



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

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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 *rppA*⁺*rppB*⁻ 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), lucasdom@visavet.ucm.es (L. Domínguez), Nicolae.Corcioni@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).

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% N₂, 10% CO₂ and 5% O₂ 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 OD₆₀₀ of 1. For oxidative stress assays, bacterial cells were exposed to H₂O₂ 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, erythromycin, 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 EUCAST 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 (Attack 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

RrpA and RrpB profile. Analysis of the *C. jejuni* strains containing the *rppA⁺rppB⁺* profile revealed that 10/16 (62.50%) were MLST clonal complex type ST-21, 3/16 (18.75%) were identified as ST-42, and 2/16 (12.50%) were noted as ST-61. One strain ZTA11/00193CPF CFB was untypable. For *C. jejuni* strains with the *rppA⁺rppB⁻* profile, the MLST clonal complex distribution was highly varied with no < 23 different ST-types and 14 different clonal complex types identified (Additional File 1). Three strains were also not designated with a clonal complex type (Additional File 1). The most prevalent MLST clonal complex identified from *C. jejuni* strains containing the *rppA⁺rppB⁻* profile was ST-206, 7/42 (16.67%). The second most prevalent MLST clonal complex type was ST-45 with 6/42 (14.29%) followed by ST-443 with 4/42 (9.52%). The five *C. jejuni* strains with *rppA⁻rppB⁻* profiles were from MLST clonal complexes ST-607, ST-464, ST-828 and ST-48.

3.3. Phenotypic characterisation with oxidative stress assays and categorisation

Phenotypic characterisation of the *C. jejuni* strains was initially performed using oxidative stress assays to assess the sensitivity of the strains. Oxidative stress assays were performed on the 63 Spanish strains and each were categorized into ‘sensitive’ (S), where growth occurred when incubated with H₂O₂ for 15 min at 25 mM, but no growth was observed at 50 mM (11/63 = 17.46% (CI 95%: 8.09–26.83)) (Fig. 1A); ‘resistant’ (R), where growth occurred when incubated with H₂O₂ for 15 min at 25 mM, but < 10⁸ colonies were observed when incubated for 15 min with 50 mM H₂O₂ (20/63 = 31.75% (CI 95%: 20.25–43.24)) (Fig. 1B); ‘highly resistant’ (HR), where growth occurred when H₂O₂ for 15 min at 25 mM and > 10⁸ colonies were observed when incubated for 15 min with 50 mM H₂O₂ (12/63 = 19.05% (CI 95%: 9.35–28.74)). The categorisation of the *C. jejuni* strains allowed us to correlate the oxidative stress results to different variables.

Table 1

Source of *C. jejuni* isolates and oxidative stress category. () data represents percentage of strains based on each category. The column “not determined” indicates the number of samples from each category that were not analyzed.

	Sensitive	Resistant	Highly resistant	Not determined	Total
Caecal content	6 (54.55)	6 (30.00)	4 (20.00)	1 (8.33)	17
Neck skin	3 (27.27)	4 (20.00)	10 (50.00)	6 (50.00)	23
Meat	0 (0.00)	8 (40.00)	6 (30.00)	5 (41.67)	19
Urban effluent	2 (18.18)	2 (10.00)	0 (0.00)	0 (0.00)	4
Total	11	20	20	12	63

where growth occurred when incubated with H₂O₂ for 15 min at 25 mM and > 10⁸ colonies were observed when incubated for 15 min with 50 mM H₂O₂ (20/63 = 31.75% (CI 95%: 20.25–43.24)) (Fig. 1C). The oxidative stress results for 12 strains were also not determined (12/63 = 19.05% (CI 95%: 9.35–28.74)). The categorisation of the *C. jejuni* strains allowed us to correlate the oxidative stress results to different variables.

