Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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GJMR-G Classification: *DDC Code: 362.10971 LCC Code: RA449*

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Association of Cyclopropane Fatty Acid Synthesis with Thermo-Tolerance of Campylobacter Survival

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The *cfa* gene was present in thermophilic isolates and absent in non-thermophilic isolates. The *C. jejuni* strains collected after the scalding process surviving 52°C showed to differ significantly in terms of survival P< 0.0420. The *C. jejuni* strains surviving high temperature at 52⁰C had higher expression of the *cfa* gene when grown at 37°C and these strains were able to survive elevated temperatures dependent on the level of the *cfa* gene expressed during the stress response.

Conclusions: The *cfa* gene has a direct role for the adaptation of *C. jejuni* during exposure to scalding tank temperatures which are in excess of 50⁰C and serves to increase the resistance to the thermal stress in these strains.

Significance and Impact of Study: The *cfa* gene plays a role in the ability of thermophilic Campylobacter spp. to grow at temperatures above 37⁰C and therefore tolerate heat stress.

Keywords: campylobacter, cfa gene, heat tolerance, survival, poultry processing.

I. Background

Campylobacter spp. are the leading cause of bacterial foodborne diarrheal disease worldwide (WHO, 2018). Poultry are believed to be the main contributor to human cases of *Campylobacter* spp. with the bacteria being found in both live and slaughtered chickens (Skirrow and Blaser, 2000, Wittenbrink, 2002). The majority of *Campylobacter* spp. associated with chicken carcasses are identified as *C. jejuni* and *C. coli* (PHE, 2015, Wieczorek and Osek, 2015) and these two species are most frequently found in human cases in developed countries. Campylobacter spp. are exposed to many stress factors such as survival in the acidity of the host gut, survival on food and in the environment (Oh et al. 2018). The organism is capable of adapting to these stresses by regulating specific gene expression in response to stress (Murphy et al., 2006), such as *dps, sodB, trxB,* and *ahpC,* oxidative stress defence genes. Gene expression of these are increased through exposure to acid stress (Birk *et al.* 2012). During exposure to cold-shock Campylobacter increases the expression of *sodB* and *Cjo358* stress response genes are increased (Stintzi and Whitworth, 2003). Depending on the stress conditions involved *Campylobacter* has different survival rates (Reid *et al.* 2008).

Many other species of bacteria have the ability to change physiologically during starvation or environmental stress; for example by modifying their membrane lipids in situ by changing the phospholipid unsaturated fatty acid (UFA) to cyclopropane fatty acids (CFA) (Grogan and Cronan, 1997). The presence of cyclopropane ring-containing lipids, especially phospholipids, has been reported for many bacterial species including *Escherichia coli* and *Salmonella typhimurium* and there is a strong correlation between acid survival and chlorosome glycolipid molecules in heat protection (Mizoguchi *et al.* 2013).

Konkel *et al.* (1998) reported that *C. jejuni* preferentially synthesises 24 proteins immediately following heat shock. One of the major heat-shock proteins is DnaJ, which enables *Campylobacter* to colonise chickens while it has been shown that *Campylobacter* DnaJ mutants cannot colonise chickens. This suggests that DnaJ (HSP 40) plays an essential role in *C. jejuni's* thermal tolerance at temperatures above 42ºC (Konkel *et al.* 1998). However, DnaK and DnaJ are both found in non-thermophilic *Campylobacter* and so cannot be a factor in determining thermotolerance (Riedel *et al.* 2020).

The presence of CFAs in bacterial fatty acid membranes has been shown to provide protection against temperature changes and it is therefore likely that lipid composition of membranes changes when the microbial growth temperatures change (Russell *et al.*1995). The aim of this study was to investigate the possession of the cyclopropane fatty acid synthase gene by thermo-tolerant and non-thermo-tolerant

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Campylobacter spp. and to examine the presence of the *cfa* gene in Campylobacter that survived the scalding stage of poultry processing and to further investigate the *cfa* gene expression at 37ºC in different *C. jejuni* strains that are able or not able to survive at 52ºC.

