Investigation of molecular mechanisms underlying tetracycline resistance in thermophilic Campylobacter spp. suggests that previous reports of tet(A)-mediated resistance in these bacteria are premature

Caoimhe Lynch
Department of Biological Sciences, Cork Institute of Technology

Kayleigh Hawkins
Department of Biological Sciences, Cork Institute of Technology

Helen Lynch
Celbridge, Co.

John Egan
Celbridge, Co.

Declan Bolton
Teagasc - Irish Agriculture and Food Development Authority

Follow this and additional works at: https://sword.cit.ie/dptbiosciart

Recommended Citation
Lynch, C., Hawkins, K., Lynch, H. et al. Investigation of molecular mechanisms underlying tetracycline resistance in thermophilic Campylobacter spp. suggests that previous reports of tet(A)-mediated resistance in these bacteria are premature. Gut Pathog 11, 56 (2019). https://doi.org/10.1186/s13099-019-0338-1

This Article is brought to you for free and open access by the Biological Sciences at SWORD - South West Open Research Deposit. It has been accepted for inclusion in Department of Biological Sciences Publications by an authorized administrator of SWORD - South West Open Research Deposit. For more information, please contact sword@cit.ie.
LETTER TO THE EDITOR

Investigation of molecular mechanisms underlying tetracycline resistance in thermophilic Campylobacter spp. suggests that previous reports of tet(A)-mediated resistance in these bacteria are premature

Caoimhe Lynch¹, Kayleigh Hawkins¹, Helen Lynch²,³, John Egan², Declan Bolton⁴, Aidan Coffey¹ and Brigid Lucey¹*⁷

Abstract

The true prevalence of tet(A), which codes for a tetracycline efflux pump, in thermophilic Campylobacter spp. requires clarification after reports emerged in Iran (2014) and Kenya (2016) of the novel detection of tet(A) in Campylobacter. During our investigation of antibiotic resistance mechanisms in a sample of Irish thermophilic Campylobacter broiler isolates, it was determined that 100% of tetracycline-resistant isolates (n = 119) harboured tet(O). Accessory tetracycline-resistance mechanisms were considered as tetracycline minimum inhibitory concentrations ranged from 4 to ≥ 64 mg/L. Primers previously reported for the detection of tet(A) in Campylobacter failed to produce an amplicon using a positive control strain (Escherichia coli K12 SK1592 containing the pBR322 plasmid) and a selection of Campylobacter isolates. Accordingly, we designed new tet(A)-targeting primers on SnapGene2.3.2 that successfully generated a 407 bp product from the positive control strain only. Further in silico analysis using BLASTn and SnapGene2.3.2 revealed that previously reported Campylobacter tet(A) sequences deposited on GenBank shared 100% homology with Campylobacter tet(O). We postulate that this gave rise to the erroneous report of a high tet(A) prevalence among a pool of Kenyan broiler Campylobacter isolates that were tested using primers designed based on these apparent tet(A) sequences. In conclusion, further work would be required to determine whether the homology between tet(A) potentially present in Campylobacter and known tet(A) genes would be sufficient to allow amplification using the primers designed in our study. Finally, the existence of tet(A) in thermophilic Campylobacter spp. remains to be demonstrated.

Keywords: Campylobacter, Tetracycline, tet(A), tet(O), Antibiotic resistance, Antimicrobial susceptibility, Antibiotic resistance mechanisms, Mobile genetic elements, Plasmids

© The Author(s) 2019. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
**Main text**

We read with interest an article reporting the novel detection of tet(A) among thermophilic *Campylobacter* spp. poultry isolates in Iran [1] and the subsequent detection of tet(A) among a pool of *Campylobacter* spp. chicken isolates in Kenya [2]. It is a timely reminder of emerging antibiotic resistance associated with the mobilisation of genes from other bacterial genera. However, we believe that it remains to be determined whether tet(A) exists among thermophilic *Campylobacter* spp.

Tetracycline-containing therapeutics are the most commonly administered antimicrobial in poultry production and animal husbandry in Ireland, used for the treatment of enteric, respiratory and dermal infections [3, 4]. Tetracycline resistance in *Campylobacter* spp. is usually mediated by a ribosomal protection protein Tet(O), which confers resistance by preventing tetracycline ribosomal binding, thus abolishing the inhibitory effect of the antibiotic by preventing bacterial protein synthesis via association of aminoacyl tRNA with the bacterial ribosome [3, 5].