3.4. *C. jejuni* strains isolated from meat and neck skin display increased resistance to oxidative stress

We next asked the question as to whether there was any variation in the level of sensitivity to oxidative stress and the source of the *C. jejuni* isolates (Table 1). *C. jejuni* strains were obtained from chicken meat (breast) (*n* = 19), neck skin (*n* = 23), faecal content (*n* = 17) from

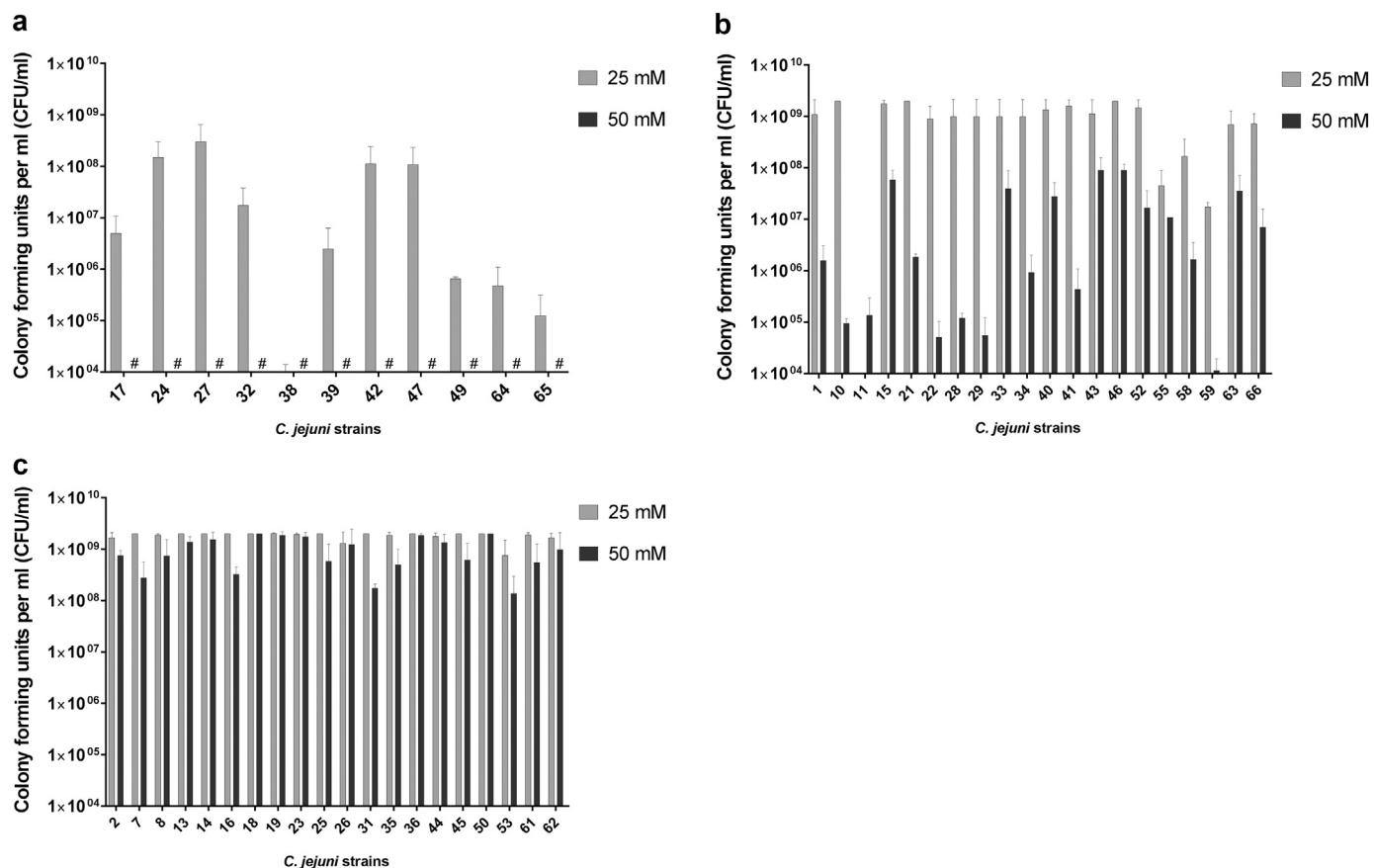


Fig. 1. Effect of oxidative stress on the survival of *C. jejuni* strains. *C. jejuni* strains were incubated with 25 or 50 mM H₂O₂ for 15 min at 37 °C followed by bacterial survival assessment. The strains were categorized into ‘sensitive’ (S) where growth occurred when incubated with H₂O₂ for 15 min at 25 mM, but no growth was observed at 50 mM (A); ‘resistant’ (R) where growth occurred when incubated with H₂O₂ for 15 min at 25 mM, but < 10⁸ colonies were observed when incubated for 15 min with 50 mM H₂O₂ (B); ‘highly resistant’ (HR) where growth occurred when H₂O₂ for 15 min at 25 mM and > 10⁸ colonies were observed when incubated for 15 min with 50 mM H₂O₂ (C). #Indicates no growth detected.

