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Korrespondenzadresse:
ali.al-sakkaf@lincolnuni.ac.nz

Institute of Food, Nutrition and Human Health, Massey University, Palmerston North 4442, New Zealand

Survival of *Campylobacter jejuni* under oxidative stress at different temperatures and atmospheres

Überleben von Campylobacter jejuni unter oxidativem Stress bei verschiedenen Temperaturen und Atmosphären

Ali Al-Sakkaf

Summary

New Zealand (NZ) has a higher rate of reported campylobacteriosis than most of the developed world. One possible reason for the higher rate is that local *Campylobacter* strains have a greater ability for survival. The objective of this study was to investigate the oxygen tolerance of local isolates. The study will focus on the most implicated isolates (ST-474, ST-48, ST-190) in human cases. These isolates tested: 1) broth culture in a flask exposed to atmospheric oxygen; 2) spread plating on agar and exposed to atmospheric oxygen; (3) plating on Muller Hinton Agar onto which filter discs inoculated with 10 µl of 1mM, 10mM, 100mM, or 1 M hydrogen peroxide (H_2O_2), after which, the inhibition zone was assessed.

C. jejuni strains exposed to air (for 1 and 2) survived longer at a lower temperature (4°C >10°C >20°C >25°C). At 4°C, the strains could survive for between three weeks to more than four weeks in broth or agar microaerobically (5% O_2, 10% CO_2 and 85% N_2) and for about three weeks aerobically. Whereas, at 10°C the survival was up to two weeks, and at 20°C or 25°C, survival was about a week or less than a week. Exposure to H_2O_2 revealed that *Campylobacter* strains tested were sensitive to all concentrations, except the lowest (1 mM). The novelty of this study is that no previous study has disproved the hypothesis of New Zealand *Campylobacter* strains that they are more oxygen tolerance than most of the other international *Campylobacter* strains. The higher NZ rate of reported campylobacteriosis compared to other developed countries is possibly due to other factors.

Keywords: Oxidative stress, air tolerance, *Campylobacter,* low temperature, survival

Introduction

Campylobacter is considered as the greatest bacterial causative agent of gastrointestinal disease in humans (Allos and Acheson 2001). *Campylobacter* strains survive well in modified atmosphere and vacuum packaging, but poorly at atmospheric oxygen concentrations (21%) (Kelly 2008). *Campylobacter* spp. are harboured in many wild and domestic warm-blooded animals. These pathogens do not survive well in the environment when compared to other bacterial foodborne pathogens, such as *Listeria* and *Salmonella*. These organisms are unable to grow in the presence of air, and traditionally, a microaerobic mix (5% O_2, 10% CO_2, and 85% N_2) and high temperature (42°C) have been used for the isolation of the thermotolerant *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*) (ICMSF 1996, Garenaux et al. 2008). Many studies have investigated the pathogen's survival under oxidative stress, which is defined as cell damage leading to cell death caused by excessive levels of oxidants and free radicals in the environment, including both free oxygen and compounds such as H_2O_2, hydroxyl radicals, and superoxide anions. This is summarized in the literature, for example, the publication of the International Commission on the Microbiological Specifications for Foods (ICMSF 1996). Studies that have investigated the growth of *Campylobacter* under different gas compositions with different techniques and substrates are in general agreement with this proposed optimum composition (Koidis and Doyle 1983, Grigoriadis et al. 1997). It remains unclear exactly how this pathogen survives the high oxygen level in the atmosphere and the low ambient temperature during its transmission to its human host.

Other studies have reported that the survival of *Campylobacter* strains on poultry and meat was not influenced by the storage atmosphere or the treatment condition (vacuum packing) at 4°C (Hanninen et al. 1984, Wesley and Stadelman 1985). Few studies reported that the presence of oxygen increased the death rate at 4°C in broth and milk (ICMSF 1996). A New Zealand study revealed that *C. jejuni* could adapt to aerobic growth after a repeated subculture. In this study, about 81% of human, 75% of water, and 65% of poultry *C. jejuni* isolates were capable of growth under aerobic conditions in Nutrient Agar, and a cocktail of isolates (human, poultry, veterinary) survived aerobically for more than four weeks in poultry meat at 5°C and for more than a week at 25°C (Chynoweth et al. 1998). It was reported that *C. jejuni* isolates (10 human and nine poultry) could survive up to 4 weeks at 4°C aerobically in Mueller Hinton Broth and sterilised chicken rinses with a variation in their resistance from one week to four weeks (Chan et al. 2001).

Yamasaki (2004) reported that one log of the initial *C. jejuni* inoculums (9.5 log) in Muller Hinton broth (MHB) decreased in *C. jejuni* concentration after the first 6 hours under aerobic conditions at 37°C followed by another one log reduction for the next six hours. Yamasaki observed that a rapid reduction by about 5 log occurred between 12 and 15 hours of incubation. Another study reported the survival in broth and agar plates for poultry and human isolates at three different temperatures (4, 25, and 42°C), but the data were collected only for a one week period (Garenaux et al. 2008). Another study also indicated that the exposure to atmospheric oxygen in broth culture for 5 and 15 hours did not affect the growth of *C. jejuni*, and the count was similar to that of *C. jejuni* incubated under microaerobic conditions (Mihaljevic et al. 2007). This is consistent with other reports regarding the growth and adaptation of *C. jejuni* in aerobic metabolism (Jones et al. 1993, Harvey and Leach 1998). One study investigated the survival of *C. jejuni* under oxidative stress in broth and agar plates but only at 37°C (Kaakoush et al. 2009). Recently, a study (Karki et al. 2019) reported the survival of *Campylobacter j.* and Coli isolates in diluted chicken liver juices, chicken juices, beef meat juices, beef liver juices, laked horse blood and (MHB) at 42°C. This study (Karki et al. 2019) indicated that all diluted retail meat, liver juices and laked hourse blood (10% v/v with MHB) significantly enhanced *Campylobacter* survival relative to (MHB) for 24 hours incubation period. Kim et al. (2021) concluded in their review about *Campylobacter* stress tolerance that the reported differences in various strains of *C. jejuni* highlight the constraints of drawing generalized conclusions from individual strain research.