In our study, during the investigation of antibiotic resistance mechanisms among a sample of 350 Irish broiler *Campylobacter* spp. isolates, we were especially interested in tetracycline resistance genes, as resistance to tetracycline was most prevalent (34%) by phenotypic sensitivity testing (Unpublished 2019). Tetracycline-resistant isolates were preliminarily screened for the presence of tet(O), using the method described by Aminov et al. [6] and it was determined that 100% of tetracycline-resistant isolates harboured the tet(O) gene. However, accessory tetracycline-resistance mechanisms were considered as minimum inhibitory concentrations ranged from 4 to ≥ 64 mg/L. Moreover, the mobilisation of tetracycline-resistant determinants is associated with the presence of tet genes on plasmids [3]. Hence, the tet(A) gene, which codes for an efflux protein and has been reported to co-exist with tet(O) for *Campylobacter*, was considered as part of the investigation [1, 2]. However, we seek clarification about the results published in the referenced articles [1, 2], and the true prevalence of tet(A) among thermophilic *Campylobacter* spp.

We tested the tet(A) primers described by Abdi-Hachesoo et al. [1] (Table 1) but they failed to produce an amplicon using the positive control strain *Escherichia coli* K12 SK1592 containing the pBR322 plasmid (DSM 3879). In addition, a selection of tetracycline-resistant thermophilic *Campylobacter* spp. isolates also failed to generate an amplicon. New tet(A)-targeting primers were thus designed on SnapGene2.3.2, based on homologous regions of the tet(A) gene from the pBR322 plasmid (GenBank J01749.1) and from the *Pseudomonas putida* strain Fars110 (GenBank JN937120.1) (Table 1)—the latter strain having been reported by Abdi-Hachesoo et al. [1] as a tet(A) positive control. A 407 bp product was successfully amplified using the new primers (Tet(A)-Camp-F and Tet(A)-Camp-R) with the *E. coli* K12 positive control strain but none of the tetracycline-resistant thermophilic *Campylobacter* spp. isolates generated a product.

With reference to the methods used in our study, DNA was extracted using PureLink™ Genomic DNA Mini Kit (Invitrogen, CA, USA). PCR mixtures (50 µL) contained 2.5U AmpliTaq™ DNA polymerase (Applied Biosystems, CA, USA), 1× buffer I (Applied Biosystems, CA, USA), 2.5 mM magnesium chloride, 0.2 mM of each dNTP (Sigma Aldrich, MO, USA), 200 µM forward and reverse primer (Table 1) and 1 µL of genomic DNA (between 50 and 100 ng/µL starting concentration). The PCR cycling conditions were: 95 °C for 2 min, 35 cycles of 94 °C for 30 s, annealing temperatures as described in Table 1 for 30 s, 72 °C for 1 min and final extension at 72 °C for 5 min. Amplified tet(O) and tet(A) products were resolved by electrophoresis in a 2% and a 1.5% agarose gel, respectively. All primers used are listed in Table 1.

The failure of the tet(A) primers listed by Abdi-Hachesoo et al. [1] to produce an amplicon with the same *E. coli* (DSM 3879 positive control strain), under less stringent conditions, prompted further investigation within our study. In the Abdi-Hachesoo et al. [1]

**Table 1** Primer used for the detection of tet(O) and tet(A)

| Primer         | Sequence (5′–3′) | Amplicon size (bp) | Annealing temperature (°C) | References |
|----------------|------------------|--------------------|----------------------------|------------|
| TetO-FW        | ACGGARAGTTATATGATACC | 171                | 52                         | [6]        |
| TetO-RV        | TGGCGATCTTATAATGTTGAC  |                    |                            |            |
| Tet(A)-F       | GTGAAACCCCAACATACCCC | 888                | 50                         | [1]        |
| Tet(A)-R       | GAGGCGAAGCAGGATGTAG  |                    |                            | [7]        |
| Tet(A)-Camp-F  | ATCGTGCCGGCGCATCACGGG | 407                | 54                         | This study |
| Tet(A)-Camp-R  | TCCTCCGCCGAAAATGACCC |                    |                            |            |
publication, the original tet(A) primer (Tet(A)-F and Tet(A)-R) reference is not listed in their bibliography, although these primers were previously reported by Maynard et al. [7] for the detection of tet(A) among Canadian swine E. coli isolates (Table 1) [1, 7].

We scanned the C. coli and C. jejuni tet(A) sequences, Shiraz3 and Shiraz4 (GenBank accession numbers JX891463.1 and JX891464.1, respectively) deposited in GenBank in the Abdi-Hachesoo et al. [1] publication against all Campylobacteraceae (taxid 72294) sequences using BLASTn. Multiple tet(O) sequences were returned with 100% identity, including C. jejuni 81-176 (GenBank NG_048260.1). Furthermore, our alignment studies using SnapGene2.3.2 demonstrated absolute homology between tet(O) (GenBank M18896.2) and Shiraz3 and 4 tet(A) sequences (GenBank JX891463.1 and JX891464.1, respectively). We propose that the true identity of the Shiraz 3 and 4 sequences are Campylobacter tet(O).

