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Abstract

In *Campylobacter* spp., resistance to erythromycin and other macrolides has typically implicated ribosomal mutations, especially substitutions in the 23S rRNA genes. However, in 2014, the macrolide resistance gene *erm*(B) was reported for the first time in *Campylobacter* and shown to be harbored by a multidrug resistance island in the chromosome of the swine-derived strain *Campylobacter coli* ZC113. *erm*(B)-*positive C. coli* and *Campylobacter jejuni* strains from the food supply have been mostly reported from China. However, *erm*(B)-positive *C. coli* isolates were also detected recently in fecal samples from turkeys in Spain. To determine whether *erm*(B) may be harbored by erythromycin-resistant *Campylobacter* from commercial turkey production in eastern North Carolina, a major turkey-growing region in the United States, we investigated a panel of 178 erythromycin-resistant isolates (174 *C. coli*, 4 *C. jejuni*) using PCR with *erm*(B)-specific primers. None of the isolates were PCR-positive for *erm*(B) and sequence analysis of a subset of these erythromycin-resistant isolates revealed that all harbored A2075G substitutions in the 23S rRNA genes. Data fail to provide evidence for infiltration of *erm*(B) into erythromycin-resistant *Campylobacter* from commercial turkey production in this region and suggest the need for continuing surveillance.

Keywords

campylobacter, erythromycin, erm(B), turkeys, antimicrobial, resistance

Disciplines

Food Microbiology | Food Processing | Food Science | Pathogenic Microbiology

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Hannah K. Bolinger,[1] Qijing Zhang,[2] William G. Miller,[3] and Sophia Kathariou[1]

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Abstract

In *Campylobacter* spp., resistance to erythromycin and other macrolides has typically implicated ribosomal mutations, especially substitutions in the 23S rRNA genes. However, in 2014, the macrolide resistance gene *erm*(B) was reported for the first time in *Campylobacter* and shown to be harbored by a multidrug resistance island in the chromosome of the swine-derived strain *Campylobacter coli* ZC113. *erm*(B)-*positive C. coli* and *Campylobacter jejuni* strains from the food supply have been mostly reported from China. However, *erm*(B)-positive *C. coli* isolates were also detected recently in fecal samples from turkeys in Spain. To determine whether *erm*(B) may be harbored by erythromycin-resistant *Campylobacter* from commercial turkey production in eastern North Carolina, a major turkey-growing region in the United States, we investigated a panel of 178 erythromycin-resistant isolates (174 *C. coli*, 4 *C. jejuni*) using PCR with *erm*(B)-specific primers. None of the isolates were PCR-positive for *erm*(B) and sequence analysis of a subset of these erythromycin-resistant isolates revealed that all harbored A2075G substitutions in the 23S rRNA genes. Data fail to provide evidence for infiltration of *erm*(B) into erythromycin-resistant *Campylobacter* from commercial turkey production in this region and suggest the need for continuing surveillance.

Keywords: campylobacter, erythromycin, *erm*(B), turkeys, antimicrobial, resistance

Introduction

Erythromycin and other macrolides constitute drugs of choice for treatment of human campylobacteriosis, and macrolide resistance determinants in *Campylobacter* are, therefore, of major public health significance. Macrolide resistance is markedly more common in *Campylobacter coli* than in *Campylobacter jejuni*, and has been largely mediated by substitutions in the 23S rRNA, especially A2075G (Bolinger and Kathariou, 2017). Until 2014, evidence was lacking for involvement of *erm* (erythromycin ribosome methylation) determinants in macrolide resistance in *Campylobacter*, although such determinants, especially *erm*(B), are widely disseminated through horizontal gene transfer among bacteria. In 2014, however, *erm*(B) was shown to mediate high-level macrolide resistance in *Campylobacter coli* ZC113, a swine-derived isolate from China (Qin *et al.*, 2014). Subsequent analyses placed the first *erm*(B)-positive *Campylobacter* isolates in 1994 (Zhou *et al.*, 2016). Analysis of multiple isolates, primarily *C. coli*, from humans and food animals in China revealed that *erm*(B) is localized on multidrug resistance genomic islands (MDRGIs) (Qin *et al.*, 2014; Wang *et al.*, 2014; Zhou *et al.*, 2016; Bolinger and Kathariou, 2017).

