The spread, persistence and survival of *Salmonella* and *Campylobacter* in the domestic kitchen

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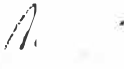## September 2002

A thesis submitted to the University of Wales in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the School of Applied Sciences at the University of Wales Institute Cardiff

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree

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Abstract

This thesis investigates the exposure risk of *Salmonella* and *Campylobacter* following the preparation of a chicken salad in domestic kitchens.

Methods were first devised, developed and validated to maximise recovery of cells, including those sub-lethally damaged, in order to accurately assess exposure routes. Isolation rates of *Campylobacter* were maximised by delaying the addition of rifampicin and polymyxin and prolonging the incubation period of broths. It was found that isolation of *Salmonella* was improved when sulphamandelate was added to the pre-enrichment broth.

Pilot work, utilising these improved isolation methodologies, was then carried out in a test domestic kitchen to determine likely exposure routes and commonly contaminated sites during the preparation of a meal by 30 participants. Using the techniques refined in the pilot study, the food preparation practices of 70 participants were studied preparing the same salad in their home. A total of 609 samples were taken and contamination by *Campylobacter* was found to be relatively common with 13% of participants contaminating the kitchen or the prepared salad. The raw chicken breasts used to prepare the salad were the most significant source of contamination; 90% were contaminated with high numbers of *Campylobacter*, 6% were contaminated with *Salmonella*. Commonly contaminated items included the salads and wiping cloths. The most common exposure routes were due to the inadequate washing / drying of hands, chopping boards and knifes.

In order to accurately assess the risks associated with the widespread contamination of *Campylobacter* spp., their ability to survive on simulated kitchen work-surfaces was compared with *Salmonella* spp.. *Salmonella* was found to survive significantly better than *Campylobacter* after two hours of air drying ($P= 0.001$). Differences in the ability of some *Campylobacter* strains to survive air drying were also found to be significant.

It is suggested that the results of this thesis could be used to determine exposure assessment and quantitative risk assessment in the domestic kitchen in order to prioritise and target food safety messages.

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Chapter 1. Introduction

Numerous studies have examined the consequences of kitchen malpractices in artificial settings but this is one of the first to combine food preparation practices in domestic kitchens with the examination of the spread of pathogens during preparation. Exposure routes associated with the preparation of a poultry-based meal were studied with specific reference to the spread, persistence and survival of *Salmonella* and *Campylobacter* spp.. These important food borne pathogens infect large numbers of people each year, and it is, therefore, important that exposure assessment data are available in order to make accurate risk assessments of the handling and preparation of poultry in the home. In Chapter two these two organisms are introduced and details about them, including symptoms, sources and routes of infection are discussed.

In Chapter three isolation methodologies for the two target organisms (*Salmonella* and *Campylobacter*) are developed. *Campylobacter*, particularly, does not survive well on foods at room temperature (Blankenship & Craven 1982) and so may only be isolated in low numbers. Even low numbers of cells have the potential to cause infection (Robinson 1981) and may still represent a risk, it is therefore, important that these cells are isolated. It is also possible that the low isolation rate of *Campylobacter* from foods may be a result of the use of unsuitable isolation methods. Cells may be sub-lethally injured and demonstrate an increased sensitivity to antibiotics present in isolation media. In Chapter three the delayed addition of antibiotics is investigated, as is the effect of prolonging the incubation period in enrichment broth, allowing maximum time for low levels of damaged cells to recover.

A number of methods are currently available to isolate *Salmonella* from food products and the environment (Anon 2001a; Fricker 1987) and in Chapter three a range of these techniques are examined. The most sensitive and specific isolation methodologies were validated using *Salmonella,* which were injured by surface drying and chilling, as well as samples heavily contaminated with other micro-flora. The use of sulphamandelate in the pre-enrichment broth is examined and results from this validation work have been published (Cogan *et al.* 2002).

Chapter four is concerned with piloting the isolation techniques developed in Chapter three and determining common exposure routes and sites of contamination. Thirty participants (10 mothers with children < 10 years old; 10 single men aged 18 – 24 and 10 older participant aged 60 - 75 years) were asked to prepare a chicken salad in a test domestic kitchen. Throughout the food preparation session the hygiene practices of the participants were observed and any potential routes of contamination recorded. All raw materials entering the test kitchen were analysed for the presence of *Salmonella* and *Campylobacter.* Numbers of *Enterobacteriaceae* and aerobic colony counts were also determined. After the preparation of the meal, selected areas of the kitchen were sampled and analysed for *Salmonella* and *Campylobacter,* and the number of *Enterobacteriaceae* present was determined on a selection of these samples. *Campylobacter* and *Salmonella* isolates were typed using standard methodologies and potential exposure routes were determined based on the microbiological and observational results. In Chapter four the exposure routes of each contaminated area or item is discussed, as are the contamination rates of the three groups of participants.

Having established appropriate sample sites and contamination routes in Chapter four, Chapter five is concerned with obtaining exposure assessment data after the preparation of a chicken salad in domestic homes. Seventy participants, from a range of socio-economic groups, were recruited and their actions during the food preparation session were recorded. Risk scores were determined for each of the participants based on kitchen malpractices. The presence of *Salmonella* on the raw chicken was assessed and numbers of *Campylobacter* present were determined. Selected sites were analysed for the presence of *Salmonella* and *Campylobacter* and strains isolated as a result of cross contamination were typed along with an isolate from the raw chicken used in the food preparation session. Potential exposure routes were confirmed based on microbiological and observational results. Contamination of individual areas / items is discussed in relation not only to the observed meal preparation but also in relation to the hygiene of the kitchen prior to the food preparation session. The additional data collected within this chapter means that, rather than concentrating on isolated incidences, the overall kitchen hygiene of participants could be examined in relation to cross contamination events. The effect that *Campylobacter* numbers on the raw chicken breasts had on cross contamination

in the domestic kitchen was also discussed. A paper containing results from this chapter has been published (Mattick *et al.* 2003).