II. Material and Methods

a) Origin of isolates

A total of 60 *C. jejuni* isolates were collected from commercial poultry processing plants in the UK. Samples were taken from carcasses early during the processing cycle, at the post-bleed, immediately after leaving the scalding tank (post-scald) and at the post-chill stages, and 21 isolates (7 from each stage of process) were chosen to be used within the framework of the study. Twenty *Campylobacter* strains for the environmental group were obtained from the laboratory culture collection and original came from different sources (water, sheep, soil). All *Campylobacter* isolates had been identified to the species level using conventional multiplex PCR (Lund et al. 2004).

b) Bacterial strains and culture conditions

The *C. jejuni* isolates were recovered from frozen storage by direct plating onto blood agar (BA; Oxoid Ltd.). Plates were incubated at 37ºC for 48 h in a MACS-MG 1000 anaerobic cabinet (MAC-Cabinet; Don Whitley Scientific, Shipley, UK) with a microaerobic gas mixture consisting of 10% CO_2, 5% O_2 and 2% H_2, balanced in N_2. These cultures were used for Deoxyribonucleic acid (DNA) extraction.

c) DNA Extraction

The DNA from the samples was extracted using the QIAamp® DNA extraction Kit (Qiagen, Crawley UK) according to manufacturer's instructions. Eluted DNA was stored at -20ºC until used.

d) PCR for detection of the cfa gene in Campylobacter isolates

DNA was extracted from *C.jejuni* isolates in order to examine the presence or absence of the *cfa* gene, PCR primers specific for this gene were designed based on the gene sequence information in the Campy database which was determined by conventional PCR using custom-designed primers (Table 1). Twenty-five µl PCR reaction mix were prepared as follows: 12.5 µl GoTag mastermix (Promega, UK), 1 µl forward primer (1:10 dilution), 1 µl reverse primer (1:10 dilution), 9.5 µl nuclease-free water and 1 µl DNA. The cycling conditions were as follows: 95ºC for 10 min and then 40 cycles of 95ºC for 60 seconds, 50ºC for 60 seconds, and 72ºC for 90 seconds. Samples were incubated at 72ºC for 5 min and held at 4ºC until processed. A 10 µl aliquot was taken from the amplified PCR products and analysed by gel electrophoresis at 100 V for 90 min using 1X TBE (0.89 M Tris borate, 0.02 M EDTA) running buffer on 2% agarose gels (Sigma-Aldrich). Gels were stained with 10 mg/ml ethidium bromide solution (Sigma-Aldrich) and visualised on a UV gel documentation system. A DNA-molecular ladder (50-bp and 100-bp ladder) (Hyper ladder, Bioline, UK) was included in each gel so that the size of products could be determined. Presence of a band of the appropriate length was taken to be a positive result. *C. fetus* (NCTC 10842) was used as a negative control and *C. jejuni* (NCTC 11168) was used as a positive control.

e) Purification of PCR products for sequencing

The PCR products were purified using the QIAquick® PCR purification Kit (Qiagen) according to the manufacturer's instructions. A 15ul aliquot of purified DNA with a concentration of 1 ng/ µl (150- 300 bp) was mixed with 2 µl of 10 pmol/ µl (10 µM) sequencing primer (cfa forward) in a microcentrifuge tube (Eurofins). Purified PCR products were sent to Eurofins Genomics, MWG Operon (Ebersberg, Germany) for sequencing. Sequence data was assembled with Multiple Sequence Alignment (Clustral Omega) and CLC Sequence Viewer 6.7.1 (CLC bio, Aarhus, Denmark) was used to align the sequences.

f) Heat tolerance

In order to determine the effect of heating on survival, 71 *C. jejuni* strains were divided into two groups: pre and post scald, 36 and 35 isolates were collected from each group respectively. As described by Hughes et al (2009) with slight modification, the isolates were recovered from frozen storage as described above. To determine survival at 52ºC, isolates were sub cultured in 50 ml Mueller Hinton broth (Oxoid), in a Bijoux and a 10µl loopful of bacteria was added and mixed thoroughly for each sample. These were incubated microaerobically at 37ºC for 48 h using the MAC-Cabinet. Following incubation 50 µl from each sample was pipetted into a 300µl microcentrifuge tube which was placed in a water bath at 52ºC for 30 mins. Serial dilutions were made in microtitre plates by pipetting 180 µl of PBS (Oxoid) into the wells of rows two to eight and 40 µl of samples into the wells of row one. Twenty µl of samples from row one was removed and mixed into the next row. This was continued across the plate. To check for the presence of bacteria in the wells 20 µl samples from each well were pipetted onto BA plates and incubated under microaerobic conditions at 37ºC for 48 h as described above. The number of bacteria that survived was determined using the following formula.