Reports of *erm*(B)-harboring *Campylobacter* from humans or the food supply outside of China have remained scarce with only four such *erm*(B)-harboring isolates reported to date. Three were *C. coli* isolated in Spain from poultry, including two from turkeys in 2014 (Florez-Cuadrado *et al.*, 2016, 2017), whereas the first *erm*(B)-harboring *C. jejuni* strain in the United States was recently identified through whole genome sequencing (WGS) of human clinical isolates collected through the National Antimicrobial Resistance

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Surveillance system. This strain was isolated in 2016 from a patient who had recently returned from international travel, and remains the only *erm*(B)-harboring *Campylobacter* strain reported in the United States (Chen *et al.*, 2018). Previously, WGS of macrolide-resistant *C. coli* and *C. jejuni* from humans, retail meat, and poultry intestinal samples in the United States revealed that all harbored substitutions in the 23S rRNA (mostly A2075G), whereas *erm*(B) was not detected (Zhao *et al.*, 2016; Whitehouse *et al.*, 2018).

Turkeys in the United States are frequently colonized by multidrug-resistant *Campylobacter* strains, with macrolide resistance being highly prevalent in *C. coli* (Luangtongkum *et al.*, 2006; Bolinger, 2017). The objective of this study was to determine whether *erm*(B) has infiltrated turkey-derived *Campylobacter* spp. in eastern North Carolina, one of the major turkey-growing regions in the United States. We hypothesized that this would be detected by targeted PCR screening of erythromycin-resistant isolates using primers derived from *erm*(B) sequences conserved in *C. coli*, *C. jejuni*, and Gram-positive bacteria (Wang *et al.*, 2014), similarly to the approach used to detect *erm*(B)-harboring isolates from turkeys in Spain (Florez-Cuadrado *et al.*, 2017).

Materials and Methods

The panel of 174 *C. coli* and 4 *C. jejuni* isolates was chosen based on their previously determined resistance to erythromycin, with erythromycin minimum inhibitory concentration (MIC) breakpoint for resistance set at ≥8 μg/mL, as described (Luangtongkum *et al.*, 2006). Erythromycin MIC was typically >128 μg/mL. The erythromycin-resistant isolates were collected between July 2013 and June 2016 for a larger project assessing antimicrobial resistance and risk factors for *Campylobacter* colonization of turkeys grown conventionally in North Carolina (Bolinger, 2017). Most isolates ($n = 159$) were from ceca which were kindly provided throughout the study period by a vertically integrated commercial turkey company, from flocks grown on different farms in eastern North Carolina. The remaining isolates were derived from flies in different turkey houses ($n = 14$) and from turkey feces ($n = 5$) in the same region (Bolinger, 2017). The preponderance of *C. coli* in the panel reflects the fact that although turkeys in North Carolina are extensively colonized with both *C. coli* and *C. jejuni*, erythromycin resistance is primarily encountered in *C. coli* (Bolinger, 2017). Erythromycin-resistant *C. jejuni* was detected only in four farms, and one isolate from each farm was included in the panel. Multilocus sequence typing revealed several sequence types (STs) among *C. coli* (STs 1067, 1101, 8086, 8212, and 8551) and two (ST-1839, ST-7729) among the four erythromycin-resistant *C. jejuni* (Bolinger, 2017).

PCR for *erm*(B) used primers ermB-F: 5′-GGGCATT TAACGACGAAACTGG-3′ and ermB-R: 5′-CTGTGGTAT GGCGGGTAAGT-3′ (Wang *et al.*, 2014). All isolates were additionally tested using PCR for the aminoglycoside-resistance cluster (ARC) from the type I MDRGI with primers cluster-F: 5′-GGATGGATTCCTATGAAAACAT-3′ and cluster-R: 5′-GGCTTTGTTCATCTTCATACTCT-3′ (Qin *et al.*, 2012). Genomic DNAs of *Campylobacter coli* ZC113 and *Campylobacter coli* NADC2A were included each time as positive controls for *erm*(B) and the ARC, respectively.

The 23S rRNA gene region typically associated with erythromycin resistance in *Campylobacter* was amplified from each strain and sequenced using Sanger sequencing. Sequenced amplicons from the erythromycin-resistant *C. coli* strain 14983A (Miller *et al.*, 2016) and the erythromycin-sensitive *C. coli* type strain ATCC 33559 were used as positive and negative controls, respectively. All sequences were assembled in SeqMan (Lasergene v. 8.0; DNASTAR, Madison, WI).

