Research in Veterinary Science 119 (2018) 170–175

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Exploring the oxidative, antimicrobial and genomic properties of *Campylobacter jejuni* strains isolated from poultry

Maria Ugarte-Ruiz[a], Lucas Domínguez[a,b], Nicolae Corcionivoschi[c], Brendan W. Wren[d], Nick Dorrell[d], Ozan Gundogdu[d,*]

[a] VISAVET Health Surveillance Centre, Universidad Complutense Madrid, Madrid, Spain
[b] Facultad de Veterinaria, Universidad Complutense Madrid, Madrid, Spain
[c] Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast BT9 5PX, UK
[d] Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

ARTICLE INFO

Keywords:
Campylobacter jejuni
Oxidative stress
Antimicrobial resistance
MarR domain

ABSTRACT

Campylobacter jejuni is the leading cause of food-borne bacterial enteritis in humans, with contaminated poultry products considered the main source of infection. To survive the food chain, *C. jejuni* utilizes multiple defense mechanisms that counter oxidative and aerobic stresses. In this study, we phenotypically characterised 63 *C. jejuni* strains with oxidative stress survival and antimicrobial susceptibility testing to investigate correlations between these two phenotypes against the source of the strains and the presence of the MarR regulators RrpA and RrpB which have a role in regulating the response to oxidative and aerobic stress. *C. jejuni* strains isolated from meat and neck skin displayed the highest resistance to oxidative stress. In addition, *C. jejuni* strains that have an *rrpA*$^{+}$*rrpB*$^{-}$ profile exhibit increased resistance to oxidative stress and to antimicrobials. Here we establish a preliminary link between the distribution of RrpA and RrpB and the increased resistance to antimicrobials. This study provides insight into how the genotypic make up of *C. jejuni* can influence the ability of the bacterium to survive within areas of high oxygen stress, such as the food chain, and subsequently can have a potential negative impact on human health.

1. Introduction

Campylobacteriosis is the most frequently reported bacterial food-borne illness in the European Union with the cost to public health systems and lost productivity estimated to be around €2.4 billion a year (EFSA, 2017). The number of human cases has been reported to be over 240,000 annually in the EU, although this is believed to be a gross under representation due to lack of reporting, with the actual numbers of infected humans believed to be nearer to nine million each year (European Food Safety et al., 2016). Approximately 80–90% of these infections are attributed to *Campylobacter jejuni* (Humphrey et al., 2007), with poultry as the most important source of human campylobacteriosis in industrialized countries (Mullner et al., 2009; Sheppard et al., 2009). *C. jejuni* necessitates low oxygen concentrations (3–15%) for growth and is sensitive to high oxygen tension under normal atmospheric conditions (Kim et al., 2015). *C. jejuni* resides in the gastrointestinal tract of poultry where low oxygen levels prevail. Once excreted from animals however, *C. jejuni* encounters various harsh environmental stress, such as high oxygen tension and it is this increased oxidative stress in the atmosphere which is a critical barrier that *C. jejuni* has to overcome during its zoonotic transmission from animals (i.e., poultry) to humans via food (Kim et al., 2015). Thus, an improved understanding of the mechanisms of response towards oxidative stress is essential for explaining the survivability of *C. jejuni* within the food chain.

C. jejuni has evolved specific defense mechanisms to survive under increased oxidative stress conditions (Fields and Thompson, 2008). The bacterium expresses a repertoire of proteins that are directly involved in the breakdown of reactive oxygen species (ROS) e.g. catalase (KatA) (Grant and Park, 1995), or regulate the response to ROS e.g. PerR (van Vliet et al., 1999). The regulators RrpA and RrpB have been implicated in both oxidative and aerobic stress responses, enhancing bacterial survival in vivo and ex vivo in the environment. Both regulators are MarR type transcriptional regulators demonstrating auto-regulatory

Abbreviations: T6SS, Type VI Secretion System; gDNA, Genomic DNA; BA, Blood agar; CI, Confidence Intervals; S, Sensitive; R, Resistant; HR, Highly resistant; HTH, Helix-turn-helix; RIF, Rifampicin; DNP, 2,4-dinitrophenol; RND, Resistance-nodulation-division

