates from outbreaks available in the database of PulseNet USA, and two isolates from raw milk purchased from the dairy farm in Pennsylvania, U.S. implicated in the 2008 outbreak from which 14 of the human samples also originated. Multi-locus sequence typing (MLST) was also done and showed related sequence types among the human, animal and milk isolates.

The importance of the pre-existing health and antimicrobial history of humans exposed to *Campylobacter* was the subject of one publication. Koningstein et. al. characterized fluoroquinolone exposure in humans as a factor conferring increased risk (odds ratio = 2.4) for contracting campylobacteriosis when antimicrobial use occurs within the year prior to *Campylobacter* exposure (Koningstein, *et al.*, 2011). The odds were even higher (3.8) for acquiring fluoroquinolone-resistant *Campylobacter* when a history of fluoroquinolone exposure was present.

***Fitness***

Two articles received full review that dealt with the ability of macrolide-resistant *Campylobacter* to replicate in poultry. One study used as its subjects broad breasted white turkeys in a single flock of approximately 30,000 with half of the birds treated with Tylan®

(tylosin tartrate), a macrolide, in the drinking water during three separate dosing periods. About 50% of isolates from the treated group tested highly resistant to erythromycin, exhibiting a minimum inhibitory concentration of > 256 µg/ml (break point ≥ 32 µg/ml) and with half of the isolates identified as *C. coli* (Logue, *et al.*, 2010). In addition, during the break periods between Tylan® treatments, in the absence of antibiotics, the prevalence of macrolide resistance decreased dramatically. In the second study, newly hatched broiler chicks were used to test the *in vivo* fitness of *Campylobacter* with and without erythromycin resistance-conferring mutations in 23S rRNA. Thus, *Campylobacter* strains that differed only by introduced mutations to cause erythromycin resistance competed to colonize the guts of broiler chicks. The erythromycin-sensitive strain consistently out-performed the resistant strains, suggesting that carrying the resistance mutations weakens the ability of *Campylobacter* to replicate and survive effectively in the host (Luangtongkum, *et al.*, 2012). In this study, the absence of antibiotic selection pressure alone did not correlate with reversion of erythromycin-resistant *C. jejuni* to drug sensitivity. The presence of erythromycin-sensitive *C. jejuni* strains was required in order for the chicks to be recolonized with sensitive strains and for resistant strains to be displaced from the enteric flora.

***Genetics***

The gene conferring tetracycline resistance in *Campylobacter*, *tet*(O), is most commonly found on plasmids that can be horizontally transferred to other bacteria and was recently reported in a chromosomal location for the first time in *C. coli* isolates from turkey drinking water and a swine fecal sample (Crespo, *et al.*, 2012). The *tet*(O) gene was determined to be carried on a chromosomal transposable unit (transposon; IS*605*) that was inserted into the *C. jejuni*-associated citrate transporter gene and likely subsequently acquired by *C. coli*. These genetic

elements may be the same ones that provide tetracycline resistance to the *C. jejuni* clone SA responsible for recent sheep, goat and cattle abortions and raw milk-perpetuated outbreaks in humans. The implication of the genetic location of this resistance gene is the stability of chromosomal location and assurance of replication from generation to generation compared to a plasmid.

Fluoroquinolone resistance is typically a result of a mutation in the *gyrA* gene. In *Campylobacter*, this single mutation caused high level resistance to both ciprofloxacin and nalidixic acid (Deckert, *et al.*, 2010). Expression of the multi-drug efflux pump gene, *CmeABC*, may also confer fluoroquinolone resistance. Quinolone resistance continues to be a concern, and the 2011 NARMS Retail Meat Annual Report showed slowly increasing resistance to ciprofloxacin (14.5% in 2003 to 22.4% in 2011) and nalidixic acid (15.1% in 2004 to 20.9% in 2011) in *C. jejuni* isolated from retail chicken despite discontinuation of quinolones in poultry in the U.S. in 2005.

Gentamicin resistance was identified in *C. coli* isolated from U.S. retail chicken and human isolates in 2007, and its prevalence increased, peaking in 2011 (CDC, 2015; Chen, *et al.*, 2013; FDA, 2015b). Whole genome sequencing allowed the identification of a novel self-transmissible plasmid-mediated resistance mechanism resulting from a phosphotransferase gene, *aph(2")-Ig* (Chen, *et al.*, 2013). Single nucleotide polymorphisms showed that the *C. coli* isolates carrying this resistance gene clustered with other poultry isolates, separate from livestock.

***DISCUSSION***

Our search parameters and reviews were designed to determine if recent published evidence was available that demonstrated a direct relationship between the use of antibiotics in

agricultural food animal production and antibiotic-resistant campylobacteriosis in humans. The search we conducted found no longitudinal studies that collected data along the entire food production pathway, including details about specific antibiotic usage on farms, identification of *Campylobacter* isolates from farm to retail packaging, and direct linkage to human disease. While conclusive evidence of a causal relationship between use of antibiotics in food animals and emergence of drug-resistant *Campylobacter* was not obtained, our search findings informed us of important concerns related to *Campylobacter* and possible nodes to focus efforts for improvements in food safety.