From all of the above studies, it is concluded that there is ambiguity about the behaviour of *C. jejuni* under oxidative stress. This may confirm the capricious nature of such bacterial organisms due to the high variation found between tests and discrepancies between the reported results of the different studies. Evidently, *Campylobacter* causes more difficulties in the standardization and handling of this pathogen than are seen in any other pathogen (Bergsma et al. 2007, Jasson et al. 2007). It is believed that New Zealand has the highest rate of reported campylobacteriosis cases in the world. In 2006, the notification rate was 422 per 100,000, thus an increase of 56% from 2001, and 14% since 2005. This is 35 times higher than the rate for the United States, four times higher than the rate for Australia, and five times higher than the rate for the U.K. One possible reason for the high rate of campylobacteriosis in New Zealand could be that local strains have a greater ability to survive under processing, storage, and handling conditions (Hansen et al. 2003).

The objective of this study was to investigate the oxygen tolerance of selected New Zealand isolates of *C. jejuni* under varying conditions of oxidative stress and temperature. The strains chosen were those commonly found in human cases of campylobacteriosis and contaminated food samples in New Zealand. Thus, the study will focus on those relevant isolates in order to provide a fast response for the scientific community and risk managers in New Zealand in regard to the isolates' aerotolerance and resistance to oxidative stress. A comprehensive study using more New Zealand isolates and international isolates is planned if those chosen isolates show aerotolerant or resistance to oxidative stress. Tough New Zealand biosecurity measures regarding the importation of biological hazards and the resulting possible delays in the administrative process have prevented the inclusion of any international isolates in this study.

Materials and method

Culture growth and enumeration

Cultures included strains isolated from poultry and humans. The multilocus sequence typing profile for these strains were ST-474, ST-48, ST-190. These three strains were implicated in more than 42% of campylobacteriosis cases in the Manawatu study (French 2008). Strains were stored at –80°C on beads (Microbank, Pro-Lab Canada); they were subcultured onto Columbia Blood Agar (CA) and incubated at 41.5°C for 48 hours in a microaerobic at-

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mosphere within a specialized work station (Forma System model 1024, USA) supplied with a gas mixture of 5% O_2, 10% CO_2 and 85% N_2. This atmosphere was used in all subsequent incubations in this study. Colonies grown on CA were subcultured on to another CA plate and incubated for 48 hours in a microaerobic atmosphere at 41.5°C to obtain a plate with prolific growth for the preparation of the inoculum. Each inoculum for each experiment was prepared by transferring all colonies from a CA plate to BHI. The culture concentration used for inoculation was in the range of 10^7 to 10^9 CFU/ml.

The determination of the inoculum concentration was conducted by serial dilution of the inoculum in Brain Heart Infusion broth (BHI) followed by plating in duplicate on modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA) using the Surface Plate method (Downes and Ito 2001). Plates were incubated microaerobically at 41.5°C for 48 hours, as described above.

Exposure to oxygen in broth

The method of Chan et al. (2001) was modified in this experiment. This experiment added the shaking of the flasks during incubation and the use of special bung with the flasks. Inoculums of a volume of 3ml were added to 27ml BHI, which was then dispensed to form a shallow layer of 30ml in a 125ml conical flask equipped with a special bung (Bug stopper, Whatman), allowing air exchange with the surrounding atmosphere whilst preventing microbial contamination. This flask was allowed to equilibrate at the appropriate temperature and atmosphere (aerobically or microaerobically) by gentle shaking using an electrical shaker (Lab-line Junior Orbit Shaker, U.K). The number of surviving organisms in samples withdrawn from each flask at predetermined intervals after exposure to the controlled atmosphere conditions at each temperature (4, 10, and 20°C) was determined by dilution in BHI, followed by plating on mCCDA. Duplicate plates were made at each dilution. The plates were incubated microaerobically within a specialized work station (Thermo scientific, USA) continuously supplied with 5% O_2, 10% CO_2, and 85% N_2 at 41.5°C for 48 hours.

Exposure to oxygen on agar plates

In order to increase the validity of the results reported by the above broth experiment and save time, resources, cost, and the complexity of conducting long studies on the survival of *C. jejuni* in food matrix as was designed in this study Garenaux et al. (2008) agar plates method to investigate oxygen tolerance was adapted in this experiment. 50 µl of the culture suspension in BHI was spread on CA plates (blood-free 16 to 40 plates for each isolate at the specified incubation temperature according to the previous primary trial data collected before the actual experiment) using a spiroplater (Don Whitley, Yorkshire, U.K) and incubated aerobically at 4°C and 25°C for 1 to 5 weeks. The spread CA control plates were simultaneously incubated under microaerobic conditions at all of the above temperatures using anaerobic plastic jars with a microaerobic atmosphere generating system (Pack MicroAero, Mitsubishi Gas Chemical Co. Inc). Two plates from each aerobic condition were sampled every day and incubated at 41.5°C for 48 hours in a microaerobic atmosphere within a specialized work station (MACS VA 500, Don Whitley Scientific U.K).

Exposure to H_2O_2

Each culture suspension prepared in BHI was spread with a swab onto Muller Hinton Agar plates and allowed to grow under microaerobic conditions for 48 hours. Subsequently, filter discs (6 mm) inoculated with 10 µl of 1 mM, 10 mM, 100 mM, or 1 M hydrogen peroxide were placed onto the plates as per the Disc Diffusion Method (Fields and Thompson 2008). These plates were then incubated at 41.5°C under microaerobic conditions for 48 hours. Three plates were prepared each time, and the experiment was repeated once for each tested isolate.