Up to this point in the thesis the cross contamination of *Salmonella* and *Campylobacter* has been associated with the actions of participants. In order for these organisms to be isolated after a contamination incident, or to cause food poisoning, they must also be able to persist in the environment. Given the great potential for cross contamination in domestic kitchens (Chapters four and five), and the sequential use of kitchens by different household members (Griffith *et al.* 1999), even limited survival could lead to the contamination of subsequent meals. In Chapter six the ability of *Salmonella* and *Campylobacter* to persist on a kitchen work surface is investigated and the differences discussed. The survival of *Campylobacter* strains isolated from areas within the kitchen after a contamination incident are compared to strains isolated from raw chicken breasts. The ability of the different strains to survive air-drying and a possible link to persistence and sero/phage type is examined.

In Chapter seven the key points from each of the individual chapters are drawn together to discuss the spread, persistence and survival of *Salmonella* and *Campylobacter*. Conclusions are drawn and ideas for future work put forward. A paper combining conclusions from this investigation with results from other projects has recently been published (Humphrey *et al.* 2001a). A report containing results from this investigation has also been submitted to the Food Standards Agency (Redmond *et al.* 2001).

Chapter 2. Literature review

2.1   Food poisoning

The Department of Health's Advisory Committee on the Microbiological Safety of Food (ACMSF) has defined food poisoning, as "any disease of an infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water" (Department of Health 1994). This definition encompasses bacterial, mycotic, viral and helminthic infections as well as bacterial or chemical toxins present in food. Due to the rarity of chemical poisoning in the UK (<1% of all food-poisoning episodes; Gilbert & Humphrey 2001) it will not be discussed in further detail.

Food poisoning incidences have risen dramatically in England and Wales in recent years from 52,543 cases in 1991 to 85,468 in 2001 (Anon 2002a). It is generally considered to be a short lived illness, causing nothing more than gastroenteritis, but it can cause substantial morbidity. Infectious intestinal disease is responsible for 35,000 hospital admissions in England and Wales annually (Djuretic *et al.* 1996). Commonly implicated food poisoning agents include *Salmonella, Campylobacter, Bacillus cereus* and the widely publicised *E. coli* 0157:H7. Viruses such as Norwalk-like viruses and rotavirus may also be transmitted via food, although the majority of cases are transmitted by person-to-person contact.

Salmonella and *Campylobacter* are the leading causes of bacterial gastroenteritis with the number of reported cases exceeding 16,000 and 56,000 respectively in 2001 (Anon 2002a), and it is due to their relatively high prevalence that they were chosen for investigation in this study. In contrast to *Salmonella* cases, which are frequently associated with outbreaks, *Campylobacter* tend to be associated with sporadic cases of food poisoning (Ryan *et al.* 1996; Tirado & Schmidt 2000). Cases of *Campylobacter* have steadily increased over the last two decades, possibly due to improved isolation methods in laboratories and an improvement in notification. An increase in the consumption of fresh, rather than frozen, chicken has also been linked to the increase (Hood *et al.* 1988). In contrast, the number of reported *Salmonella* cases appear to be declining. This has not always been the case; in the mid 1980's and 90's the numbers

of reported cases, in England and Wales, were on the increase predominantly due to an increase in the isolation rate of *Salmonella* Enteritidis PT4 (Humphrey 2000), commonly associated with poultry meat and eggs. Although this organism still remains problematic, the slaughter of breeding flocks infected with *Salmonella* Enteritidis, and widespread vaccination of egg laying flocks is believed to have resulted in a reduction of *Salmonella*-positive poultry carcases and eggs entering consumer kitchens (Anon 2001b; Humphrey 2001a) which is reflected in the decrease in the number of cases. Although numbers of *S.* Enteritidis PT4 appear to be decreasing the ubiquitous nature of *Salmonella* and the large number of different sero- and phage types means that the problems of *Salmonella* infection are far from being under control. *Salmonella* strains resistant to multiple antibiotics, notably *S.* Typhimurium, are emerging in the UK (Frost *et al.* 1995) probably as a result of the over use of antibiotics in food animals as growth promoters and for prophylactic use. Such anti microbial resistance is not confined to *Salmonella*; in the early 1990's fluoroquinolone resistance was recorded in *C. jejuni* isolates in Europe (Altekruse *et al.* 1998) and antimicrobial-resistant infections are common in travellers retuning from developing nations.

A recent study (Wheeler *et al.* 1999) has indicated that the majority of bacterial food borne cases are under reported and that the actual figures may be much higher; three fold for *Salmonella* and up to eight fold higher for *Campylobacter*. These discrepancies are believed to be due to the proportion of participants seeking medical advice, only a subset of which will submit a specimen for analysis, and relies on the laboratory to isolate and identify the pathogen and the subsequent submission of an accurate report by laboratories (Tompkins *et al.* 1999).