The literature from 2010 through July 2014 mirrored U.S. NARMS surveillance programs in overall prevalence of *Campylobacter* and drug resistance, identified retail chicken and unpasteurized milk as food products carrying highest risks for exposure to *Campylobacter*, confirmed zoonotic transmission of tetracycline-resistant *Campylobacter* to humans through raw cow's milk, and demonstrated the emergence in turkeys and swine of drug-resistant *Campylobacter* phenotypes during exposure to antibiotics. Differences in the fitness and rapidity of reversion in macrolide- and fluoroquinolone-resistant *Campylobacter* compared to sensitive isolates and comparisons of conventional and ABF swine production systems showed that simply removing antibiotics from food animal production will not predictably reduce the prevalence of antimicrobial-resistant *Campylobacter* on the farm or on the fork.

The literature we reviewed was consistent with recent NARMS annual reports surveying animals at slaughter (USDA, 2014) and retail meat products (FDA, 2015b) for overall prevalence and antimicrobial resistance of *Campylobacter*. While prevalence in fresh fecal samples from cattle (37-55%) and swine (56%) was significant in the reports we reviewed, the contamination

of most retail products was exceedingly low, < 5% (Noormohamed, *et al.*, 2013; Zhao, *et al.*, 2010). In fact, retail ground beef and pork chops have carried such low levels of *Campylobacter* contamination that the FDA NARMS retail meat surveillance program ceased testing for *Campylobacter* in these products after 2007 (FDA, 2013). In our study, retail ground turkey was found to have prevalences of *Campylobacter* of eight percent or less (Thakur, *et al.*, 2010; Zhao, *et al.*, 2010). Exceptions to these low contamination rates are retail packaged chicken and organ meats (beef and poultry), particularly liver. One explanation for high organ meat prevalence of *Campylobacter* may be bacterial translocation of intestinal flora through the portal circulation with capture during first pass through the liver. Proper preparation and cooking minimizes consumer risk from these products.

The evidence we reviewed supported retail packaged chicken meat as a moderate risk food product for exposure to *Campylobacter*. The chill step of processing chicken may be less successful at reducing *Campylobacter* contamination than with pork, where post chill prevalence of *Campylobacter* was 1.6% or less (Quintana-Hayashi, *et al.*, 2012; Tadesse, *et al.*, 2011). Achieving significant reductions in post-chill prevalence of *Campylobacter* on chicken meat is important for both organic and conventional products. Our literature search uncovered high prevalence of colonization of organic chickens in Canada (Thibodeau, *et al.*, 2011), increased contamination of post-chill organic broiler meat over conventionally raised Danish chickens (Rosenquist, *et al.*, 2013), and common contamination of post-chill broiler meat from alternative small-scale pasture-raised production units in the southeastern U.S. (Trimble, *et al.*, 2013). Thus, consumers assuming that organic chicken meat products have less bacterial contamination than

conventionally produced ones have a false sense of security as relates to *Campylobacter*. Further research into reducing post chill *Campylobacter* prevalence in chicken meat is warranted.

In addition to chicken meat, raw milk was identified as a high risk food product. It was the only food product for which our limited 4.5-year period of literature search found firm evidence for transmission of antimicrobial-resistant *Campylobacter* to humans (Sahin, *et al.*, 2012). The increase in outbreaks of human cases of campylobacteriosis linked to nonpasteurized milk and its resultant products (e.g., queso fresco) was further documented after the period of our literature search concluded (Mungai, *et al.*, 2015), and the Interagency Food Safety Analytics Collaboration among CDC, FDA and USDA ascribed 74% of *Campylobacter* illnesses to dairy (66%) and chicken (8%) (IFSAC, 2015). Solutions to reducing unnecessary cases of campylobacteriosis linked to consumption of nonpasteurized milk include public education about the safety benefits of pasteurization, public policy such as laws preventing the sale of raw milk, and enforcement of existing laws to prevent transport of nonpasteurized milk from states where its sale or cow-sharing programs are legal to neighboring states where they are not (Mungai, *et al.*, 2015). In 2014, the USDA's National Animal Health Monitoring System initiated the sixth national dairy study in seventeen participating states, which in part collected data on antibiotic usage and prevalence of antimicrobial resistance patterns in foodborne pathogens like *Campylobacter* (APHIS, 2013). Results of this work will inform agricultural decision making for ensuring animal welfare and milk safety.

Only one study was identified in our review that appeared to have directly linked an animal source of drug-resistant *Campylobacter* to human foodborne illness. This was the report of tetracycline-resistant *C. jejuni* clone SA that was found in a human foodborne outbreak where

raw milk samples from the dairy source were also tested and found to be genetically similar to isolates from ill individuals (Sahin, *et al.*, 2012). However, no information was available on antibiotic usage on the implicated dairy farm, so it is unknown whether farm practices contributed to the presence of the tetracycline-resistant *Campylobacter*.