Statistical analysis

All bacterial counts were log10 transformed prior to statistical analysis. The dataset was analysed using R software (version 2.9.1). Statistical differences between isolates, incubation atmosphere, time, and temperature were undertaken by ANOVA, with P = 0.05 used as the statistical threshold for significance for only the survival experiment in BHI, due to the level and amount of data generated from the experiment. The survival data then fitted to the conventional log linear linear model by Equation (1). using R software programme (the R code is provided in the appendix).

$$\frac{N_0}{N_t} = \frac{t}{D} \tag{1}$$

Where N_0 is the initial count of bacteria, N_t is the log of *C. jejuni* counts at time t (days) and D is the decimal reduction time. This is the time required to reduce the bacterial count by a factor of 10 and is equal to the number of days for the survival curve to traverse one log cycle.

In addition to the log linear model, the Weibull model expressed by Equation (2). (Cunha et al. 1998, Fernandez et al. 1999, Mafart et al. 2002, Peleg 2002) fitted to the survival data. The Weibull model has been the most widely studied and has been successfully tested using a variety of published survival studies in which it was concluded that the Weibull model was both more suitable and simpler for describing non-log-linear survival curves than other models (van Boekel 2002).

$$\log_{10} N_t = \log_{10} N_0 - \left(\frac{t}{\delta}\right)^{\eta} \tag{2}$$

where, δ is the storage time (days) for the first log reduction, and η is the shape parameter. The shape parameter gives a convex curve when η>1, or a concave curve when η<1. When η is equal to 1, a straight line corresponding to the log-linear model is obtained. A detailed comparison between the log-linear and the Weibull by using some statistical criteria such as the likelihood ratio test (LRT) was performed in the analysis. The LRT is considered as the best comparision test statistically as small p-value shows that the improvement in going from the simple model (log linear) to the more complex model (Weibull) is statistically significant. The Akaike information criterion (AIC) for both models fitting comparison is reported because AIC accounts for the difference in the number of parameters in each model and the classical R^2 values for each model was presented.

Results

Exposure to oxygen in broth

The results from exposure to oxygen in BHI from the duplicate plates at all temperatures (4°C, 10°C and 20°C) are

shown in Figure 1–3. All constructed linear model and Weibull models for each data set were shown in figures 4–6. The output of ANOVA analysis is summarised in Table 1. The ANOVA analysis confirms that temperature has significant effect in *Campylobacter* survival where the atmosphere has less effect on *Campylobacter* tested isolates survival. At every temperature, the poultry isolate survived longer than the human isolates. The human isolates did not show differential survival between the atmospheres especially at 4°C suggesting they were less sensitive to oxygen than the poultry isolates. The *D* values reported in Table 2 for human at 4°C were similar to *D* values of poultry isolates.

The parameters of both fitted models and the comparison of goodness of fit criteria were reported in Tables 2–4. The Weibull model produced better fits in most of the survival data than the log-linear model based on the goodness of fit criteria employed such as the likelihood ratio test (LRT). It northworthy to state that *p* value in most cases was < .001. The Akaike information criterion (AIC) also has indicated that Weibull fits better for the most of the survival data in tables 2–4. The Weibull parameter p (the shape parameter) in most curves were >1 indicating convex survival curves.

Exposure to oxygen on agar plates

The results from exposure to air in agar plates indicated that survival was significantly longer at low temperatures than high, but on agar, both poultry and human isolates survived longer microaerobically than aerobically. The data are summarised in Table 5. There was no significant difference between the survival of the poultry and human isolates when differences in the initial concentrations are accounted for.

Exposure to H_2O_2

The exposure to H_2O_2 revealed that all the selected NZ *C. jejuni* strains (ST-474, ST-48, and ST-190) tested are sensitive to all H_2O_2 concentrations tested, except for the lowest concentration of 1 mM (Figure 7).

Discussion

Most of the data reported and the *Campylobacter* survival studies in the ComBase database for predictive microbiology were either for a short period exposed to a specified temperature or were investigating more than two factors (temperature, atmosphere) targeted by this study, such as the addition of NaCl, CO_2, and other stress factors.

Therefore, the comparison of our results to the international data which used similar experimental conditions to this study is limited. The results obtained in this study

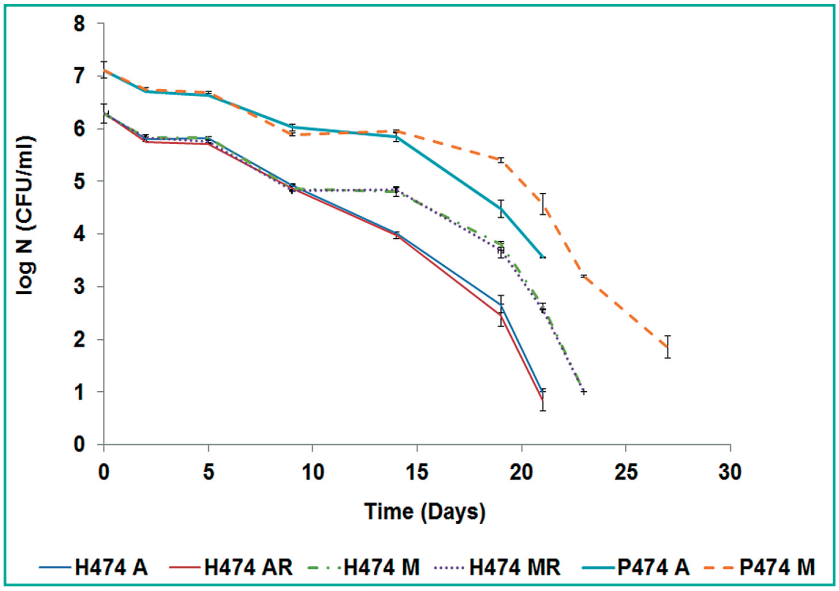

FIGURE 1: *Survival of C. jejuni isolates at 4°C (A: aerobic incubation, M: microaerobic AR/MR: aerobic/ microaerobic replicate data).*