It is all too easy to assume that the high incidence of food poisoning is due to poor hygiene practices in the kitchen. It should be remembered, however, that hygiene errors would not cause infection if the foodstuffs entering the kitchen were free from pathogens. The government and industry must all take some responsibility to ensure that all reasonable steps have been taken to reduce or eliminate contamination (Anon 1997) and consumers also need to be aware that many foods are not pathogen-free and that once purchased some responsibility lies with them.

2.2   The organisms

2.2.1   Salmonella

Salmonella are a genus of the *Enterobacteriaceae* group of bacteria consisting of Gram- negative rod shaped cells.   They have an ability to grow over a wide range of temperatures ranging, 7 to 48 °C, and from pH 4 to 9 (Baird-Parker 1991; Mattick & Humphrey 2000).   They are facultative anaerobes and grow in aerobic and anaerobic conditions on standard growth media. In order to distinguish them from other *Enterobacteriaceae* a wide range of selective media has been developed. Direct inoculation onto selective solid media may be sufficient to isolate *Salmonella* from samples containing high levels of cells, such as faeces, but for those in which cells are likely to be present in low numbers or which have suffered sub-lethal injury, isolation may involve several steps. These include pre-enrichment in a nutritious, non-selective broth aimed to promote maximum recovery, enrichment in a selective broth, which allows growth of *Salmonella* but suppresses that of competitors, and isolation by streaking onto a selective agar.

Salmonella can be presumptively identified based on their biochemical characteristics (D'Aoust 1997), being oxidase negative and catalase positive and using citrate as their sole carbon source.   The majority of strains produce acid and gas (a few exceptions produce only gas) from glucose and mannitol and usually from sorbitol. They do not hydrolyse urea and most organisms form H_2S on triple sugar iron agar. White (1926) developed the first sero-typing scheme based on antigenic variation of the cell surfaces and flagella. This scheme was later expanded by Kaauffmann (1966). Of the 2399 serotypes identified approximately 2000 are capable of causing disease in humans (Anon 2001c). The majority of cases in England and Wales in 2001 were caused by *Salmonella* enterica serovar Enteritidis (*S.* Enteritidis; 65%) and *Salmonella* enterica serovar Typhimurium (*S.* Typhimurium 13%; Anon 2002a).

2.2.1.1   Symptoms

Symptoms of *Salmonella* infection can vary depending on host factors, the ingested dose and strain characteristics. The incubation period is generally between 12 and 72 hours, although there have been cases were it may extend to one week. Common acute symptoms of *Salmonella* (occurring in the majority, but not necessarily all cases) include diarrhoea, abdominal pain, fevers, nausea and muscle pain. Other less common symptoms can include vomiting, headaches and, in a minority of cases, blood in stools (Humphrey 2000). These acute symptoms usually last four to seven days and recovery usually occurs without treatment (Anon 2001d). Bacteraemia occurs in about 1% of cases and subsequent infection of organs, including bone, aorta and kidneys has been reported. Although such infections can be treated with antibiotics the increase in mortality associated with these cases is significant. Chronic symptoms, generally occurring three weeks after infection, include post-enteritis arthritis and Reiter's syndrome, a form of arthritis commonly involving the joints of the spine and the sacroiliac joints (where the spine attaches to the pelvis).

2.2.1.2   Infectious dose

The number of *Salmonella* cells required to cause human illness is an area of considerable variability and uncertainty. It may be influenced by a number of factors including the general health and immune status of the host, the food matrices, strain virulence characteristics and the physiological condition of cells. For example, the very young (< 1 year old) and persons aged over 60 appear to suffer from a higher incidence of the disease than any other age group presumably due to their immature/ reduced immune responses (Blaser & Newman 1982). The gastric acidity of the host may also affect the infectious dose and anything which increases the stomach's pH, such as foods with a high buffering capacity, will decrease the cells' exposure to stomach acid and, therefore, result in more cells surviving passage through the gut. Similarly it has been suggested that fatty foods, where cells are protected within fat droplets, results in a lower infectious dose as do some foods which expose cells to

sub-lethal levels of acid and induce acid resistance (Blaser & Newman 1982; Buchanan *et al.* 2000). Given the range of factors involved, estimates of the infectious dose have been highly variable. A review of outbreak data (Blaser & Newman 1982) suggested that the infectious dose for *Salmonella* was less than 1000 cells; in some outbreak cases this figure can be as low as 10 cells (Kapperud *et al.* 1990). Infectious dose rates calculated from volunteer studies tend to be higher ($> 10^4$ cells; Blaser & Newman 1982) presumably due to use of healthy volunteers and the strains examined.

2.2.2 Campylobacter

Campylobacter spp. are a group of Gram-negative slender curved rods consisting of about twenty species and sub-species, eight of which have been shown to cause human enteritis (Solomon & Hoover 1999). Other members of the Campylobacteriaceae family are the genera *Helicobacter* and *Arcobacter*. *Helicobacter pylori* is commonly associated with duodenal and gastric ulcers whereas *Arcobacter* has been associated with livestock abortion and gastroenteritis (Solomon & Hoover 1999). Both organisms are Gram-negative spiral rods and given that *Arcobacter* may be isolated from similar sources as *Campylobacter*, using the same isolation procedures, care must be taken to ensure correct identification.

Campylobacter jejuni and *Campylobacter coli* are responsible for the majority of infections with the former being the most prevalent, causing between 90 and 95% of cases (Humphrey 1995a). They have a limited growth range (30 – 48 °C; pH 4.9 – 9; Anon 1995a; Solomon & Hoover 1999) and are unable to replicate at average UK room temperatures. *Campylobacter spp.* are microaerophilic, growing best in an atmosphere of reduced oxygen concentration such as 5% O_2, 10% CO_2 and 85% N_2. They are biochemically inert and, therefore, relatively hard to identify. Colonies are oxidase-positive and can generally be recognised, on solid media, by their shiny, grey and spreading appearance. Microscopy can be used for presumptive identification and growth in air, at 25 °C, can distinguish between *Arcobacter* and *Campylobacter* spp. (Humphrey 1995a).