We reviewed evidence of antimicrobial resistance developing rapidly and being directly associated with antibiotic exposure in swine (Quintana-Hayashi, *et al.*, 2012; Tadesse, *et al.*, 2011) and turkey poults (Logue, *et al.*, 2010). Reversion from macrolide-resistant to macrolide-sensitive phenotype occurred when drugs were not in use (Logue, *et al.*, 2010). The acquired mutations that allow *C. jejuni* to resist macrolides are detrimental to the bacterium (Luangtongkum, *et al.*, 2012); therefore, removal of macrolides from the agricultural environment would be expected to result in decreases in resistant phenotypes on farms. The findings of the detrimental fitness cost of macrolide resistance in *Campylobacter* are in contrast to the history of persistence of fluoroquinolone resistance in the U.S. and Denmark following bans on the use of fluoroquinolones in poultry (FDA, 2013), consistent with previously published work showing that fluoroquinolone resistance can confer a fitness advantage for *Campylobacter* (Luo, *et al.*, 2005). Therefore, removal of antibiotics has little to no effect on promoting reversion back to the quinolone-sensitive phenotype. In fact, even in the example of macrolide resistance reversion in poultry, complete displacement of resistant strains from the feces of broiler chicks required the presence of a sensitive *Campylobacter* strain for successful recolonization. The sole removal of macrolides from chicks colonized with pure cultures of resistant *Campylobacter* did not result in reversion to sensitivity (Luangtongkum, *et al.*, 2012).

Findings in two comparison studies between conventional and ABF swine farms showed that the presence or absence of antimicrobials did not affect the overall prevalence of *Campylobacter*, but the prevalence of antimicrobial resistant phenotypes in the farm environment and on carcasses was higher in conventionally reared pigs (Quintana-Hayashi, *et al.*, 2012; Tadesse, *et al.*, 2011). Persistence of antimicrobial resistance was observed from the farm environment to the slaughter house regardless of production system used. As mentioned previously, there were no differences between the very low prevalences of *Campylobacter* isolated from pork post chill. Therefore, there may be farm level benefits in reducing antimicrobial usage in swine, but the practical implications for how the reduction of antimicrobial-resistant *Campylobacter* ultimately affects pork safety are not entirely clear.



***Review Limitations***

Limitations of this review included its narrow focus on domestically acquired disease, *Campylobacter* origin limited to animal-derived foods, foodborne route of transmission and relatively short 4.5-year period. Antimicrobial-resistant *Campylobacter* infections are often acquired when traveling internationally, can be contracted from contaminated water or vegetables, and may result from direct contact with the environment and animals, including pets. While we excluded these articles, they are indeed important factors in the study of campylobacteriosis and warrant further investigation. We chose to limit our search to a recent 4.5-year time span (2010 through July 2014) due to the project start date of August 2014 and the volume of articles we encountered in our preliminary search.

Challenges encountered when critically evaluating the *Campylobacter* literature included multiple and evolving laboratory methodologies and interpretations of drug susceptibility or

resistance, incomplete information regarding antibiotic usage on farms, and molecular testing limitations. Each of these items alone may confound interpretation of research results and when combined made it virtually impossible to compare literature over time. Molecular methods for studying genotypes are useful, but there is not a direct relationship between PFGE results using a single restriction enzyme and the antibiotic resistance phenotype, nor is there always agreement with other molecular tests, such as MLST.

Finally, complete data for the use of antimicrobials on farms was often inadequate to fully assess the research findings. In trying to evaluate the contribution of agricultural use of antibiotics to the overall drug resistance dilemma, it was not possible to estimate the scope of use of antibiotics in either animals or humans since usage data is not tracked in the U.S. at this time.

***Current Action***

In 2014, a report was prepared by the U.S. President's Council of Advisors on Science and Technology (PCAST) addressing the urgent need to address antimicrobial resistance (PCAST, 2014). The PCAST recommendations included expanding antibiotic stewardship programs broadly and facilitating collection of prescribing and usage data that is needed for making evidence-based decisions in the future, both in human and veterinary medicine, and including these topics in professional education. The report was followed by Presidential Executive Order 13676 and a National Action Plan for Combating Antibiotic-Resistant Bacteria (NAP)(House, 2015). The NAP outlines specific and interrelated goals for action by the U.S. government by 2020 in conjunction with partners in healthcare, public health, veterinary medicine, agriculture, food safety and academic, federal and industrial research. As part of the action plan, the FDA strategies for implementing the Veterinary Feed Directive final rule (FDA,

2015a) and Guidances for Industry (GFI) 209 and 213 are detailed (House, 2015). Together, these initiatives will eliminate the use of medically important antibiotics for production purposes, and those needed for the prevention, treatment and control of animal disease will be used under veterinary supervision. Full implementation is targeted for December 2016.