poultry and urban effluents ($n = 4$). The majority of *C. jejuni* strains classified as sensitive were obtained from faecal content (6/11; 54.55%); whilst a high proportion of *C. jejuni* strains classified as resistant were from chicken meat 8/20 (40.00%). Finally, 10/20 (50.00%) of *C. jejuni* strains classified as highly resistant came from neck skin. Comparison between different sources of *C. jejuni* strains and the oxidative stress resistance pattern revealed a statistical significant difference ($p < 0.05$) between the proportion of *C. jejuni* strains classified as sensitive that came from faecal content (6/11) vs. the proportion of sensitive strains obtained from chicken meat (0/11). In addition, a higher proportion ($p < 0.05$) of resistant or highly resistant strains from neck skin or chicken meat (28/40; 70%) were obtained compared with faecal content results (10/40; 25%). Of the four urban effluent samples, none were classified as highly resistant, with an equal proportion classified as either sensitive or resistant (Table 1). These results suggest that the *C. jejuni* strains isolated from the meat and neck skin display increased resistance to oxidative stress.

3.5. *C. jejuni rrpA⁺rrpB⁻* strains display increased resistance to oxidative stress

Following this, we assessed whether the distribution of RrpA and RrpB were correlated to the oxidative stress sensitivity of the *C. jejuni* strains (Table 2). The proportion of bacteria classified as *rrpA⁺rrpB⁻* was higher than *rrpA⁺rrpB⁺* strains for resistant or highly resistant bacteria (30/37, 81.1%) compared to sensitive ones (7/11; 63.6%), although the difference observed was statistically not significant ($p > 0.05$). These data suggests that *C. jejuni* strains containing the *rrpA⁺rrpB⁻* profile have a greater proportion of resistant or highly resistant oxidative stress profile, however greater number of strains are required with the *rrpA⁺rrpB⁺* profile to fully elucidate if there is a significant difference between the different RrpA and RrpB profile types.

3.6. *C. jejuni rrpA⁺rrpB⁻* strains show increased resistance to antimicrobials

We next assessed the antimicrobial susceptibility of the *C. jejuni* strains. Data was attained for 60 of the *C. jejuni* strains in this study (Table 3). A large number of the *C. jejuni* strains were resistant to ciprofloxacin 50/60 (83.33%; CI 95%:73.90–92.76), tetracycline 46/60 (76.67%; CI 95%:65.96–87.37) and nalidixic acid 49/60 (81.67%; CI 95%:71.88–91.46) while all strains were sensitive to gentamicin, erythromycin and chloramphenicol. Considering the strains with *rrpA⁺rrpB⁺* profile ($n = 16$), only 3/16 (18.75%) were resistant to three or more antimicrobials. In contrast, 33/42 (78.57%) of strains with an *rrpA⁺rrpB⁻* profile were resistant to three or more antimicrobials. The difference observed between the two groups was statistically significant ($p < 0.05$). Comparison of oxidative stress resistance to antimicrobial results showed that for strains classified as highly resistant to oxidative stress, the number of strains that were resistant to three or more antimicrobials was 13/20 (65%). For strains classified as resistant to oxidative stress, the number of strains that were resistant to three or more antimicrobials was 16/20 (80%). For strains classified as sensitive to oxidative stress, the number of strains that

were resistant to three or more antimicrobials was 6/11 (54.55%). These data suggest that *C. jejuni* strains with the *rrpA⁺rrpB⁻* profile were more resistant to three or more antimicrobials.

3.7. Correlation between *C. jejuni* strains containing a T6SS and resistance to oxidative stress

Finally, whole genome sequence analysis had previously shown that the full 13 gene T6SS loci was present in 9 of the 63 *C. jejuni* strains (14%) (Ugarte-Ruiz et al., 2015b). Here we have investigated the resistance of these strains to oxidative stress. 8/9 (88.89%) of the strains containing the full 13 gene T6SS loci were designated as resistant or highly resistant. In addition, 7/9 strains that contained the full 13 gene T6SS loci were resistant to three or more antimicrobials (77.78%). These results show that the T6SS containing *C. jejuni* strains may also have a role in increased oxidative or antimicrobial stress.