Campylobacter spp. are generally present in faeces of infected people in high numbers but because of their slow growth can only be isolated using selective techniques, before incubation in a micro-aerobic environment. This procedure relies on the presence of antimicrobials in the selective media, to which *C. jejuni* is resistant, and an elevated incubation temperature (42 °C) is commonly used in clinical laboratories to reduce the number of competing organisms. Isolation of *Campylobacter* from foodstuffs is much more difficult. Cells are generally present in much lower numbers and may also be sub-lethally damaged. Sub-lethal damage can manifest as an inability of cells to grow under culture conditions suitable for uninjured cells with cells demonstrating an increased sensitivity to antibiotics, hydrogen peroxide and elevated temperatures (Humphrey *et al.* 2001b). Selective enrichment broths containing oxygen-quenching agents, to protect cells from the toxic effects of oxygen derivatives, and antimicrobials, to prevent out growth by competing microorganisms, are the preferred method for the enrichment of such cells before inoculation onto solid media and incubation in a microaerobic environment. Delayed addition of antimicrobials has been found to improve isolation rates as has the incubation of broths at 37 °C rather than 42 °C (Martin *et al.* 1996).

2.2.2.1 Symptoms

After an incubation period of between two and ten days the predominant symptoms of *Campylobacter* are diarrhoea, which is often bloody, fever and abdominal pain (Humphrey 2001b). Other symptoms can include malaise and vomiting. Symptoms generally last for seven to ten days, although relapses occur in approximately 25% of cases. In a minority of cases septic arthritis and haemolytic uremic syndrome can occur and following bacteraemia infection of almost all organs has been reported (Anon 2001c). The mortality rate has been estimated to be one death per 1000 cases, occurring primarily in the very young or old and patients with an underlying disease.

Guillain-Barre syndrome (GBS) is a rare sequalae of infection, with approximately one case occurring for every 1000 diagnosed cases. It is a disease of the nervous symptom which can lead to paralysis that can last several weeks and usually requires intensive care. Approximately 20% of cases of GBS are left with some disability and

the mortality rate is 5% (Altekruse *et al.* 1999). *Campylobacter* has also been associated with Reiter's syndrome (affecting ~ 1% of cases).

2.2.2.2 Infectious dose

As with *Salmonella* (section 2.2.1.2) the infectious dose rate for *Campylobacter* is difficult to determine due to all of the pre-mentioned factors. Matters are further complicated by the lack of outbreak data available for *Campylobacter* on which to base calculations. Volunteer studies have suggested that the infectious dose for *Campylobacter* can be low, with 50 – 800 cells capable of causing disease in healthy adults (Black *et al.* 1988; Robinson 1981).

2.2.3 A comparison of *Salmonella* and *Campylobacter*

Throughout this section the two most common food poisoning organisms *Salmonella* and *Campylobacter* have been discussed. A summary comparing the two organisms is shown below (Table 2-1).

Table 2-1 A comparison of the characteristics of *Salmonella* and *Campylobacter*.

		Salmonella	*Campylobacter*
Growth conditions	Temperature (physiological limits)	7 – 48 °C	30 – 47 °C
	pH (physiological limits)	4 – 9	4.9 – 9
	Atmosphere	Facultative anaerobes	Microaerophilic
Common symptoms		Diarrhoea, abdominal pain, nausea, fever, muscle pain	Diarrhoea, abdominal pain
Incubation period		12 – 72 hours	2 – 10 days
Infectious dose		< 1000 cells	50 - 800 cells
Reported cases in England and Wales in 2001		16, 000 (Frequently associated with outbreaks)	56, 000 (Outbreaks are rare; usually associated with sporadic cases)

2.3   Sources of infection

Although *Salmonella* and *Campylobacter* are thought of as food borne diseases they are primarily zoonotic in origin and other vehicles of infection, including direct animal contact, water, and humans have all been reported.

2.3.1 Food vehicles

Salmonella has been associated a wide range of food vehicles, reflecting not only its large number of animal reservoirs but also its ability to survive a range of environmental stresses. Commonly implicated foodstuffs include egg and egg dishes (including desserts made from raw egg) and raw meat (poultry and red meat). Fish, shellfish, milk and milk products and salad, fruit and vegetables (presumably a result of cross contamination) and other cross-contaminated foodstuffs have also been shown to cause infection (Humphrey 2000).

Many of the vehicles of infection for *Salmonella* are also common for *Campylobacter*, although raw or under cooked poultry are believed to be most important in the UK. Additional sources of infection for *Campylobacter* include other raw or undercooked meats, cross-contaminated foodstuffs and unpasteurised milk. Bird pecked milk has also been implicated as a vehicle for infection (Humphrey 1995a; Leach 1997). As yet there has been no convincing evidence for the contamination of eggs by *Campylobacter*.

2.3.2 Direct animal contact

Direct contact with farm animals and their faeces can be an important route of infection for both *Salmonella* and *Campylobacter*. Farmers, veterinarians, slaughterhouse workers, poultry processors and butchers, who all have occupational exposure to livestock, suffer a higher incidence of infection than members of other

occupations. It has been found that repeated exposure of long term workers to *Campylobacter* results in immunity to this bacterium (Blaser *et al.* 1983) and this could explain why, in a recent case control study, Adak *et al.* (1995) found that occupational contact with livestock or their faeces resulted in a significant decrease in the risk of becoming ill with *Campylobacter*.