The global nature of today's world means that the U.S. will continue to import food from lower income economies that may manage antibiotics and agricultural animal practices differently from that to which the U.S. is accustomed. We encourage continued and increased transparency in food labeling so that consumers may make informed decisions about the country of origin of all of their food choices. Continued public education about consumer responsibility for safe food handling, avoiding cross-contamination and cooking to adequate temperatures are also necessary. Young et. al. suggested from a survey of Canadian broiler chicken producers that education about foodborne illness and how to minimize its transmission may encourage a culture of safe poultry farm practices (Young, *et al.*, 2010). Important to providing the safest retail meat possible are initiatives such as the U.S. Department of Agriculture Food Safety and Inspection Service-initiated 2011 performance standards to reduce contamination rates of *Salmonella* and *Campylobacter* on chicken and turkey carcasses in slaughter establishments (FSIS, 2013).

Antibiotic stewardship efforts are important in human healthcare as well as in animal food production as evidenced by the increased risk for *Campylobacter* infection and quinolone-resistant *Campylobacter* infection within the year after humans have been treated with antimicrobials and quinolones, respectively (Koningstein, *et al.*, 2011) The antimicrobial exposure history of patients is a responsibility of physicians, and all may not be aware of the

consequences that their antimicrobial prescribing habits may have related to increasing risk for campylobacteriosis.

***Conclusions***

The findings of our review demonstrate the sobering reality that there is no one-size-fits-all answer to whether removing antibiotics from agricultural production will result in loss of drug resistance. For some bacteria, and for some antimicrobials, this may be the case. However, for some drugs, once they are used and resistance develops, it may be impossible from a biological perspective to reverse the effect. The need exists in the U.S. for a more robust national data collection system and heightened publication expectations for transparency in antibiotic usage in both animals and humans in order to assess which factors contribute to the persistence of antimicrobial resistance and to what degree. There is great need to start with studies that follow *Campylobacter* and other foodborne bacteria along the entire pathway from farm to fork to human illness.

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**Conflict of interest**

McCrackin MA, Helke KL, Galloway AG, Poole AZ, Salgado CD, Marriott BP, no conflicts of interest.

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**Table 1.** Scoring system for generating a grade for articles that were kept for full article review based on the GRADE system [1]

| Category | Scoring range |
|---|---|
| **Quality** Evidence of statistical analysis | 0 = no evidence +2 = evidence |
| Probability of bias or design limitations | 0 = none -1 = some -2 = high |
| **Directness** Methods and Results presented were clear and straightforward | -2 = not direct -1 = some uncertainty +1 = direct |
| Consistency - results and conclusions presented appeared to be consistent with methods Guyatt, G. H., et al,(2011) | -2 = important inconsistency -1 = some inconsistency+1 = consistent |

**Table 2.** Preliminary and Final Search terms, 2005-July 2014

| **Preliminary Search Strings**Search # | Search String | Number of Articles Retrieved |
|---|---|---|
| 1 | campylobacter AND "antibiotic resistan*" | 829 |
| 2 | campylobacter AND "antibiotic resistan*" OR "drug resistan*" | 935 |
| 3 | campylobacter AND "antibiotic resistan*" OR "drug resistan*" OR resistan* | 1287 |
| 4 | campylobacter AND resistan* OR antimicrobial | 1514 |
| 5 | campylobacter AND resistan* OR antimicrobial AND agri* | 55 |
| 6 | campylobacter AND resistan* OR antimicrobial AND agri* AND food | 35 |
| 7 | campylobacter AND resistan* OR antimicrobial AND food | 536 |
| 8 | campylobacter AND resistan* OR antimicrobial AND food OR meat | 588 |
| 9 | campylobacter AND resistan* OR antimicrobial AND food OR meat OR | 588 |
| 10 | campylobacter AND resistan* OR antimicrobial AND food OR meat OR | 595 |
| 11 | campylobacter AND resistan* OR antimicrobial AND food OR meat OR | 613 |
| 12 | campylobacter AND resistan* OR antimicrobial AND food OR meat OR | 614 |
| 13 | campylobacter AND resistan* OR antimicrobial AND "food animal" | 64 |
| 14 | campylobacter AND resistan* OR antimicrobial AND "food production" | 19 |
| 14b | campylobacter AND resistan* OR antimicrobial AND "food production" OR | 54 |
| 15 | campylobacter AND resistan* OR antimicrobial AND environment* | 216 |
| 16 | campylobacter AND resistan* OR antimicrobial AND "animal production | 0 |
| 16b | campylobacter AND resistan* OR antimicrobial AND "animal production" | 19 |
| 16c | campylobacter AND resistan* OR antimicrobial AND "animal production" OR | 30 |
| 17 | campylobacter AND resistan* OR antimicrobial AND slaughter* | 96 |
| 18 | campylobacter AND resistan* OR antimicrobial AND process* *(for processing* | 169 |
| 19 | campylobacter AND resistan* OR antimicrobial AND pack* *(for packaging* | 21 |
| 20 | campylobacter AND resistan* OR antimicrobial AND "food prep*" | 2 |
| 20b | campylobacter AND resistan* OR antimicrobial AND "food prep*" OR prep* | 40 |
| 21 | campylobacter AND resistan* OR antimicrobial AND gene* | 645 |
| 22 | campylobacter AND resistan* OR antimicrobial AND gene* OR plasmid | 652 |
| 23 | campylobacter AND resistan* OR antimicrobial AND gene* OR plasmid OR | 653 |
| 24 | campylobacter AND resistan* OR antimicrobial AND gene* OR plasmid OR | 661 |
| 25 | campylobacter AND resistan* OR antimicrobial AND gene* OR plasmid OR | 678 |
| 26 | campylobacter AND resistan* OR antimicrobial AND MDRGI OR "multidrug- | 1 |
| 27 | campylobacter AND resistan* OR antimicrobial AND human | 953 |
| 28 | campylobacter AND resistan* OR antimicrobial AND human AND ill* OR | 299 |
| 29 | campylobacter and "zoonotic disease" | 38 |
| 30 | campylobacter and "zoonotic disease" OR zoonotic disease | 173 |
| 31 | campylobacter and "animal reservoir" | 15 |
| | Total | 13,687 |