4. Discussion

The ability of *C. jejuni* to survive both oxidative and aerobic stress is central to the ex vivo and in vivo stages of this bacterial pathogens life cycle. This characteristic allows *C. jejuni* to survive in the food chain and so give rise to cross contamination whereby handling and eating raw and undercooked poultry have consistently been shown to be important risk factors for *Campylobacter* infection (Epps et al., 2013; Wilson et al., 2008). There is an urgent need to reduce both the incidence and levels of carcass contamination (Humphrey et al., 2007). With this in mind, we wanted to investigate how the genotype of *C. jejuni* strains could influence the phenotypic properties and thus influence the ability to survive within areas of high oxygen stress, such as the food chain. In this study, we performed phenotypic characterisation of 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 has identified correlations which may give *C. jejuni* an advantage in survivability, specifically relating to oxidative stress and antimicrobial resistance.

In this study, we investigated the prevalence of RrpA and RrpB that have been linked to regulation of the oxidative and aerobic stress responses in *C. jejuni* (Gundogdu et al., 2015; Gundogdu et al., 2011). Previously a study of 4232 *Campylobacter* genomes showed that *rrpA* was present in over 99% of *C. jejuni* strains, whilst the presence of *rrpB* was restricted and appears to correlate with livestock-associated MLST clonal complexes, predominantly ST-21 and ST-61 (Gundogdu et al., 2016). Analogous to these previous observations, here we also observe that the majority of *C. jejuni* strains contain *rrpA*, whereas the presence of *rrpB* appears more restricted. In addition the majority of *C. jejuni* strains with a *rrpA⁺rrpB⁺* profile in this study were MLST clonal complex type ST-21 and this correlates with published data that a large majority of livestock-associated MLST clonal complexes include ST-21 and ST-61 and contain both RrpA and RrpB regulators (Gundogdu et al., 2016). We also observed that for *C. jejuni* strains with a *rrpA⁺rrpB⁻* profile, the MLST clonal complex distribution was highly varied with no < 14 different MLST clonal complexes identified. The most prevalent MLST clonal complex identified from *C. jejuni* strains containing the *rrpA⁺rrpB⁻* profile was ST-206. These observations correspond with published data that *C. jejuni* strains with the *rrpA⁺rrpB⁻* profile have a wide range of possible MLST clonal complexes such as ST-45 and ST-443 and come under the classification of water & wildlife-associated lineages (Gundogdu et al., 2016). However, prominent MLST clonal

Table 2
Distribution of *rrpA* and *rrpB* in *C. jejuni* strains based upon oxidative stress resistance profile.

	<i>rrpA⁺rrpB⁺</i>	<i>rrpA⁻rrpB⁻</i>	<i>rrpA⁺rrpB⁻</i>	<i>rrpA⁻rrpB⁺</i>
Sensitive	4	0	7	0
Resistant	3	1	16	0
Highly resistant	4	2	14	0
ND	5	2	5	0
Total	16	5	42	0

Table 3

Antimicrobial susceptibility of *C. jejuni* strains. ECOFF is the epidemiological cut-off value. Strains were considered resistant when the obtained MIC value was above the ECOFF.

Antimicrobial agents	MIC Range (mg/L)	ECOFF (mg/L)	Number of strains with a MIC (mg/L) of:										Number of resistant strains (%)	
			0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	
Gentamicin	0.12–16	2		51	9	1								0 (0)
Ciprofloxacin	0.06–4	0.5		11				2	18	30				50 (83.33)
Tetracycline	0.25–16	1			12	3			2	1	43			46 (76.67)
Erythromycin	0.5–32	4				58	2	1						0 (0)
Nalidixic acid	2–64	16						6	3	3	14	21	14	49 (81.67)
Chloramphenicol	2–32	16					55	3	1	2				0 (0)
Streptomycin	1–16	4					57	1	1		2			2 (3.33)

complexes such as ST-48 and ST-206, which still have the *rrpA*⁺*rrpB*[−] profile, interestingly still come under the classified livestock associated lineages (Gundogdu et al., 2016). The five *C. jejuni* strains with *rrpA*[−]*rrpB*[−] profiles were from MLST clonal complexes ST-607, ST-464, ST-828 and ST-48.