Household pets have also been implicated as sources of infection. Contact with cats and dogs has been found to be associated with both *Salmonella* and *Campylobacter* infections (Deming *et al.* 1987; Kapperud *et al.* 1992; Kist & Freitag 2000) and numerous workers have linked *Salmonella* infections with the keeping of exotic pets, such as turtles, iguanas, snakes and hedgehogs (Schutze *et al.* 1999; Woodward *et al.* 1997).

2.3.3 Water

Salmonella and *Campylobacter,* originating from the faeces of infected animals, are common contaminants of rivers, lakes and other surface waters (Baudart *et al.* 2000; Skirrow 1991). These bodies of water represent a source of infection where there is a recreational involvement or when they are used as a source of drinking water but it is the distribution of unchlorinated or inadequately treated drinking water which is of more concern. Contaminated drinking water can be responsible for infecting hundreds and even thousands of people during outbreaks (Angulo *et al.* 1997; Blaser *et al.* 1983; Vogt *et al.* 1982).

2.3.4 Person to person spread

Person-to-person infections occur when the faeces of an infected person are inadvertently ingested by another, possibly due to contamination of ready to eat foods in the kitchen. Infected infants and children are believed to be particularly important when this mode to transmission is involved (Blaser *et al.* 1981).

Although person-to-person transmission is common in cases of *Salmonella* it is rarely reported for *C. jejuni*. Blaser *et al.* (1983) proposed that this could, in part, be due to the rarity of asymptomatic excretion of *Campylobacter*. The poor ability of *Campylobacter* to withstand environmental stresses and to multiply on contaminated foodstuffs would also reduce transmission by this method.

2.4 Difficulties in determining vehicles of infection

Although cases of *Salmonella* and *Campylobacter* are routinely identified using relatively straightforward microbiological methods, the vehicle of infection can be much harder to determine. Foodstuffs are often disposed of before the onset of symptoms, and the isolation of pathogens from the implicated foodstuff is uncommon.

Sporadic cases of infection are generally not investigated but when investigations are carried out the majority of infections remain unexplained. The majority of *Campylobacter* cases are sporadic and, as such, the sites and routes of infection are often undetermined.

It is generally easier to determine the source of infection in an outbreak situation, the majority of which are caused by *Salmonella* and are commonly associated with commercial catering settings, including restaurants, hotels, pubs/ bars, halls/caterers and canteens (Tirado & Schmidt 2000). Outbreaks due to *Campylobacter* spp. are rare and were identified in only 3% of the outbreaks representing only 0.04% of the total number of reported cases (Ryan *et al.* 1996).

Ryan *et al.* (1996) examined 101 outbreaks of infectious disease associated with domestic catering and, although specific food vehicles were suspected in 74 of the outbreaks, a pathogen was only actually isolated from a foodstuff in 12 (16%) cases. When foodstuffs are available for sampling cells may no longer be viable or may be sub-lethally injured. Sub-lethally damaged cells may not be recovered using standard isolation procedures and specialised enrichment procedures must be carried out to ensure isolation. Care must also be taken to ensure adequate volumes of the foodstuffs are analysed. Low infectious doses of both *Salmonella* and *Campylobacter*

have been reported (Kapperud *et al.* 1990; Robinson 1981) and, given the large number of competing organisms in some products, low numbers of pathogens may not be isolated. Typing of recovered isolates can provide valuable data, not only confirming the vehicle of infection but also identifying infective strains. Although typing is commonly used to identify all *Salmonella* isolates the typing of *Campylobacter* isolates is relatively new and not yet routine. This lack of typing for *Campylobacter* isolates and the sporadic nature of infection outbreaks not only means that foodstuffs may not be microbiologically implicated but also that some outbreaks may not be identified.

The majority of food vehicles are implicated using case-control investigations, where infected persons are questioned about recent food consumption patterns and their responses compared to controls. Once a vehicle for infection has been identified the contributing factors, which led to the contamination incident, can then be assessed. Such factors can be difficult to identify since those who prepare food may be unwilling to disclose any lapses in hygiene or they may not even associate some of their actions with unhygienic behaviour. Inspection of restaurants after contamination events may be useful in assessing hygienic behaviour. For example, Anon (1998b) reported that inspection of a restaurant after a contamination event determined that the counter surface was too small to separate raw poultry and other foods adequately during preparation.

2.5 Risk assessment

Risk assessment provides a means to identify the probability of adverse health effects due to a potentially contaminated foodstuff (Lammerding & Fazil 2000). It is a process involving four steps; hazard identification, exposure assessment, hazard characterisation and risk characterisation. In the case of microbial risk assessment, the hazard is usually identified before initiation of the risk assessment due to the short period of time between cause and effect. An exposure assessment determines how likely it is that an individual or population will be exposed to a microbial hazard and what number of the micro-organism are likely to be ingested (Lammerding & Fazil 2000). These data can be difficult to accumulate, particularly when domestic food

handling is involved. Numerous factors need to be taken into account including the number of organisms on a contaminated product, how the product is prepared and ideally include cross contamination data and consumption patterns of the product (Anon 2000a). In particular, cross contamination data are extremely scarce and is one area recommended for further work (Anon 2000a). The third step in developing a risk assessment is hazard characterisation, which involves the response of a human population to exposure to a food borne pathogen. The factors, which may affect a populations response, are complex and, to a small degree, have already been discussed in sections 2.2.1.2 and 2.2.2.2 (Infectious doses of *Salmonella* and *Campylobacter*). In the final stage of a risk assessment the results of the exposure assessment and hazard identification are combined to determine the likelihood that the population will suffer an adverse affect as a result of the hazard (Buchanan *et al.* 2000).