Results and Discussion

All 178 erythromycin-resistant *Campylobacter* isolates were PCR-negative for *erm*(B), as well as for the ARC from the type I MDRGI. On the other hand, analysis of the 23S rRNA gene sequences of a subset of isolates (45 *C. coli*, 4 *C. jejuni*) selected to include different farms and STs revealed that all harbored the A2075G transition in the 23S rRNA typically associated with macrolide resistance in *Campylobacter* (Bolinger and Kathariou, 2017). Thus, our findings suggest that, as of 2016, *erm*(B) had not yet infiltrated the genomes of the macrolide-resistant *C. coli* or *C. jejuni* from conventionally grown turkeys in this region.

As *erm*(B) has already been identified in *Campylobacter* from food production animals in at least two locations, China and Spain, we consider it likely that it will be eventually encountered in *Campylobacter* from other regions as well, including the United States. Although not detected in our survey, *erm*(B) may emerge in the U.S. turkey production in response to yet unidentified selective pressures. Our investigation focused on *Campylobacter* from turkeys grown in eastern North Carolina, one of the leading turkey-growing regions in the United States, and needs to be complemented by similar investigations from other major turkey-producing regions. Nonetheless, our findings and those from other surveys (Zhao *et al.*, 2016; Whitehouse *et al.*, 2018) suggest a relatively slow pace of acquisition and spread of the *erm*(B) determinant in *Campylobacter* in the United States, where, with the sole exception of the one clinical *C. jejuni* strain already discussed (Chen *et al.*, 2018), macrolide resistance continues to involve 23S rRNA substitutions. The current findings will contribute to worldwide efforts to monitor macrolide resistance and the presence of *erm*(B) in *Campylobacter* from food animal production and other sources.

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Disclosure Statement

No competing financial interests exist.

References

Bolinger H. *Emerging Trends of Antibiotic Resistance and Risk Factors for* Campylobacter *spp. in Commercially Produced Turkey Flocks*. PhD Dissertation. Raleigh, NC: North Carolina State University, 2017.

Bolinger H, Kathariou S. The current state of macrolide resistance in *Campylobacter* spp.: Trends and impacts of resistance mechanisms. Appl Environ Microbiol 2017;83:e00416-17.

Chen JC, Tagg KA, Joung YJ, *et al.* Report of *erm*(B)$^{+}$ *Campylobacter jejuni* in the United States. Antimicrob Agents Chemother 2018;62:e02615-17.

Florez-Cuadrado D, Ugarte-Ruiz M, Meric G, *et al.* Genome comparison of erythromycin resistant *Campylobacter* from turkeys identifies hosts and pathways for horizontal spread of *erm*(B) genes. Front Microbiol 2017;8:2240.

Florez-Cuadrado D, Ugarte-Ruiz M, Quesada A, *et al.* Description of an *erm* (B)-carrying *Campylobacter coli* isolate in Europe. J Antimicrob Chemother 2016;71:841–843.

Luangtongkum T, Morishita TY, Ison AJ, *et al.* Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. Appl Environ Microbiol 2006;72:3600–3607.

Miller WG, Huynh S, Parker CT, *et al.* Complete genome sequences of multidrug-resistant *Campylobacter jejuni* strain 14980A (turkey feces) and *Campylobacter coli* strain 14983A (housefly from a turkey farm), harboring a novel gentamicin resistance mobile element. Genome Announc 2016;4:e01175-16.

Qin S, Wang Y, Zhang Q, *et al.* Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in *Campylobacter coli*. Antimicrob Agents Chemother 2012;56:5332–5339.

Qin S, Wang Y, Zhang Q, *et al.* Report of ribosomal RNA methylase gene *erm*(B) in multidrug-resistant *Campylobacter coli*. J Antimicrob Chemother 2014;69:964–968.

Wang Y, Zhang M, Deng F, *et al.* Emergence of multidrug-resistant *Campylobacter* species isolates with a horizontally acquired rRNA methylase. Antimicrob Agents Chemother 2014; 58:5405–5412.

Whitehouse CA, Young S, Li C, *et al.* Use of whole-genome sequencing for *Campylobacter* surveillance from NARMS retail poultry in the United States in 2015. Food Microbiol 2018;73:122–128.

Zhao S, Tyson GH, Chen Y, *et al.* Whole-genome sequencing analysis accurately predicts antimicrobial resistance phenotypes in *Campylobacter* spp. Appl Environ Microbiol 2016;82:459–466.

Zhou J, Zhang M, Yang W, *et al.* A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. Int J Infect Dis 2016;42:28–33.

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