* Corresponding author.
E-mail addresses: maria.ugarte@visavet.ucm.es (M. Ugarte-Ruiz), lucasdo@visavet.ucm.es (L. Domínguez), Nicolae.Corcionivoschi@afbini.gov.uk (N. Corcionivoschi), Brendan.Wren@lshtm.ac.uk (B.W. Wren), nick.dorrell@lshtm.ac.uk (N. Dorrell), Ozan.Gundogdu@lshtm.ac.uk (O. Gundogdu).

https://doi.org/10.1016/j.rvsc.2018.06.016
Received 11 October 2017; Received in revised form 25 April 2018; Accepted 13 June 2018

activity, typical of MarR-type transcriptional regulators (Gundogdu et al., 2015; Gundogdu et al., 2011). RrpA has also been shown to bind upstream of *katA*, suggesting that RrpA directly influences the expression of catalase (Gundogdu et al., 2015). MarR family of transcriptional regulators includes proteins that control virulence factor production, responses to both oxidative stress and antibiotics (Alekshun and Levy, 1999; Wilkinson and Grove, 2004; Wösten et al., 2008). Antibiotic resistance has been demonstrated in *Escherichia coli* mutants by decreasing influx and/or increasing efflux of toxic chemicals from the cell (Alekshun and Levy, 1999; Cohen et al., 1989). Increased efflux has been shown to be attained by increased synthesis of the AcrAB-TolC multidrug efflux system (Fralick, 1996; Okusu et al., 1996). The regulator of multiple antibiotic resistance (MarR) in *E. coli*, a member of the MarR family of regulator proteins, modulates bacterial detoxification in response to diverse antibiotic (Hao et al., 2014). To date, the antimicrobial response of RrpA and RrpB has not been analyzed.

In this study, we phenotypically characterised 63 *C. jejuni* strains with oxidative stress survival and antimicrobial susceptibility testing to investigate correlations between these two phenotypes against the source of the strains and the presence of the MarR regulators RrpA and RrpB which have a role in regulating the response to oxidative and aerobic stress. The genotypic make up of *C. jejuni* can influence the ability of the bacterium to survive within areas of high oxygen stress, such as the food chain, and subsequently have a potential negative impact on human health.

2. Methods and methods

2.1. Sample collection

In this study, we utilized 63 *C. jejuni* strains from previous studies Ugarte-Ruiz., 2012 and 2015a. Briefly, strains were collected from poultry at the slaughterhouse and, in a limited number of cases, from retail chicken meat in Spain between 2010 and 2011. *C. jejuni* was identified from faecal samples directly after evisceration, neck skin immediately after chilling and skinless packaged breast meat at the end of the processing line (Ugarte-Ruiz et al., 2012). Additionally, *C. jejuni* was also identified from urban effluents obtained at a wastewater treatment plant between 2010 and 2012 (Ugarte-Ruiz et al., 2015a). The 63 *C. jejuni* strains were classified as 17 from faecal content, 23 from neck skin, 19 from meat and 4 from urban effluents (Ugarte-Ruiz et al., 2015a; Ugarte-Ruiz et al., 2012). Genomic DNA (gDNA) was isolated using PureLink® Genomic DNA Mini (Thermo Fisher Scientific, U.S.A.).

2.2. Genome sequencing and genetic searches

Genomic data (ENA - PRJEB10936) was utilized from Ugarte-Ruiz et al., 2015b. For full sequencing details, please refer to Ugarte-Ruiz et al., 2015b.

2.3. Bacterial strains and growth conditions

C. jejuni strains were grown at 37 °C in a microaerobic chamber (Don Whitley Scientific, U.K.) or using microaerobic generators (CampyGen, Thermo Fisher Scientific), containing 85% N_2, 10% CO_2 and 5% O_2 either on blood agar (BA) plates containing Columbia agar base (Thermo Fisher Scientific), supplemented with 7% (v/v) horse blood (TCS Microbiology, U.K.) and *Campylobacter* Selective Supplement (Thermo Fisher Scientific) or in Brucella broth (Thermo Fisher Scientific) shaking at 75 rpm. *C. jejuni* strains were grown on BA plates for 24 h prior to use in all assays unless otherwise stated. For antimicrobial susceptibility testing sheep blood (BioMérieux, France) was used with no supplement.