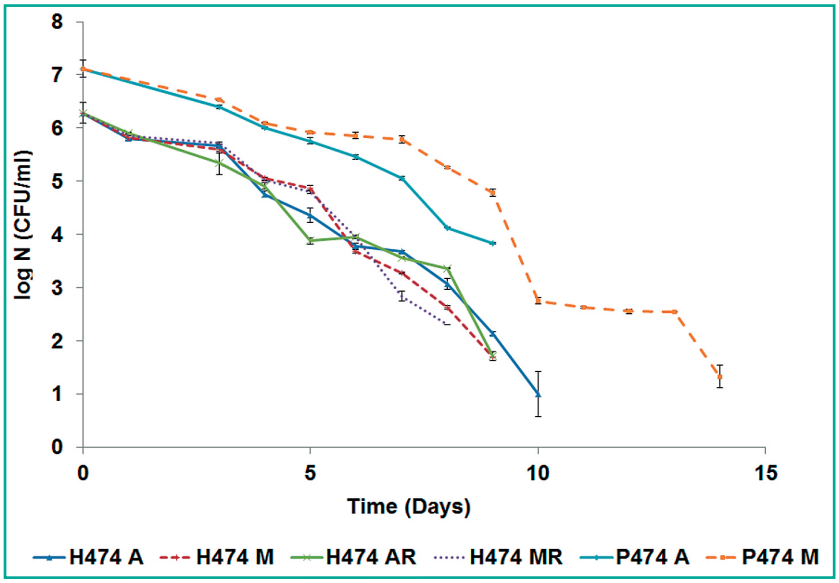

FIGURE 2: *Survival of C. jejuni isolates at 10°C (A: aerobic incubation, M: microaerobic AR/MR: aerobic/ microaerobic replicate data).*

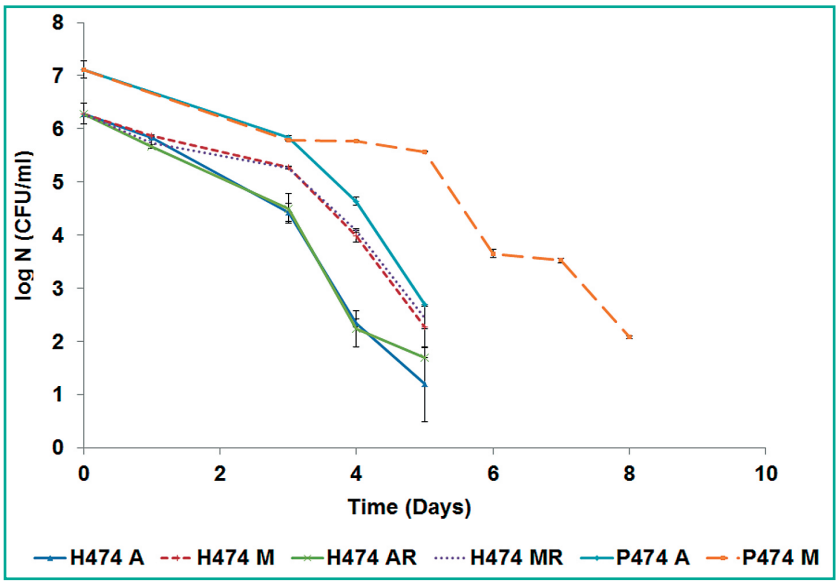

FIGURE 3: *Survival of C. jejuni isolates at 20°C (A: aerobic incubation, M: microaerobic AR/MR: aerobic/ microaerobic replicate data).*

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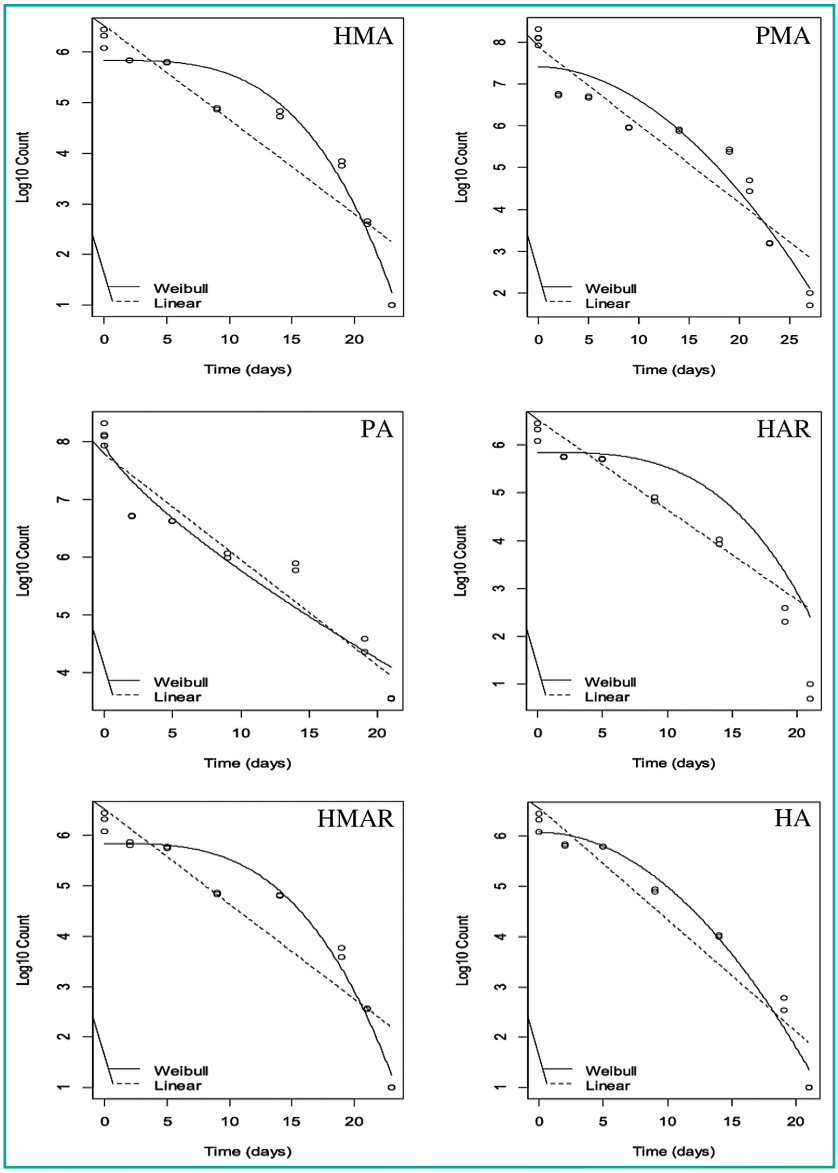