Number of bacteria= $N \times D \times 50 \times {}^{d}10$, in which: N= number of bacteria per spot, D and d = dilution number

g) Gene expression analysis

Twenty-four different *C. jejuni* strains were recovered from frozen storage as described above. Following recovery, they were plated onto BA and

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incubated at 37ºC for 48 h in a microaerobic atmosphere using the Campygen gas generating system (Oxoid). A 10µl loopful of culture was inoculated into 7 ml Mueller Hinton broth (Oxoid) in 7 ml Bijoux and incubated under microaerobic conditions at 37ºC for 48 hours as described previously. Prior to extraction a 100µl aliquot of culture broth was pipetted into MH broth in a 25 cm^2 tissue culture flask at 37ºC for 24 hours. Following incubation 2 ml from each culture was pipetted into 2.0 ml microcentrifuge tubes. The tubes were centrifuged at 8000 g for 10 minutes. To the resulting pellet, 800 µl of tri reagent (Sigma) was added and the tubes left at ambient temperature for 10 minutes. This was followed by the addition of 200 µl chloroform (Sigma) and the tubes were then centrifuged for 10 min at 13000 g. The upper aqueous layer was removed while avoiding the interphase and this was transferred to a clean 2.0 ml microcentrifuge tube (Fisher Scientific). A Qiagen® RNeasy Mini kit was used for RNA extraction following manufacturer's instructions. The extracted RNA was frozen immediately at -80 ºC for further analysis.

h) Real Time -Polymerase Chain Reaction

Quantitative RT- PCR was performed to verify the gene expression level of *cfa* at 37ºC in *C. jejuni* strains able or not able to survive at 52ºC. The following primer sequences were used for detection of *cfa* and *16S* rRNA genes in *C. jejuni* isolates: The forward cfa-RT 5' ACTATGAGCTATTCTTGCGCT 3' (21) reserve cfa-RT 5' AACCCCAGCCACCAACCTATA 3' (20), the forward 16S rRNA CCAGCAGCCGCGGTAAT (17) and the 16S rRNA GCCCTTTACGCCCAGTGAT (19) using the QuantiTect SYBR Green RT-PCR kit (QIAGEN) according to the manufacturer's recommendations. The comparative threshold (Ct) value corresponds to the PCR cycle at which the first detectable increase in fluorescence associated with the exponential growth of PCR products occurs, using comparative threshold cycle ($\Delta\Delta C_T$) (Livak and Schmittgen, 2001). The relative expression of each gene was determined three times in each of three experimental RNA samples, normalised to the *16S* rRNA reference gene and expressed as fold difference in quantity of cDNA molecules present in *C. jejuni* that could survive (+ve) at 52ºC to that present in *C. jejuni* that could not survive (-ve) at 52ºC.

i) Statistical analysis used in this study

For determination of *P*-value in heat tolerance experiments.

Fisher's analysis, a 2x2 contingency table and one-tailed *P*-value was used. *P* values were considered to be significant <0.05.

P-values for the gene expression work were determined using SPSS, Mann-Whitney U test (*P* value <0.05 was considered to be significant) using Graph Pad Prism (V.6.0) software package for the graph Mac (Graphpad, San Diego, USA).

III. Results

a) Presence of Campylobacter cyclopropane fatty acid synthase gene

The presence of the *cfa* gene in *Campylobacter* strains grouped by source was determined by PCR (Table 1). The *cfa* gene was present in all *C. jejuni* strains isolated from the poultry abattoir (Table 2), whereas, in the non-abattoir associated strains, the cfa gene was absent in *C. fetus, C. helveticus, C. sputorum* and *C. fecalis* (Table 2). The strains isolated from abattoirs originally came from chickens and were adapted to 42ºC (chicken body temperature). In strains isolated from environmental sources (non-chicken), where the temperature was below 42ºC the gene was absent (Table 2).