2.4. Oxidative stress assays

Oxidative stress assays were performed as described previously (Gundogdu et al., 2015). Briefly, bacterial cells were harvested from a 24 h BA plate and resuspended into 1 ml PBS and diluted to an OD_{600} of 1. For oxidative stress assays, bacterial cells were exposed to H_2O_2 at final concentrations of 25 mM and 50 mM for 15 min at 37 °C under microaerobic conditions. Serial dilutions were prepared and 10 µl of the 10^{-1} to 10^{-6} dilutions spotted onto BA plates, incubated for 48 h and colonies counted.

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using broth microdilution for all strains. *C. jejuni* strains were tested against seven antimicrobials; gentamicin, ciprofloxacin, tetracycline, erythromicin, nalidixic acid, chloramphenicol and streptomycin. Strains were grown on sheep blood plates and incubated for 48 h. Bacterial suspensions were then added to cation adjusted Mueller-Hinton broth (11 ml) with TES buffer (TREK Diagnostics Systems, U.S.A), corrected for turbidity equal to a 0.5 McFarland standard, and supplemented with lysed horse blood (600 µl) freshly prepared in house from defibrinated horse blood (Oxoid). Finally, this mix was distributed onto EUCAMP microdilution plates (TREK Diagnostics Systems) and incubated for 48 h. *C. jejuni* strain ATCC 33560 was used as a quality control. The interpretation of the quantitative data was performed as described by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, n.d).

2.6. Statistical analyses

The data is presented as mean + SD. All experiments represent at least two biological replicates performed with two technical replicates. Data were analyzed using SPSS (19.0 IBM, Armonk, NY, U.S.A.). Statistical significance of differences (p-value < 0.05) was assessed by Pearson's chi-square and Fisher's exact test using R Software (R Development Core Team, n.d). Confidence Intervals (CI) at 95% were calculated using the online tool developed by WinEpi (Blas, 2006).

3. Results

3.1. Investigating the presence of rrpA and rrpB in C. jejuni strains

To characterise the 63 Spanish *C. jejuni* strains, we initially utilized the whole genome sequences from Ugarte-Ruiz et al., 2015b. We investigated the presence of the oxidative and aerobic stress regulators RrpA and RrpB using their respective amino acid sequences from *C. jejuni* strain NCTC 11168. Analysis of the 63 Spanish *C. jejuni* strains identified 58/63 (92.06%; CI 95%:85.39–98.74) of strains containing *rrpA* and 16/63 (25.40%; CI 95%:14.65–36.15) of strains as containing *rrpB*. The number of *C. jejuni* strains with an $rrpA^+rrpB^-$ profile was 42/63 (66.67%; CI 95%: 55.03–78.31) and those with an $rrpA^+rrpB^+$ profile was 16/63 (25.40%; CI 95%:14.65–36.15). All *C. jejuni* strains that were $rrpB^+$ were also $rrpA^+$. In addition, 5/63 *C. jejuni* strains (7.94%; CI 95%:1.26–14.61) had a $rrpA^-rrpB^-$ profile. We also investigated the prevalence of transcriptional factors that play a role in response to oxidative or aerobic stress. These included PerR, Fur, CosR, CsrA, CprRS and RacRS, which are typically conserved amongst all *C. jejuni* and *C. coli* wild-type strains (Atack and Kelly, 2009; Hwang et al., 2012; Palyada et al., 2009; van Vliet et al., 1999). Bioinformatic analysis revealed that all 63 *C. jejuni* strains contained the sequence for the respective encoding genes (> 90% amino acid similarity).

3.2. Correlating the presence of rrpA and rrpB to MLST

To investigate further the genome sequences, we identified the MLST clonal complex type of each strain and correlated these with the