FIGURE 4: *The log linear and Weibull model fits to survival data of Campylobacter jejuni 474 isolates in broth stored at 4°C.*

are in agreement with Garenaux's result (Garenaux et al. 2008) especially at 25°C and at 4°C for the first week of our agar plate data experiment, and also with Chan's et al. and Gozález et al.results (Chan et al. 2001, González et al. 2009) at 4°C. The results obtained in our study at 4°C are in agreement with the results obtained by Yoon (2004) in broth at the same temperature aerobically in terms of the first reduction, which occurred after nine days of incubation. However the same reduction occurred after 11 days of incubation in the Yoon study, and this marginal difference is possibly due to the agitation process in this study and the absence of the agitation process in Yoon's study. As it is known that agitation creates strictly aerobic conditions, which may enhance the pathogen death rate in this study (Butzler 2014, Meredith et al. 2014). Yoon's study was planned to simulate semi aerobic conditions or mixed aerobic and anaerobic conditions. The storage time (days) for the first log reduction (δ) at 4 °C in this study in broth is in agreement with results obtained by Oyarzabal et al. (2010) on chicken meat. However, δ at 10°C in this study in broth (2–4 days) differs from the values (5–8 days) obtained by Oyarzabal et al. (2010) at 12°C on chicken meat.

Moreover, our results are in agreement with New Zealand data for chicken (Chynoweth et al. 1998) although the chicken meat as a substrate or a medium may partially influence the results of that study (Chynoweth et al. 1998) . In fact, New Zealand strains survive less as well than the international strains, especially in the range of 20–5°C. One study reported that survival at 23°C in sterilised ground chicken was for more than three weeks (Blankenship and Craven 1982), whereas New Zealand strains investigated in our study survived aerobically at 25°C only for 4 to 5 days in the agar plates, and up to 7 days at the same temperature in microaerobic environments. This can be explained by the use of a different strain for that study or the use of a different matrix.

In general, there were not many differences in hydrogen peroxide sensitivity between the poultry and human isolates of each strain. These results are in agreement with a study published internationally with regards to the diameter of the inhibition zone (Fields and Thompson 2008). Despite the report that *C. jejuni* has a good adaptive ability to hydrogen peroxide stress (van Vliet et al. 2002), our results confirmed that the tested NZ *C. jejuni* strains are sensitive to hydrogen peroxide by the method used in this study. The results confirmed that the tested NZ *C. jejuni* strains are sensitive to hydrogen peroxide.

The investigation of the New Zealand strains tested in this study by three methods revealed that the air tolerance of New Zealand strains was not different from that of

TABLE 1: *Results of two-way analysis of variance for effects of storage atmosphere, isolates, and temperature on survival of C. jejuni in broth.*

	numDF	denDF	F-value	p-value
(Intercept)	1	228	20015	<.0001
Isolates	1	54	160	<.0001
Atmosphere	1	54	2	0.1638
Temperature	2	54	40	<.0001
Time	1	228	1411	<.0001
Isolates:Atmosphere	1	54	3	0.1145
Temperature:Time	2	228	219	<.0001
Isolates:Time	1	228	14	0.0002

the international strains (ICMSF 1996, Chan et al. 2001, Oyarzabal et al. 2010, Oh et al. 2019) and the previously tested strains of a previous study in New Zealand (Chynoweth et al. 1998). The results of this study did not support the hypothesis that the survival of *C. jejuni* was not influenced by the storage atmosphere aerobically or anaerobically, as the survival of *C. jejuni* was shorter in the agar and the broth experiments under aerobic conditions than under the microaerobic conditions at all temperatures tested, except in the broth experiment at 10°C. Whereas after the fifth day, the survival rate was more or less similar at aerobic or anaerobic conditions. This is consistent with other reports regarding the growth and adaptation of *C. jejuni* to aerobic metabolism (Oh et al. 2015). However, the best known and fundamental hypothesis that *Campylobacter* is a microaerophilic microorganism that requires specific gas composition for its growth and survival (ICMSF 1996, Kelly 2008, Gharst et al. 2013, Butzler 2014).