b) Heat tolerance survival

Thirty-six samples of *C. jejuni* collected before and 35 collected after the scalding stage process 'both groups were exposed to 52ºC' showed to differ significantly in terms of survival $P< 0.0420$ (Table 3), with the proportion of strains collected after the scalding process surviving 52ºC being roughly twice as high as before the scalding process.

c) The cfa gene expression

Two groups of *C. jejuni* isolated from chicken abattoirs, previously identified to contain a *cfa* gene using PCR, were exposed to 52ºC in a waterbath. The results obtained by QRT-PCR analysis using the *cfa* as the gene targets showed that expression of *cfa* mRNA relative to *16S* mRNA differed. When cultured at 37ºC, *C. jejuni* strains turned out to have the ability to survive at 52ºC and had a significantly higher expression of the *cfa* gene compared to *C. jejuni* strains that appeared not to survive at 52 ºC (Figure 1).

IV. Discussion

The ability of *C. jejuni* to tolerate conditions found during processing can be considered an important factor associated with their survival (Oh *et al.* 2018). To understand the mechanisms involved, *Campylobacter* spp. isolates from the environment and *C. jejuni* from various location in the chicken slaughter line were compared for their ability to produce the *cfa* gene, which has been shown previously to be associated with increased survival at elevated temperature (Grogan and Cronan, 1997, Zhang and Rock, 2008). In the present study, *C. jejuni* strains isolated from processing plants are more likely to possess the *cfa* gene, whereas the *cfa* gene was not present in *C. fetus, C. helveticus* or *C. sputorum* isolated from non- abattoir sources (Table 2). The source was seen to have a great influence on the presence of the

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cfa gene and the presence of this gene could explain thermophilic survival temperatures above 37°C (Table 2). The upregulation of the *cfa* gene was seen when the bacterial cell started to enter the stationary phase in E. coli (Chang et al. 2000b) and under thermal resistance they modified the profile of their phospholipid fatty membrane (Annous *et al.*1999; Zhang and Rock, 2008). The biosynthesis of CFAs effected stability and integrity of the cell membrane at high temperatures (Guzzo, 2011; Dufourcet al. 1984). This change supports the role of CFAs in the stress tolerance which is encoded by the *cfa* gene and enables cells to physiologically adapt to the condition of heat stress (Guzzo, 2011). Interestingly, all isolates obtained from the processing plants had the *cfa* gene present (Table 2), suggesting that Campylobacter strains colonising birds were able to withstand the challenge of the heat during processing plant regardless of *cfa* gene synthase.

During slaughter, Campylobacter spp. in and on chicken carcasses are subjected to temperatures higher than 50°C (Osiriphun *et al.* 2012) and this is a form of stress because under normal conditions Campylobacter does not grow at temperatures higher than 42°C (Park, 2002). To mimic circumstances of heat tolerance survival and maintenance of the survival response in *C. jejuni* strains were examined for survival of 52°C (Table 3). This experiment demonstrated a higher capacity of post scald *C. jejuni* strains to adapt to and survive heat (Table 3). A temperature of 52°C was selected as the whole chicken carcass is subjected to a scald tank with a water temperature of approximately 52 to 55°C to remove the feathers (Lehner *et al.,* 2014).In the present study the differences in survival between isolates collected pre and post scald stage were significant ($P < 0.05$). A large proportion of the *C. jejuni* strains from the post scald stage can survive higher temperatures compared to those from the pre scald stage (Table 3). The ability to adapt to higher temperatures correlates with the upregulation of specific genes, including *groEL* and *rpoD* that enhance survival of heat-stress (Klancniket al. 2008). The heat-stress response mechanisms of *C. jejuni* resulted in changes in morphology and protein profile when exposed to 48°C and to 55°C for a short time and their culturability and viability correlated with an altered protein profile and decreased virulence properties (Klancnik *et al.* 2014).