**Table 3**. Final Search strings used for the *Campylobacter* search with number of resulting citations, 2005-July 2014**Search**

| **Search String** | **Number of Articles Retrieved per Library** |
|---|---|
| SCOPUS SEARCH **Campylobacter AND resistan* OR antimicrobial** AND food OR meat OR seafood OR egg OR milk OR cheese OR dairy OR poultry OR agri* | **234** |
| **Campylobacter AND resistan* OR antimicrobial** AND human AND animal OR agri* | 133 (97 duplicates) **36** additional to library |
| **TOTAL** | **270 citations** |
| AGRICOLA SEARCH**Campylobacter AND resistan? OR antimicrobial** AND food OR meat OR seafood OR egg OR milk OR cheese OR dairy OR poultry OR agri? | **225** |
| **Campylobacter AND resistan? OR antimicrobial** AND human AND animal OR agri? | 29 (21 duplicates) **8** additional to library |
| **TOTAL** | **233 Citations** |

^Bolded terms appear in abstract



 

**Table 4.** Summary of studies investigating antimicrobial resistant *Campylobacter* by animal species

| Reference | Country | GRADE | *Campylobacter* species | Sample size/type | Location of samples | Antimicrobial use[1] | Organic vs. Conventional | Timeframe |
|---|---|---|---|---|---|---|---|---|
| ***Poultry*** | | | | | | | | |
| (Logue, *et al.*, 2010) | U.S. | 4 | *C. jejuni C. coli* | n = 3,252 total samples; farm | North Dakota, U.S. Turkey production facilitySlaughterhouse | a, e | conventional | March 24, 2007-July 30, 2007 |
| (Luangtongkum, *et al.*, 2012) | U.S. | 3 | *C. jejuni* | 10-15 newly hatched broiler chickens per group; cloacal swabs | Ames, Iowa, U.S.Poultry farm setting | e | not specified | 10-14 day in vivo studies |
| (Chen, *et al.*, 2013) | U.S. | 2 | *C. coli* | Banked isolates from retail meat samples; 2 isolates used for genetic studies | California andTennessee, U.S. | none | not specified | 2008 TN isolate; 2011 CA isolate |
| (Scheinberg, *et al.*, 2013) | U.S. | 2 | not specified | n = 100 whole chicken rinsate samples from farmers | Pennsylvania, U.S. Farmers' markets and supermarkets | none | organic and conventional | not specified |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ' markets, n = 50 conventionally processed organic, n = 50 nonorganic chicken samples resulting in 90, 14, and 26 positive samples, respectively | | | | |
| (Young, *et al.*, 2010) | Canada | 2 | n/a | n = 1,932 questionnaires distributed; n = 642 responses | Canadian broiler chicken producers | n/a | not specified | 2008 |
| (Rosenquist, *et al.*, 2013) | Denmark | 1 | *C. jejuniC. coli* | 10 g post-chill neck skin; n = 228 conventional, n = 208 | Danish conventional and organic broiler meat samples | not specified | organic and conventional | Organic: April 2009-March 2010Conventional: Jan.-Dec. 2009 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | organic broiler chicken samples resulting in prevalence of 54% in organic and 20% conventional samples | | | | |
| (Thakur, *et al.*, 2013) | U.S. | 1 | *C. jejuni* | n = 400 fresh pooled fecal samples collected from floor with gloved hand, n = 500 environmental samples; resulting in 118 positive fecal and 4 environmental samples | North Carolina, U.S.10 commercial broiler chicken houses | a, c, e | conventional | October 2010-March 2011 |