Information on the biological aspects and mechanisms responsible for microaerobic growth or better survival at a molecular level, in order to understand what takes place from a physiological and genetic perspective, is not yet completely understood. It is assumed that due to the inhibition of enzymes by a higher concentration of O_2 (more than the maximum level 10%), the vulnerability to reactive oxygen species (ROS) and/or metabolic generation of free radicals are capable of reacting with molecular components of cells and disturbing their function as well as producing more toxic components (Krieg and Hoffman 1986, Kaakoush et al. 2009). The poultry isolates survived longer than the human isolates, especially at the incubation temperatures of 10 and 20°C, and there was no significant difference between the survival of the poultry and human isolates when differences in the initial concentrations are accounted for at incubation at 4°C.

It is revealed from this study that *C. jejuni* survival under oxidative stress is more influenced by the temperature than the incubation atmosphere. The low temperature at 4°C had less effect on the survival of *C. jejuni,* and it was able to survive more than four weeks in both the broth experiment and agar experiment. This was assumed by Gareneux et al. (2008) and Hazelegar et al (1998) to be due to a less active metabolism and a decrease in catalytic activity, or it may be that the oxygen may become less toxic for cells at 4°C. Despite the fact that this study was not conducted

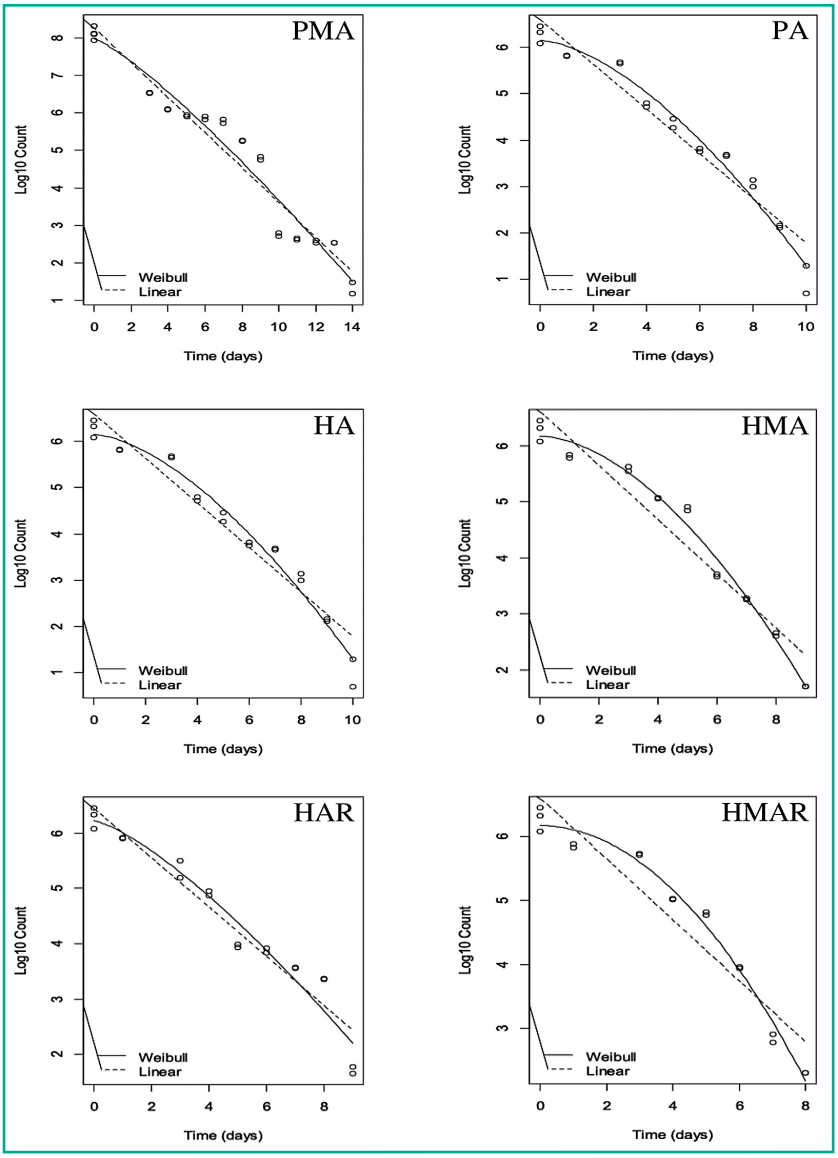

FIGURE 5: *The log linear and Weibull model fits to survival data of Campylobacter jejuni 474 isolates in broth stored at 10°C.*

TABLE 2: *Weibull and log linear models parameters for survival of Campylobacter jejuni isolates in broth stored at 4°C.*

	Weibull				Linear			LRT	
	Delta (days) SE	P SE	R^2	AIC	D (days) SE	R^2	AIC		
HMA	14.6668 1.0636	3.3876 0.5633	0.95	22.75	5.3610 0.5363	0.87	37.82	2.26E-04	***
PMA	11.2477 1.8213	1.9037 0.3629	0.91	42.18	5.3415 0.4388	0.89	44.14	0.07	
PA	3.5591 1.2688	0.7625 0.1452	0.93	24.19	5.4683 0.4247	0.92	23.51	0.31	
HAR	14.2950 1.0693	3.2102 0.5194	0.96	21.87	5.3079 0.5140	0.88	37.05	2.15E-04	***
HMAR	14.2950 1.0693	3.2102 0.5194	0.96	21.87	5.3079 0.5140	0.88	37.05	2.15E-04	***
HA	9.5886 0.9536	1.9810 0.2464	0.98	11.92	4.4859 0.3433	0.93	26.48	3.55E-04	***