The lack of availability of appropriate data for use in any risk assessment can lead to difficulties in modelling individual stages and result in associated uncertainty (Anon. 2000a). As already mentioned, such data, particularly when domestic handling of the foodstuff is involved, can be difficult to determine and the routes of infection and numbers of bacteria causing disease are often not resolved. One of the aims of this project is to provide data on kitchen malpractices, particularly cross contamination, for use in exposure assessment. The study also examines the ability of *Salmonella* and *Campylobacter* to survive and persist in the kitchen environment, which are also important factors to take into account in an exposure assessment.

2.6 Sensitivity of *Salmonella* and *Campylobacter* to commonly encountered stresses

The ability of food borne pathogens to cause infection relies not only on contamination by *Salmonella* and/or *Campylobacter* but also on the ability of cells to survive any environmental stresses they are exposed to. Depending on the route of infection these stresses can be numerous and include not only environmental stresses but also food processing methods. Common stresses which may be encountered include chilling, freezing, heat, drying, and exposure to cleaning chemicals. A

number of workers (Doyle & Roman 1982a; Humphrey *et al.* 1995; Mattick & Humphrey 2000) have demonstrated that differences in the experimental procedures (e.g. culture conditions, suspending medium, whether or not the cells are attached) and the strains used can all affect the perceived tolerance of the species. To avoid confusion only broad differences in the abilities of *Salmonella* and *Campylobacter* to survive these stresses are described below.

2.6.1 Chilling

Chilling is likely to be one of the more common stresses cells are exposed to whether in the natural environment, such as a stream, or after the processing of a foodstuff which is subsequently chilled. Chilling is commonly used as a means to increase the shelf life of products due to its ability to prevent, or at least delay, the growth of pathogens. *Salmonella* and *Campylobacter* are, however, well adapted to survive periods of chilling and will often outlast the shelf life of the product. *Salmonella* can survive indefinitely under chill conditions (Mattick & Humphrey 2000) but *Campylobacter* is more sensitive and its survival time, under suitable conditions, is measured in weeks rather than months (Blaser *et al.* 1980).

2.6.2 Freezing

Although numbers of *Salmonella* and *Campylobacter* have been found to drop during freezing (Barrell 1988; Humphrey 1986a) it is likely that a population of cells will still be viable (but possibly sub-lethally injured) even after long periods of freezing. Beuchat (1987) found that *Campylobacter* could still be isolated from chicken meat after 12 months storage at −18 °C and numerous studies have isolated both pathogens from frozen products.

2.6.3 Heating

Heating or cooking is probably the most common means of rendering potentially contaminated foodstuffs safe for consumption and is very effective when adequately carried out. *Campylobacter* have been found to be more sensitive to heat than other Gram-negative pathogens including *Salmonella* and *E. coli* 0157 (Solomon & Hoover 1999). Yang *et al.* (2001) found that during 5 minutes exposure to scald water, obtained from a poultry processing plant, *C. jejuni* was sensitive to a temperature range between 50 and 55 °C (with almost all the cells dying at 55 °C) whereas for *Salmonella* Typhimurium the sensitive temperature range was 55 to 60 °C; 5 °C higher. A comparison of *Salmonella* and *Campylobacter* D-values (the time taken for 90% of the cells to die) confirms the heat sensitivity of *Campylobacter* which had a D-value of 1.23 minutes on lamb meat at 55 °C compared a D-value of 30 minutes for *Salmonella* on chicken breast meat at the same temperature (Kodis & Doyle 1983; Murphy *et al.* 2000).

2.6.4 Drying

Drying on surfaces is a common stress for both *Salmonella* and *Campylobacter* at every stage of food production, at the farmyard, the processing plant on machinery and / or on carcases or in the kitchen, possibly as a result of a cross contamination incident. It has been demonstrated that *Salmonella* is better able to survive the stress of surface dying than *Campylobacter*. Work by Humphrey *et al.* (1994b) found that *C. jejuni* were unable to survive in blood droplets at room temperature once the droplets had dried (approximately 2 h) and in similar experiments using *Salmonella* isolates even the less tolerant strains were still viable after 24 h drying (Humphrey *et al.* 1995). The sensitivity of *Campylobacter* to drying has also been demonstrated in the domestic kitchen and Cogan *et al.* (2000) found *Salmonella* spp. were isolated more frequently than *Campylobacter* spp. after a meal preparation correlating with the ability of *Salmonella* to survive better on surfaces.

2.6.5 Exposure to cleaning chemicals

Salmonella and *Campylobacter* are likely to encounter cleaning chemicals and disinfectants at various points within food production when there is an attempt to control their spread. If used correctly these chemicals can be very effective in controlling, or at least reducing, viable cells although the danger is that they can give a false sense of security. Josephson *et al.* (1997) reported that casual use of antimicrobial agents is unlikely to affect the presence of infectious agents in the domestic kitchen but when a targeted cleaning approach is taken bacterial contamination is likely to be reduced. Rusin *et al.* (1998) similarly found that the implementation of a cleaning regimen, with the incorporation of hypochlorite household products, led to a reduction in the number of bacteria isolated.