Furthermore, in 2016, a second study reporting a high prevalence of tet(A) among thermophilic Campylobacter spp. isolated from extensively reared Kenyan broilers was published by Nguyen and coworkers [2]. In that study, the tet(A) primers included the same primers as those used by Abdi-Hachesoo et al. [1] (but they produced an anomalous amplicon size) and a second set of in-house designed tet(A) primers (designated tet-A-1 and tet-A-2) [2]. However, the primers designed by Nguyen et al. [2] were based on the Shiraz 3 and 4 sequences (GenBank JX891463.1 and JX891464.1, respectively) [1], which we clarified above as tet(O). To confirm this, we performed an in silico PCR using SnapGene2.3.2 with the tet-A-1 and tet-A-2 primers [2] and Campylobacter tet(O) sequences (GenBank M18896.2 and NG_048260.1). A 486 bp product was predicted, which correlates to the amplicon length reported by Nguyen et al. [2]. We believe that this reported high prevalence of tet(A) among this subset (n=53) of thermophilic Campylobacter isolates is erroneous. Our opinion also explains why clusters of tet(A) harbouring Campylobacter spp. isolates are not described in any database, to our knowledge.

In conclusion, further study would be required to determine whether the homology between tet(A) potentially present in Campylobacter and known tet(A) genes would be sufficient to allow amplification using the primers designed in our study. The investigation of alternative Campylobacter-associated tetracycline resistance mechanisms is certainly worthwhile, but the presence of tet(A) in Campylobacter spp. is an open question.

Acknowledgements
The authors wish to thank the Irish poultry industry for providing isolates.

Authors’ contributions
CL is the primary author of the paper, directly co-supervised by BL, DB and AC. BL and AC guided the writing of the manuscript. KH co-conducted the laboratory and investigative work. The laboratory strain isolation was provided by HL and JE. All authors read and approved the final manuscript.

Funding
The project was funded by the Department of Agriculture, Food and the Marine, Food Institutional Research Measure (Ref. 15/F/641) and Teagasc Walsh Fellowship (Ref. 2017265)

Availability of data and materials
The datasets generated and/or analysed during the current study are available in the GenBank repository, https://www.ncbi.nlm.nih.gov/genbank/

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland. 2 NRL Campylobacter, Backweston Laboratory Complex, Young’s Cross, Celbridge, Co. Kildare, Ireland. 3 University College Dublin, Belfield, Dublin 4, Ireland. 4 Teagasc Food Research Centre, Ashtown, Dublin, Ireland.

Received: 21 August 2019   Accepted: 1 November 2019

Published online: 09 November 2019

References
1. Abdi-Hachesoo B, Khoshbakht R, Shariﬁyazdi H, Tabatabaee M, Hossein zadeh S, Asa i K. Tetracycline resistance genes in Campylobacter jejuni and C. coli isolated from poultry carcasses. Jundishapur J Microbiol. 2014. https://doi.org/10.5812/jjm.132129
2. Nguyen TNM, Hotzel H, Njeru J, Mwtturia J, El-Adawy H, Tomaso H, et al. Antimicrobial resistance of Campylobacter isolates from small scale and backyard chicken in Kenya. Gut Pathogen. 2016. https://doi.org/10.1186/s13099-016-0121-5.
3. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev. 2001;65:232–60.
4. HPRA. Report on consumption of veterinary antibiotics in Ireland during 2016. Dublin: HPRA; 2016.
5. Wieczorek K, Osek J. Antimicrobial resistance mechanisms among Campylobacter. Biomed Res Int. 2013;2013:340665. https://doi.org/10.1155/2013/340665.
6. Aminov RI, Garrigues-Lejean eau N, Mackie RI. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl Environ Microbiol. 2001;67:22–32. https://doi.org/10.1128/AEM.67.1.22-32.2001.
7. Maynard C, Fairbrother JM, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, et al. Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149K91 isolates obtained over a 23-year period from pigs. Anti-microb Agents Chemother. 2003;47:3214–21. https://doi.org/10.1128/AAC.47.10.3214-3221.2003.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abbreviations
Bp: base pair; C. jejuni: Campylobacter jejuni; C. coli: Campylobacter coli; dNTP: deoxyribonucleoside triphosphates; E. coli: Escherichia coli; PCR: polymerase chain reaction; spp.: species.