 

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Thibodeau, *et al.*, 2011) | Canada | 1 | *C. jejuni* | n = 300 fresh ceca, n = 30 fresh pooled fecal samples; resulting in 54 isolates | Quebec, Canada Slaughterhouse | e | organic | August and September 2009 |
| (Trimble, *et al.*, 2013) | U.S. | 1 | not specified | Post-chill broiler carcass rinsates: n = 120 (on-farm), n = 100 (USDA-inspected slaughter facility), n = 50 (mobile processing unit) resulting in prevalences of 70%, 82%, 100% | Independent, small-scale, pasture-raised broiler farms Southeastern U.S. | not specified | not specified | One calendar year |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Roberts, *et al.*, 2013) | U.S. | -1 | none | n = 192 poultry house litter samples tested; n = 0 *Campylobacter* positive | Mississippi, U.S.Commercial chicken broiler farm with 8 houses | a, e | conventional | June-December, 2008 |
| (Agunos, *et al.*, 2013) | Canada | -2 | not specified | Databank culture results from fresh retail chicken, beef, and pork samples collected by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) *Campylobacter* spp. | British Columbia,Maritimes,Ontario,Quebec,Saskatchewan, Canada | e | not specified | 2003-2010 |



 

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | prevalence: British Columbia (since 2007, 225 isolates/536 samples [42%]), Maritimes (since 2008, 117/444 [26%]), Ontario (since 2003, 845/2288 [37%]), Québec (since 2003, 683/2215 [31%]), and Saskatchewan (since 2005, 276/884 [31%]) | | | | |



 

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Deckert, *et al.*, 2010) | Canada | -2 | *C. jejuni* *C. coli* *C. lari* | N = 1,256 retail packaged chicken samples; 25-g skin from legs (670 packages), thighs (271), drumsticks (128), quarters (122), breasts (48), halves (12), wings (4), and backs (1) | Ontario, Canada | e | not specified | July 2001-January 2004 |
| (Just, *et al.*, 2012) | Canada | -3 | not specified | n = 60 air samples (30 each from cage-housed and floor-housed operations) tested by | Saskatchewan, Canada Cage-housed and floor-housed poultry operations | d, e | conventional | not specified |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | culture-independent methods | | | | |
| (Wesley, *et al.*, 2011) | U.S. | -3 | not specified | n = 446; 0-33-day-old turkey poult ceca, intestines, and yolk sacs homogenized and cultured; cecal contents from 138-day-old birds | Turkey brooder houseSlaughterhouse | a, e | conventional | Maximum of 138 days in study |
| (Noormohamed, *et al.*, 2012) | U.S. | -6 | *C. jejuniC. coli* | n = 202 total samples from retail chicken livers (159) and gizzards (43); resulting in n = 293 isolates | Tulsa, Oklahoma, U.S. Grocery stores | e | not specified | January-June 2010 |
| ***Swine*** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Wells, *et al.*, 2010) | U.S. | 3 | not specified | Serial rectal swabs from n = 256 weaned piglets weeks 0 and 2; rectal massage with gloved hand week 4 | Clay Center, Nebraska, U.S.Swine nursery facility | d[2] | conventional | February-March, 2005 |
| (Quintana-Hayashi, *et al.*, 2012) | U.S. | 3 | *C. coli* *C. jejuni* | n = 6,579 total samples resulting in 2,908 isolates Fresh feces-sterile fecal loops for piglets-gloved hands for nursery & finishing pigs Environmental samples - | North Carolina, U.S.Swine farm-live pigs-water, feed, soil, pen, floor, structure Slaughterhouse -carcass swabs & mesenteric lymph nodes-trucks-lairage | a, c, e | antimicrobial-free (ABF) farms and conventional farms | October 2008-December 2010 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | drags-swabs | | | | |
| (Wells, *et al.*, 2013) | U.S. | 1 | not specified | Rectal swab or fecal grab samples weeks 4, 8, 9, 10, 12; 1500 $cm^2$ skin swabs | Clay Center, Nebraska, U.S.Swine growing/finishing barn pens | d | ABF, conventional | 8-12 week study |
| (Tadesse, *et al.*, 2011) | U.S. | 1 | *C. coli* | n = 2011 total samples resulting in 1257 isolates Fresh feces-gloved hand for market age pigs Carcass swabs | Slaughterhouse-live pigs-carcass | a, c, e | ABF, conventional | 2002-2005 |
| (Létourneau, *et al.*, 2010) | Canada | -2 | not specified | n = 551 isolates cultured from air | Eastern Canada18 swine growing/finishing buildings | c, d | conventional | Winters of 2005-2007 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | samples collected in 18 swine confinement buildings resulting in n = 0 *Campylobacter* positive; 10 of 18 barns positive by PCR;n = 62 human nasal swab samples resulting in 1 *Campylobacter* positive | | | | |
| (Abley, *et al.*, 2012) | U.S. | -4 | *C. coli* | 11 finishing pigs initially with *C. coli* isolates from each of 1)farm, 2)post-eviscer | Farm, location unspecified | e | not specified | October 2006-March 2007 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ation, 3) hide, 4) carcass, and 5) rib meat; of n = 55 isolates, n = 43 recoverable from cryopreservation for study | | | | |
| ***Beef*** | | | | | | | | |
| (Rao, *et al.*, 2010) | Canada | 4 | *C. jejuniC. coli* | Fresh manure samples collected from pen floor; n = 1,183 isolates recovered | Alberta, CanadaFeedlots | a, b, c, e | conventional | March-December 2004 |
| (Hurd, *et al.*, 2010) | U.S. | 3 | not specified | Probabilistic risk assessment | n/a | a | n/a | n/a |
| (Sanad, *et al.*, 2011) | U.S. | 0 | *C. jejuniC. coli* | n = 944 fresh fecal samples collected from colon | Seven beef processing plants in four regions of the U.S. | e | conventional | Summer/early fall 2008 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | of cattle on convey er belt, resultin g in 181 positive s | | | | |
| (Cook, *et al.*, 2011) | Can ada | -4 | *C. jejuniC. coli* | N = 438 fresh packag ed grain-fed veal sample s resultin g in 6 isolates | Southern Ontario, CanadaRetail food outlets | e | conve ntiona l | February 2003-May 2004 |
| ***Seafoo d*** | | | | | | | | |
| (F. Wang, *et al.*, 2011) | U.S . | 1 | *C. jejuni* | n = 171 retail salmon, shrimp, and tilapia sample s | Baton Rouge, LA, U.S. | e | farm raised, wild caught or unkno wn | July 2009-January 2010 |