Cogan *et al.* (2002) found that cleaning was less effective for the removal of *Salmonella* from contaminated surfaces than for *Campylobacter*. They suggested that this might, in part, be due to the better attachment of *Salmonella* due to the possession of surface structures such as SEF 17. These fimbrial structures project from the bacterial cell surface and are believed to be involved in the attachment of cells to inanimate surfaces (Austin *et al.* 1998).

2.7   Contributing factors to foodborne contamination

Although the kitchen is often seen as the last control point in preventing food poisoning, the majority of consumers demonstrate a substantial lack of knowledge about safe home preparation practices (Institute of Food Technologists' expert panel on food safety and nutrition 1995) and the home has been identified as a major source of food poisoning. A number of factors, which have been found to commonly contribute to outbreaks of food poisoning, have been identified including inadequate storage of food, under-cooking and cross contamination (Bryan 1987). The role of these factors in cases of food poisoning, particularly those originating from the home, are difficult to determine. Data on domestic food handling are often based on self-reporting, which may differ from actual practices (Worsfold & Griffith 1997a). In

addition food poisoning is rarely a result of just one error but an accumulation of errors, which are often not determined in retrospect.

One of the aims of this study was to determine how these contributing factors affected the spread and persistence of *Salmonella* and *Campylobacter*, with particular attention focused on the adequacy of cooking raw chicken and cross contamination incidences. Control of these exposure routes is critical in reducing the number of food poisoning incidences and as Panisello *et al.* (2000) stated if they could be managed correctly a quantifiable reduction in risk would result.

2.7.1 Inadequate cooking

Inadequate cooking allows survival of pathogens in the food, which would otherwise have been killed by the heat. Adequate cooking of large chicken or turkey carcases may be problematic due to contaminated visceral cavities, which may be insulated from the heat, particularly if a stuffing has been used. Barbeques are also commonly associated with undercooked food and have been shown to carry an increased risk of infection (Oosterom *et al.* 1984). Although Kapperud *et al.* (1992) could not significantly associate the consumption of sausages with an increased risk of infection with *Campylobacter*, they found a strong association with the eating of sausages at barbeques.

Inadequate cooking of eggs, particularly, has been linked to a large number of *Salmonella* outbreaks and has also been identified a risk factor in a number of case-control studies (Kist & Freitag 2000; Schmid *et al.* 1996).

2.7.2 Inadequate storage

When inadequate cooking is also associated with inadequate storage the potential for infection by *Salmonella* is increased. Bryan (1987) and Humphrey (2000) reported that the inadequate cooling of food between preparation / cooking and ingestion, and the subsequent multiplication of *Salmonella* to numbers sufficient to cause an

infectious dose, is the cause of most *Salmonella* outbreaks in the UK and USA. Common storage errors include prolonged storage of foods at room temperature and storage of foods in large, deep containers, which prevents the rapid cooling of the food even when placed in a refrigerator.

Inadequate storage is not a feature of *Campylobacter* infections, due to its inability to grow at temperatures of less than 30° C and the specific atmospheric requirements it has for growth.

2.7.3 Cross contamination

The term cross contamination refers to any action involving the transfer of pathogens from one material to another. It encompasses such actions as the dripping of contaminated chicken rinse onto areas or materials in the kitchen (direct contamination) to the drying of inadequately washed hands, previously contaminated by raw chicken, on a hand towel (termed indirect contamination).

Although cross contamination is believed to be an important way in which ready to eat foods are contaminated by *Campylobacter* it is often difficult to determine as a contributing factor in food poisoning. Cross contamination is generally a result of a series of sequential events which occur over time, which can be further complicated by the sequential use of domestic kitchens (Griffith *et al.* 1999). Such use can potentially result in an occupant contaminating their food as a result of an action by a previous kitchen user. It is likely that retrospective epidemiological investigations underestimate cross contamination, food handlers are unlikely to recall routes of contamination and may not even realise that their behaviour constituted a risk (Griffith 2001). Indeed a survey by Williamson & Gravani (1992) reported that 37% of participants questioned would only rinse a cutting board and knife used to prepare fresh meat before using the same chopping board for vegetables, a procedure which would lead to cross contamination but which the consumers considered acceptable. Consumers are much more likely to remember that, for example, the chicken appeared to be undercooked, than to remember an event which they did not consider important. It has been suggested (Rodrigues *et al.* 2000) that the majority of sporadic infections

might be a result of cross contamination from kitchen hygiene practices usually regarded as acceptable. The home is believed to be a significant source of sporadic infection and a recent study by the Food Standards Agency found that 71% of consumers believed their food borne illness was caused by food prepared in the home (Anon 2002b). It is likely that a large majority of these sporadic cases are caused as a result of cross contamination but, because sporadic cases are generally not investigated, these cross contamination incidents are likely to remain under reported.

Poultry is frequently contaminated with large numbers of *Campylobacter*, (Hood *et al.* 1988 reported more than 10^8 cells on carcases) and de Boer & Hahne (1990) demonstrated the ease with which *Campylobacter*, and to a lesser extent *Salmonella*, could be transferred from raw chicken products to cutting boards, plates and hands. They were also able to isolate these organisms from cooked chicken products and vegetables in contact with contaminated plates.