In this study, one of our major goals was to investigate the oxidative stress sensitivity of each strain and to assess whether there were any correlations with the source of the *C. jejuni* strain. Comparison between different sources of *C. jejuni* strains and the oxidative stress resistance pattern revealed a statistically significant difference between the proportion of *C. jejuni* strains classified as sensitive that came from faecal content vs. the proportion of sensitive strains obtained from chicken meat. In addition, there was a greater number of *C. jejuni* strains isolated from neck or chicken meat that were classified as resistant or highly resistant when compared to *C. jejuni* strains from faecal content that were designated as resistant or highly resistant. Even though the sample numbers are relatively small, it is interesting to note that the *C. jejuni* isolates from chicken neck or meat were generally classified as resistant or highly resistant towards oxidative stress. In addition, a greater number of sensitive strains were isolated from the faecal content. The *C. jejuni* identified in the chicken neck or meat may represent strains that are present and surviving in the food chain, hence the higher resistance towards oxidative or aerobic stress. In addition, of the four urban effluent samples, none were classified as highly resistant, with an equal proportion classified as either sensitive or resistant. It is difficult to make any overarching statements, but it would be interesting in future work to have an extensive number of environmental samples and assess the response to oxidative stress and other factors discussed in this study. Another aspect would be to study the impact of oxidative and aerobic stress on *C. jejuni* survivability whereby altering the bacterium from a culturable form to a viable but non-culturable (VBNC) form, as previous reports have shown this is an important aspect of *C. jejuni* survival and persistence (Jang et al., 2007; Jones et al., 1991). In this study, we also investigated the response of *C. jejuni* strains to antimicrobials and a large majority of *C. jejuni* strains with an *rrpA*⁺*rrpB*[−] profile were resistant to three or more antimicrobials. Here we have not investigated the mechanism for this phenotype although it is interesting to note that the *C. jejuni* strains with a *rrpA*⁺*rrpB*[−] profile display increased resistance to oxidative stress and antimicrobial resistance. MarR family of transcriptional regulators include proteins that control virulence factor production, responses to both antibiotics and oxidative stress and catabolism of environmental aromatic compounds (Alekshun and Levy, 1999; Wilkinson and Grove, 2004; Wösten et al., 2008). Antibiotic resistance has been demonstrated in *E. coli* mutants by decreasing influx and/or increasing efflux of toxic chemicals from the cell (Alekshun and Levy, 1999; Cohen et al., 1989). To date, the

antimicrobial response of RrpA and RrpB have not been analyzed and this would be for future research. The original study by Ugarte-Ruiz et al., 2015b had investigated the presence of T6SS within the genomes of the *C. jejuni* strains. The identification of T6SS in *C. jejuni* strains is becoming more common (Corcionivoschi et al., 2015; Marasini and Fakhr, 2016; Ugarte-Ruiz et al., 2015b). In addition to the classical mechanisms described above to counter aerobic and ROS stresses, more recently T6SS have been identified as a potential mechanism for bacteria to counter oxidative stress (Goldova et al., 2011; Wang et al., 2015). The majority of strains containing the full 13 gene T6SS loci were designated as resistant or highly resistant. Although the sample size is relatively small, it is interesting to note the relatively high percentage of *C. jejuni* strains that are resistance to oxidative stress. In addition, the majority of strains which were contained the full 13 gene T6SS loci were resistant to three or more antimicrobials. Further investigations are required to analyze whether T6SS act as potential mechanism for oxidative stress control and antimicrobial resistance.

To survive the food chain, *C. jejuni* utilizes multiple defense mechanisms that counter oxidative and aerobic stresses. In this study, we presented data from the phenotypic characterisation of 63 *C. jejuni* strains that correlate the distribution of the MarR regulators RrpA and RrpB with increased resistance to oxidative stress and antimicrobial stress. We observed that *C. jejuni* strains isolated from meat and neck skin display increased resistance to oxidative stress. Future studies will include the utilization of a greater number *C. jejuni* strains from a variety of sources to confirm if the results obtained are mirrored on a larger scale. In addition, future studies will include the investigation of the mechanistic cause of the antimicrobial resistance associated with the RrpA and RrpB prevalence.

Ethics approval and consent to participate

No applicable.

Consent for publication

Not applicable.

Availability of data and material

Genomic data utilized in this study can be found on the <http://www.ebi.ac.uk/ena> website with the ENA code PRJEB10936. This data was previously published in Ugarte-Ruiz et al., 2015b.

Competing interests

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Author's contributions

LD, BW, ND, NC, MU and OG conceived and designed the study. MU performed the experimental work and statistical analysis. Preparation of the manuscript was mainly performed by MU, ND, BW and OG. Next generation sequencing and bioinformatics analysis was completed by OG. All authors contributed to editing the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rvsc.2018.06.016>.

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