Heat stress is a key feature of poultry processing and only the bacteria that survive these abattoir stresses can reach human hosts. Since high temperature causes a physical change in the composition of the bacterial membrane lipids (Zhang and Rock, 2008), it has been demonstrated that the *cfa* gene has evolved responsively to this change (Chang *et al.* 2000). In the present study the thermophilic *C. jejuni* strains that survived at 52^{0}C and cultured at 37^{0}C were significantly more likely to increase the level of the relative *cfa*/rRNAgene expression compared to the control strain of *C. fetus* (Figure 1). This suggests that the *cfa* gene alters the fatty acid composition significantly in order to stabilise the membrane (Dufourc *et al.* 1984, Chang and Cronan, 1999). *C. fetus* does not have the *cfa* gene (Table 2) so is not able to grow at temperatures higher than 37^{0}C. This confirms the involvement of *cfa* in cyclopropane fatty acids biosynthesis. The findings presented here show that there is significant variation in the relative *cfa*/rRNA levels expressed between strains surviving at 52^{0}C and strains unable to survive at this temperature when both groups are cultured at 37^{0}C (Figure 1). *C. jejuni* strains surviving at 52^{0}C expressed the *cfa* gene and are adapted to survive when cultured at 37 0C as evidenced by its increased response characteristics of altered CFA during temperature growth. Similar studies observed that the *cfa* gene was regulated under stress conditions in other pathogens. For example, E. coli expressed the *cfa* gene under acid adaptation (Grandvalet *et al.* 2008) and *S. typhimurium* induced the expression of the *cfa* gene under acid stress (Kim et al. 2005).

The proportion of *cfa* mRNA transcripts increases with the increasing amount of the CFA in the bacterial membrane (Chang and Cronan, 1999). In the present study different expression was seen with *C. jejuni* strains that were able to survive at 52^{0}C, where some strains had a low level of expression of mRNA in the *cfa* gene (less than 0.2 relative *cfa/ 16S* rRNA levels of gene expression) (Figure 1). This suggests that not all strains express *cfa* to the same extent. In previous studies the low level of CFA synthesis was linked to the level of conversion of UFAs to CFAs and to the substrate specificity of the CFA synthase (Grandvalet *et al.* 2008).

Although the two major shock proteins DnaK and DnaJ are found in non-thermophilic *Campylobacter,* their role is small and limited. Some non-thermophilic *Campylobacter* are unable to colonise the chicken gut (Kempf *et al.* 2006) as the chicken's body temperature is 41.7 ºC. Results suggest that the *cfa* gene may in some way play a role in the ability of *C. jejuni* to colonise chickens and/or to persist in the chicken gut. This to suggest that the lack of the *cfa* gene could be a factor in determining thermo-tolerance. Thermo-tolerant bacteria are able to colonise chickens, as the presence of the gene will allow cells to adapt to survive above 37 ºC. Campylobacter isolates that can colonise birds were able to withstand the challenge of the processing plant regardless of *cfa* gene synthase (Table 2). Absence of the *cfa* gene was associated with the inability of the organism to colonise birds (Hermans *et al.* 2011).

This study also found that the source of Campylobacter species and the presence of the *cfa* gene could explain the difference in ability of strains to tolerate high temperatures. These data support the role of the *cfa* gene in the thermophilic pathogen for promoting its survival as reported in other studies (Chen *et al.* 2014; Chang *et al.* 2000).

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Temperatures above 42^0C may not be optimal for *Campylobacter* spp. to grow (Stintzi, 2003), but *C. jejuni* strains are adapting their survival at 52^0C by expressing the *cfa* gene. The expression level of *cfa* synthase involved in levels of different heat stress response mechanisms was described by Klancnik *et al.* (2006) with *C. jejuni* having elevated level of gene products in response to heat stresses. The change of the fatty acid membrane composition depends on the biosynthetic reactions that use modified lipid acyl components (Russell, 2002). The expression of the *cfa* synthase gene is reported to be important in the survival of *S. typhimurium* during the stationary phase, as this bacterium expresses high levels of this gene when entering this stage and the expression of *cfa* mutants was absent (Kim *et al.* 2005).