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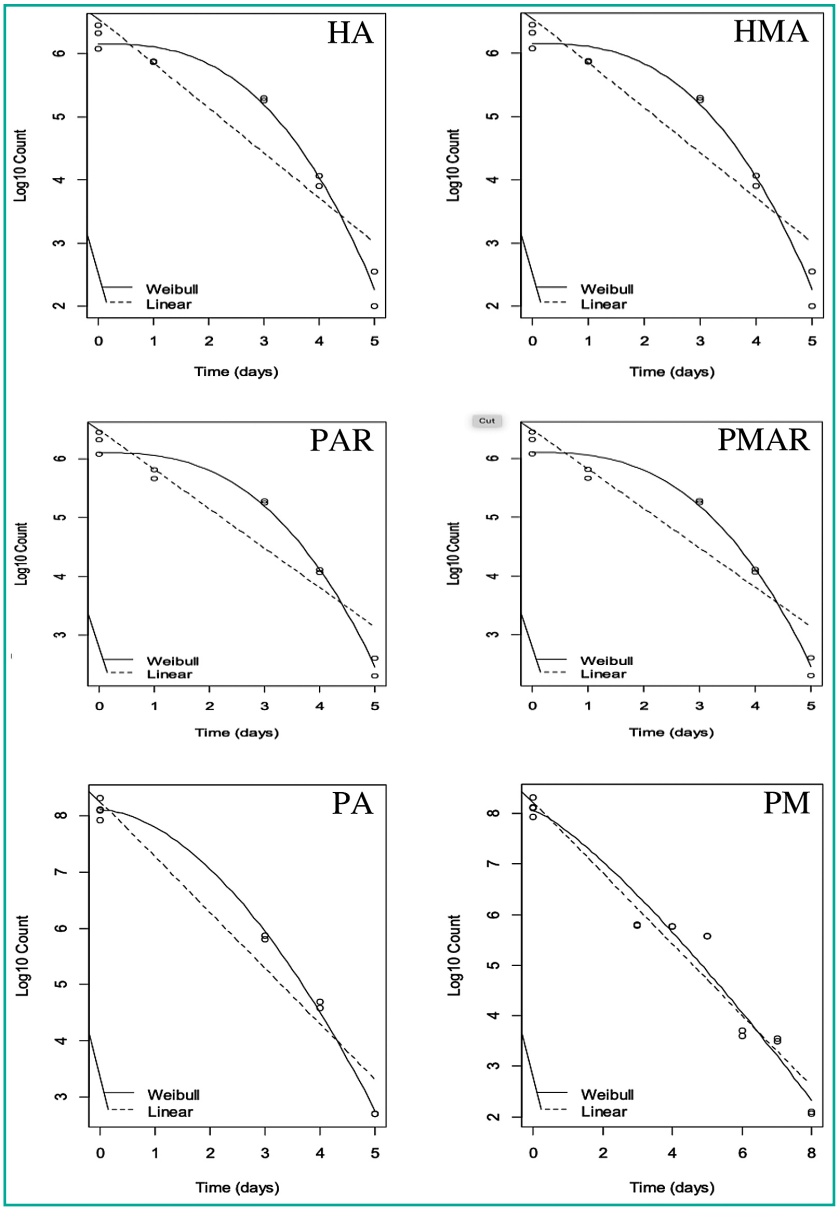

FIGURE 6: *The log linear and Weibull model fits to survival data of Campylobacter jejuni 474 isolates in broth stored at 20°C.*

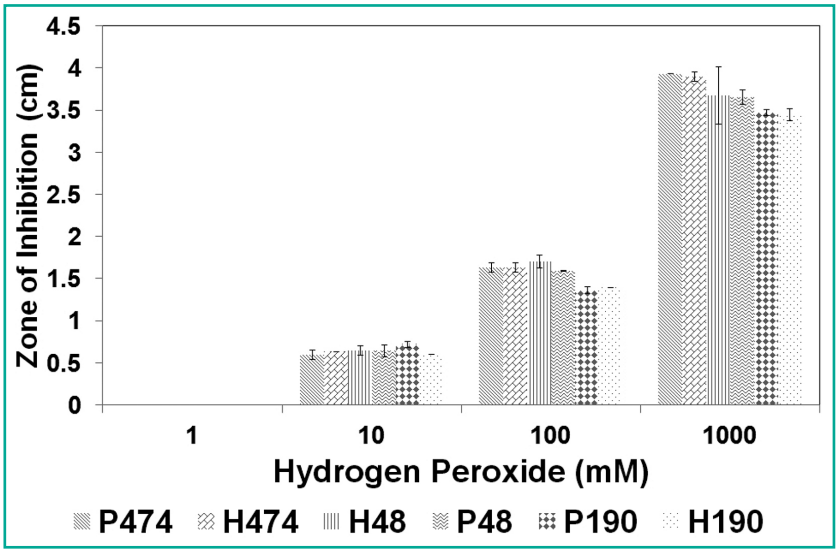

FIGURE 7: *Susceptibility of C. jejuni isolates to different hydrogen peroxide concentrations in filter discs on MH agar.*

on chicken meat, the combination of three methods to address the oxidative stress on *C. jejuni* has increased the validity of the results reported by this study and saved time, resources, and cost.

Practically, most international data is on the survival of *C. jejuni* for short periods and especially for above freezing temperatures, for example; for two days (Solow et al. 2003, Zhou et al. 2011) or for one week to two weeks (Bhaduri and Cottrell 2004, Davis and Conner 2007, Garenaux et al. 2008, Oyarzabal et al. 2010). Moreover, the results obtained by this study were in agreement with the results obtained in New Zealand with chicken mince and chicken nuggets (Chynoweth et al. 1998). For example, few studies (Byrd et al. 2011, Meredith et al. 2014) evaluated the effects of modified storage atmosphere and chilling on the survival of naturally occurring *Campylobacter* on raw poultry. The modified packaging atmosphere applied for the stored chicken at 2°C was 100% O_2, normal air, 85% N_2+10% CO_2+5% O_2, and 100% CO_2 (Byrd et al. 2011). *Campylobacter* was not detected after 14 days of storage in 13 chicken samples out of 16 chicken samples, which were stored with 100% O_2. Similarly, in 12 chickens, *Campylobacter* was detected in the chicken treated with normal air. *Campylobacter* was detected in nine chicken samples treated with 85% N_2+10% CO_2+ 5% O_2. However, only in 7 chicken samples, was *Campylobacter* was detected when the chicken was treated with 100% CO_2 (Byrd et al. 2011). Therefore comparison of the results of this study is limited to the very few international data which used similar experimental conditions to this study.