[1]a: treatment, b: control, c: prevention, d: growth, e: resistance measured

[2]carbadox was used: a synthetic growth-promoting feed additive with putative antimicrobial properties

**Table 5.** Summary of miscellaneous studies investigating antimicrobial resistant *Campylobacter*

| Reference | Country | GRADE | *Campylobacter* species | Sample size/type | Species | Location of sample | Antimicrobial Use* | Timefrme |
|---|---|---|---|---|---|---|---|---|
| ***Multiple Species*** | | | | | | | | |
| (Zhao, *et al.*, 2010) | U.S. | 4 | *C. jejuni C. coli C. lari* | n = 24,566 packaged retail meat rinsates resulting in 3,190 positive for *Campylobacter* | Chicken,Turkey,Cattle, Swine | FoodNet sites in 10 U.S. states | e | 2002-2007 |
| (Thakur, *et al.*, 2010) | U.S. | 3 | *C. jejuni, C. coli* | n = 168 isolates from ill humans; n = 814 packaged retail meat samples resulting in 192 isolates | Human,Cattle, Chicken,Turkey,Swine | Iowa, U.S. | e | June 2001-June 2002 |
| (Crespo, *et al.*, 2012) | U.S. | 0 | *C. coli* | DNA from 2 isolates recovered from turkey drinking water and swine feces | Swine, Turkey | North Carolina, U.S. conventional farms | e | not specified |



 

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (Sanad, *et al.*, 2013) | U.S. | 0 | *C. coli*, *C. jejuni* | fresh fecal samples: n = 227 dairy cows, n = 113 starlings resulting in 83 and 57 isolates, respectively | Dairy cattle, wild starlings | Northeastern Ohio, U.S. conventional farm | not specified | Summer/early fall 2008 |
| (Sahin, *et al.*, 2012) | U.S. | -1 | *C. jejuni* clone SA | n = 106 total *C.jejuni* isolates; n = 48 healthy sheep, n = 29 from ill humans, n = 29 ruminant abortion products | Human, Cattle,Goat,Sheep | Diagnostic laboratories in California, Colorado, Iowa, Kansas, North Dakota, South Dakota, Wyoming, U.S. Slaughter house | e | 2003-2010 |
| (Noormohamed, *et al.*, 2013) | U.S. | -1 | *C. coli*, *C. jejuni* | n = 97 retail beef samples (50 livers) resulting in 39 positives (liver only); n = 100 retail pork samples resulting | Cattle, Swine | Tulsa, Oklahoma, U.S. | e | January-June, 2010 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | in 2 *C. coli* isolates | | | | |
| (Y. Wang, *et al.*, 2014) | U.S. | -1 | *C. coliC. jejuni* | n = 1154 *Campylobacter* isolates -- genetic testing done in U.S. | Human, Chicken,Duck,Swine | Chinese CDC hospital cases, Farm | e | 2001-2011 |
| | | | | | | | | |
| **Reference** | **Country** | **GRADE** | ***Campylobacter* species** | **Sample size/type** | **Species** | **Location of sample** | **Antimicrobial Use*** | **Timeframe** |
| ***Other*** | | | | | | | | |
| (Koningstein, *et al.*, 2011) | Denmark | 4 | not specified | n = 31,669 total laboratory confirmed cases of campylobacteriosis | Human | Danish databases | a, e | 1995-2005 |
| (Ge, *et al.*, 2013) | U.S. | 3 | not isolated | n = 201 byproduct feed ingredients (n = 122 animal, n = 79 plant) | Animal feed | Rendering plants Oilseed industry | e | 2002-2003 |
| (Ricotta, *et al.*, 2014) | U.S. | 3 | not specified | n = 42,202 total laboratory confirmed cases of campylobacteriosis | Human | FoodNet Data | e | 2005-2011 |