The present study shows that there is a degree of variation seen in the relative *cfa*/rRNA levels expressed in the strains surviving at 52^0C (Figure 1). Some *C. jejuni* strains have a higher *cfa* gene expression than other strains also surviving at 52^0C and this indicates that these strains may alter their fatty acid composition in a different way during survival to stabilise their membrane and to cope with higher temperatures. This finding is supported by Hughes et al. (2009) who found that *C. jejuni* altered different fatty acids due to temperature challenge. The difference in the *cfa* gene expression levels has also been observed in several strains of *E. coli* when the bacteria is induced to express the *cfa* gene and the subsequent synthesis of CFA in cell membrane phospholipids that increases to protect the cells from death (Chang and Cronan, 1999). As shown in Figure 1 some *C. jejuni* strains surviving at 52^0C show lower levels of mRNA expression at 37^0C and this may account for a lack of the major change in fatty acid composition, but these strains are still able to express the *cfa* gene which is required in order to alter their fatty acid composition to tolerate high temperatures. The findings indicate that the *cfa* synthesis gene was present in abattoir strains but not in the non-poultry abattoir group, suggesting that *cfa* contributes to the ability of Campylobacter to grow at temperatures above 37^0C. Strains of *C. jejuni* that survived passage through the abattoir scald tank were more likely to be able to survive at elevated temperatures than pre-scald strains.

This study shows that the change in gene expression induced by *C. jejuni* survival at 52 0C is evidence to suggest that the expression of the *cfa* gene is an important factor for survival at elevated temperatures and allows the consequential survival of *Campylobacter* spp. throughout the poultry processing chain which ultimately leads to infection in humans (Skarp *et al.* 2016).

In conclusion, these results provide evidence that thermophilic *C. jejuni* cells induced the *cfa* gene that is important for acquisition of heat resistance. A link of this thermo-tolerance to the *cfa* gene expression associated synthesis of high levels of cyclic fatty acids impacts the survival during the scalding stage of poultry processing. Understanding the mechanism of Campylobacter during poultry abattoir processing may help in improving control measures to reduce the burden of Campylobacter and implementing strategies to prevent disease.

Declaration of conflicting interests: The authors declared no potential conflicts of interests with respect to publication of this article.

Authors' contributions: H. M. designed and carried out the experiment and wrote the manuscript. L K. W., E. VK and T. C. helped in analysis and interpretation of the data andprovided critical feedback and helped shape the research.

Acknowledgements

The authors acknowledge the staff of the Food Standard Agency funded project at the Veterinary School, University of Bristol for their cooperation and assistance in collection of the samples from processing facilities. We also acknowledge the University of Bristol for financial support.

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Table 1: Primers used in this study for detection of the *cfa* gene in *Campylobacter* isolates

The number of bases of each primer is indicated in brackets after the sequence.

Target gene	Primers		Sequence (5'-3')
Cfa	*cfa1*	Forward	GTWTTTTGGGATMAAGAAG (19)
		Reverse	TCCAACAACCTTAACTCC (18)
Cfa	*cfa2*	Reverse	GCAGARGCACAAGAACGAAG (20)
		Forward	GGGGTTGGCTTTCTATTATG (20)
Cfa	*Cfa3*	Forward	TTGATTTAAAAGAAGGAGAAAAGC (24)
		Reverse	CATGTTCAAACATACCCACAGA (22)

Table 2: Presence of the *cfa* gene in *C. jejuni* isolates from the poultry abattoirs and non-abattoir*. The PCR was used to detect the gene, using a specific primer sequence. Twenty samples from pre and post scald were chosen to represent all PFGE profiles.

Samples	Pre and Post Scald stage	PFGE profile	Sources*	*Cfa* presence
*C. jejuni*3A3/2	Pre	C7	Abattoir	YES
*C. jejuni*2A5/2	Pre	C6	Abattoir	YES
*C. jejuni*1A4/2	Pre	C23	Abattoir	YES
*C. jejuni*1B3/1	Post	C2	Abattoir	YES
*C. jejuni*1A3/2	Pre	C1	Abattoir	YES
*C. jejuni*1B1/1	Post	C4	Abattoir	YES
*C. jejuni*1A2/3	Pre	C4	Abattoir	YES
*C. jejuni*2A4/2	Pre	C3	Abattoir	YES
*C. jejuni*1A1/3	Pre	C7	Abattoir	YES
*C. jejuni*1A1/4	Pre	C3	Abattoir	YES
*C. jejuni*1A5/2	Pre	C27	Abattoir	YES
*C. jejuni*3B4/2	Post	C6	Abattoir	YES
*C. jejuni*2B2/3	Post	C7	Abattoir	YES
*C. jejuni*2B5/1	Post	C7	Abattoir	YES