Similarly, the results obtained by Garnaux (Garenaux et al. 2008) using the Colombia agar plates were similar to the results obtained by Bhaduri and Cottrell (2004) at 4°C using chicken mince or chicken skin. In fact, the implications of the spoilage of chicken meat samples or chicken skin samples during the storage of the samples above 4°C hinders the use of a food matrix in long survival studies. Moreover, the use of irradiated skin or chicken meat samples eradicates the microflora (pseudomonads, micrococci, Staphylococci), which are found naturally on poultry, and inhibits the growth of *Campylobacter* (Mai 2003). The conclusion from such studies is that irradiated chicken meat must be treated with caution. Thus, the results of this study are considered as acceptable in rejecting the hypothesis investigated in this study that the high rates of campylobacteriosis in New Zealand may be due to the emergence of unusual new strains with more oxygen tolerance or that they have a unique survival ability under storage or handling temperatures (4, 10, 20, 25°C).

Given the infection dose of illness is about 500 cells (Robinson 1981) and the length of time that *Campylobacter* can survive, as revealed by this study for New Zealand strains, this is a significant finding from a health perspective as the shelf life of a fresh chicken is eight days at 4°C and *Campylobacter* can survive for more than four weeks at that temperature. Thus, the contamination level of fresh poultry products will remain without any significant reduction until introduction into a consumer's kitchen, and this may pose a risk to consumers. Poultry processing plants should apply the necessary intervention to ensure that chicken carcasses and poultry products be released to the retail market with as low a contamination level as possible or practical, and theoretically, the counts should not exceed 500 cells per serving of chicken portion.

The linear model constructed by this study is acceptable given the empirical nature of the Weibull model with its two parameters (shape η and scale δ). The results obtained from all the oxidative stress conditions are similar and do not indicate that the New Zealand strains differ in oxygen tolerance or survival from most of the other internationally reported strains at the investigated temperatures. At 20 or 25°C, New Zealand selected strains survived in media only up to one week aerobically, but at 4°C, they survived aerobically for more than three weeks. New Zealand's higher rate of reported campylobacteriosis compared to other developed countries is possibly due to other factors that are explored in other studies (Al-Sakkaf 2012, Al-Sakkaf 2013, Al-Sakkaf 2013, Al-Sakkaf 2020).

TABLE 3: *Weibull and log linear models parameters for survival of Campylobacter jejuni isolates in broth stored at 10°C.*

	Weibull				Linear			LRT	
	Delta (days) SE	P SE	R^2	AIC	D (Days) SE	R^2	AIC		
PMA	2.9927 0.4988	1.2111 0.1243	0.95	44.26	2.1475 0.1017	0.94	45.59	0.09	
PA	1.9282 0.2268	0.9268 0.0701	0.98	-3.02	2.1513 0.0696	0.98	-3.91	0.34	
HA	3.7221 0.3359	1.5990 0.1410	0.98	10.63	2.0768 0.1184	0.94	26.40	1.16E-04	***
HMA	3.8352 0.2402	1.7534 0.1248	0.99	-2.43	2.0649 0.1330	0.93	23.93	1.43E-06	***
HAR	3.1775 0.4658	1.3351 0.1791	0.95	17.05	2.2470 0.1396	0.94	19.33	0.06	
HMAR	3.9767 0.2375	1.9815 0.1644	0.98	-1.38	2.0931 0.1713	0.91	24.38	1.43E-06	***

TABLE 4: *Weibull and log linear models parameters for survival of Campylobacter jejuni isolates in broth stored at 20°C.*

	Weibull				Linear			LRT	
	Delta (Days) SE	P SE	R^2	AIC	D (Days) SE	R^2	AIC		
HA	3.0321 0.1754	2.72 0.31	0.98	3.21	1.4050 0.1822	0.87	23.31	9.37E-05	***
HMA	3.0321 0.1754	2.7180 0.3077	0.98	3.21	1.4050 0.1822	0.87	23.31	9.37E-05	***
PAR	3.1006 0.1909	2.7090 0.3406	0.98	4.08	1.4902 0.1872	0.88	21.32	2.70E-04	***
PMAR	3.1006 0.1909	2.7090 0.3406	0.98	4.08	1.4902 0.1872	0.88	21.32	2.70E-04	***
PA	1.93881	1.77150	1.00	-5.65	1.00910	0.96	16.95	5.50E-05	***
	0.10034	0.09894			0.07315				
PM	1.9840 0.3416	1.2522 0.1539	0.96	23.33	1.42038 0.08292	0.95	24.48	0.12	

TABLE 5: *Effect of temperature and storage atmosphere conditions on survival (in days) of selected New Zealand C. jejuni strains on agar plates.*

Isolates/strain	At 4 °C		At 25 °C	
	Aerobic	Microaerobic	Aerobic	Microaerobic
ST-474$_H$	21	>32*	6	7
ST-474$_P$	18	23	4	7
ST-190$_H$	22	>32	5	6

* The experiment was ended at 32 days. (ST-474$_H$ refers to human isolate and ST474$_P$ refers to poultry isolate)

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Conflict of interest

There is no conflict of interest exist.

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Address of corresponding author:
Ali Al-Sakkaf
Faculty of Agriculture & Life Sciences
PO Box 85084, Lincoln University
7646 Christchurch
New Zealand
ali.al-sakkaf@lincolnuni.ac.nz