 

*a: treatment, b: control, c: prevention, d: growth, e: resistance measured



 

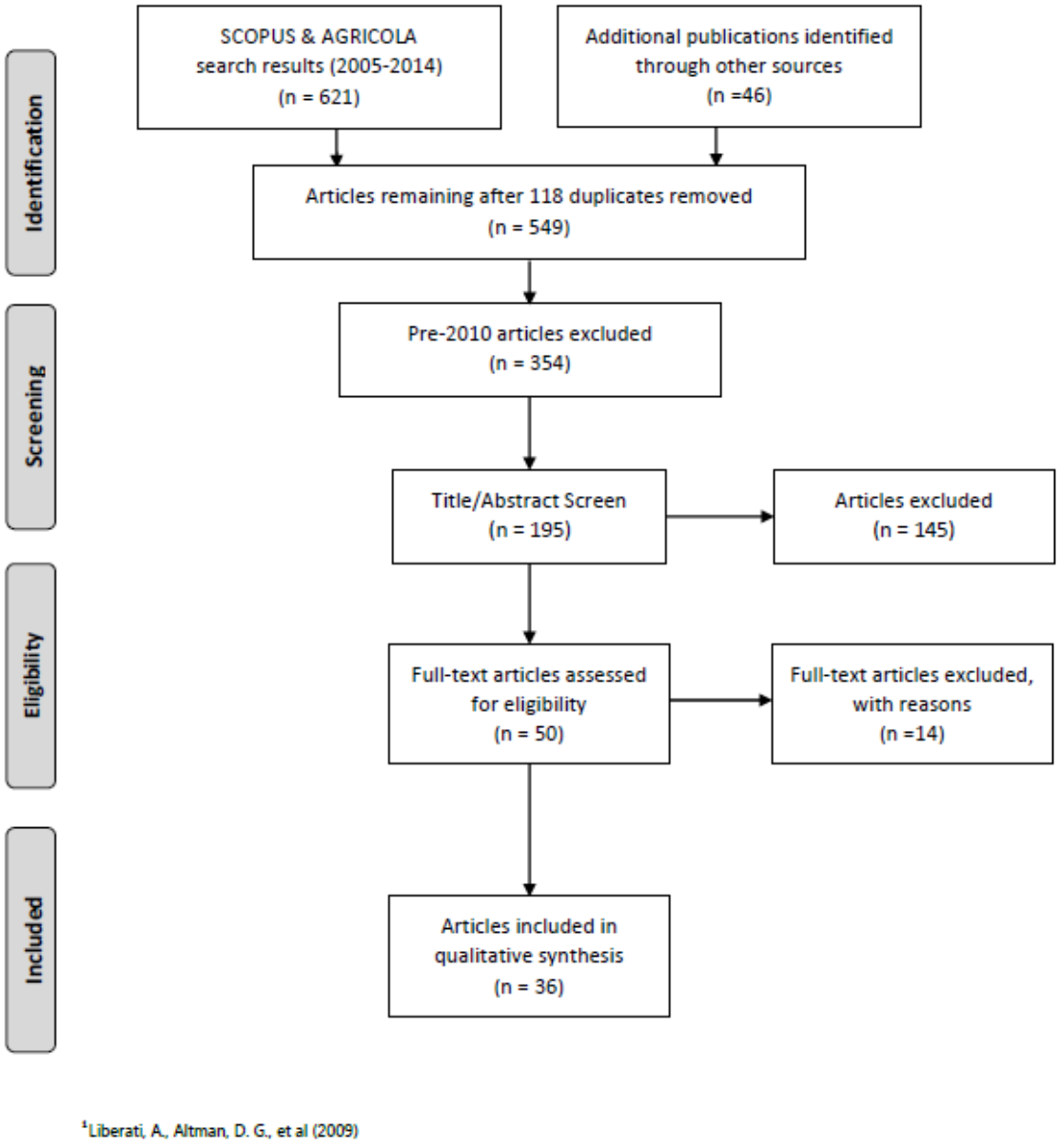


**Figure 1** Literature Search Results and Work Flow Diagram[1] [1]Liberati, A., Altman, D. G., et al (2009)