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*C. jejuni*1A2/3	Pre	C4	Abattoir	YES
*C. jejuni*1A3/3	Pre	C12	Abattoir	YES
*C. jejuni*3A4/2	Pre	C5	Abattoir	YES
*C. jejuni*1B5/1	Post	C23	Abattoir	YES
*C. jejuni*3B2/3	Post	C5	Abattoir	YES
*C. jejuni*3B3/2	Post	C7	Abattoir	YES
C.sputorumss.sputorum NCTC 11528	-	-	Non-abattoir	NO
C. sputorum ss. fecalis NCTC 11367	-	-	Non-abattoir	NO
C. jejuni ss doylei NCTC 11951	-	-	Non-abattoir	NO
C. sputorum ss. fecalis NCTC 11415	-	-	Non-abattoir	NO
C. helveticus NCTC 12470	-	-	Non-abattoir	NO
C. fetus ss fetus NCTC 10842	-	-	Non-abattoir	NO

**Abattoir samples: thermophilic C. jejuni; Non-abattoir: other Campylobacter species*

Table 3: Heat survival of *C. jejuni* strains at 52 ºC from abattoirs isolated before and after exposure to the scald tank. Fisher's exact test was used to determine the differences between the heat tolerance groups. *P*-values less than 0.05 were considered significant.

	N. of sample	+ve survive 52 ºC	-ve survive 52 ºC	P value
Pre-scald	36	7	29	0.0420
Post-scald	35	15	20	

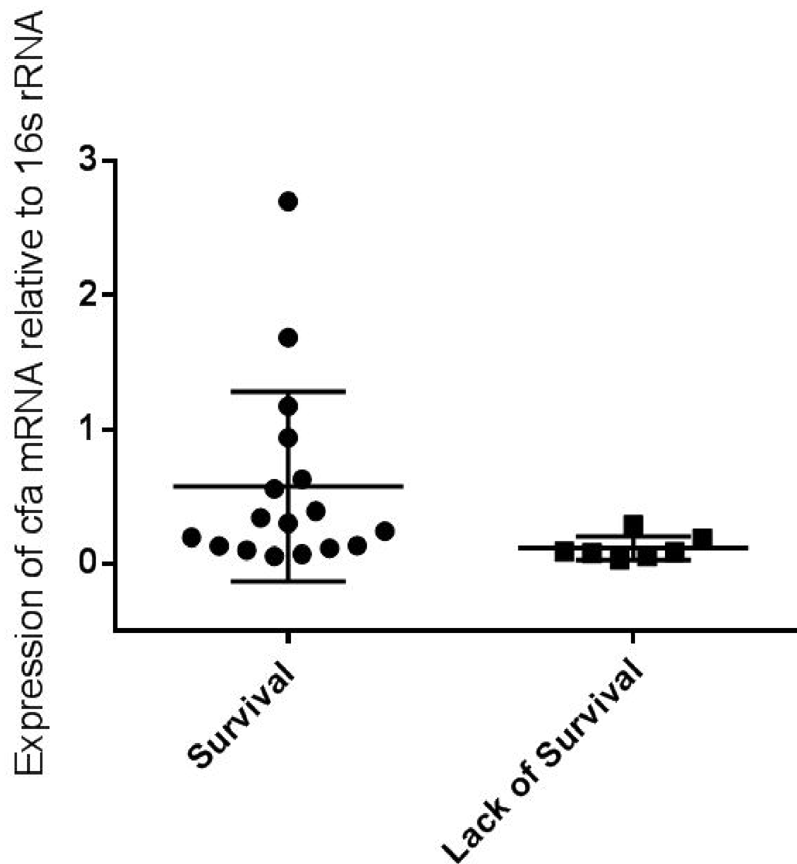

Figure 1: Relative *cfa/ 16S* rRNA levels of gene expression at 37 ºC after *C. jejuni* strains were exposed to 52 ºC, showing either survival (+ve) or lack of survival (-ve) at this temperature. Both groups were assessed by real-time PCR (control strain of *C. fetus* which lacks the *cfa* gene). Twenty-four different *Campylobacter* spp. isolates were used. Error bars show the standard deviations, experiments were carried out in triplicate, using Mann-Whitney U test (*P* value <0.05)

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