Numbers of *Salmonella* on poultry are generally lower than those of *Campylobacter* (Jorgensen *et al.* 2002) and, although cross contamination still represents an exposure route, a period of multiplication would probably be needed, in the majority of occasions, before an infectious dose is present. Cross contamination from eggs infected with *Salmonella* may be more problematic with large numbers of *Salmonella* ($> 10^4$ g^{-1} of egg contents) frequently isolated from inadequately stored eggs (Humphrey 2000). Humphrey *et al.* (1994a) demonstrated the ease with which cross contamination from eggs could occur when they isolated *Salmonella* from a work surface over 40 cm away from a bowl used to whisk eggs. In a later experiment Bradford *et al.* (1996) demonstrated that *Salmonella* present in dried egg droplets could be transferred to beef or melon slices in contact with the egg for more than one minute. Rapid growth of *Salmonella* on these foodstuffs stored at 20 °C was also observed indicating the difficulties in the control of cross contamination in the kitchen.

As well as the studies discussed above there have been numerous others carried out to determine the extent of cross contamination during meal preparations. These can be broadly divided into laboratory studies, where laboratory workers repeat commonly reported food preparation errors in a laboratory setting, observational studies, where

the behaviour of participants, including any possible cross contamination incidents, is recorded and prevalence studies, where the prevalence of bacterial pathogens in kitchens are assessed.

2.7.3.1 Laboratory studies

A number of laboratory based studies have examined transfer of organisms by both direct and indirect routes of cross contamination (Chen *et al.* 2001; Scott & Bloomfield 1990; Zhao *et al.* 1998). These studies have been able to quantify the probability of cross contamination and can be useful in determining which actions may carry a particularly high risk of cross contamination. They can also be used to study the most effective measures to prevent cross contamination and allow the investigators to study specific factors so results are not over complicated with the involvement of different environmental factors. For example Chen *et al.* (2001) found that transfer rates of *Enterobacter aerogenes* B199A among hands, food and kitchen surfaces were highly variable. In the domestic setting these data would be much harder to determine due to the numerous variables involved between the period of contamination and sampling. Disadvantages of this type of study include the differing physiological states of cells used in the laboratory and those present in kitchens and differences in the presence of competing organisms.

2.7.3.2 Observational studies

Observational studies have reported extensive opportunities for cross contamination in domestic kitchens and have reported that it represents a significant proportion of all unhygienic food practices (Jay *et al.* 1999, Worsfold & Griffith 1997b).

Inadequate hand washing had been found to one of the major causes of cross contamination (Jay *et al.* 1999, Worsfold & Griffith 1998), which is often compounded by the lack of separate hand towels, which could lead to contamination of tea-towels after the drying of inadequately washed hands.

Other commonly observed causes of cross contamination resulting in indirect contamination include inadequate cleaning of kitchen and kitchen equipment, especially work surfaces, chopping boards and draining boards, and a lack of facilities for the segregation of raw and cooked foods (Worsfold & Griffith 1998).

This type of study is advantageous in that data can be collected in a much more natural setting although it is possible that observations of participants may lead to a change in behaviour, either because of a wish to impress or anxiousness. In either case participants would be more likely to demonstrate more hygienic behaviour than they might otherwise use and thus the data obtained from such studies are likely to represent the most hygienic scenario. Not all participants realise that their actions are unhygienic and however much they wish to impress these actions may still be repeated. A big disadvantage of this type of study is that although opportunities for cross contamination were observed, no method was used to establish if contaminants were actually transferred.

2.7.3.3   Prevalence studies

This type of study includes those in which domestic kitchens are visited and specific areas sampled, to determine which sites in the kitchen are frequently contaminated, and those where participants are asked to prepare specific meals before the sampling of specified sites, to determine which sites have been contaminated during the preparation of the meal.

Sampling of specific sites in kitchens has demonstrated that the highest concentrations of bacteria tend to be present on moist sites, such as dishcloths and kitchen sinks (Josephson *et al.* 1997; Speirs *et al.* 1995) and on areas which are frequently touched such as the tap handles, and fridge / freezer handles (Kassa *et al.* 2001; Rusin *et al.* 1998). Dishcloths, particularly, can be contaminated with extremely high numbers of bacteria (> 10^{11} cfu per cloth; Wilson *et al.* 1998) suggesting that they may not only act as reservoirs but also, because of their multiple uses in the kitchen, as disseminators of infection (Scott 1999). These studies demonstrate the high levels of bacteria, including potential pathogens, which can be isolated from the domestic

kitchen but the routes by which these areas were contaminated cannot be determined and the number of contamination events, which resulted in this level of contamination cannot be assessed.

Studies in which workers have asked participants to prepare specific meals before sampling, have reported extensive cross contamination as a result of just one meal preparation event. de Wit *et al.* (1979) examined cross contamination events which occurred after participants prepared frozen chickens contaminated with an indicator organism (*E. coli* K12). They found that cross contamination occurred in a high proportion of the kitchens and in a number of cases that the indicator organism was still present even after 'cleaning'.

This study highlighted the large number of sites, within a kitchen, which can become contaminated after only one meal preparation event but relied on the use of an indicator organism, which may have different attachment and survival characteristics to organisms naturally contaminating chickens including *Salmonella* and *Campylobacter*.

Studies carried out by Cogan *et al.* (2000; 2002) examined sites in domestic kitchens, for *Salmonella* and *Campylobacter* contamination, after the preparation of naturally contaminated chicken. The focus of these studies was to determine the effectiveness of cleaning regimes for preventing cross contamination and although *Salmonella* and *Campylobacter* were frequently isolated from the kitchens no attempt was made to link the contamination of specific sites with particular hygiene practices.

2.8   Aims of the study

The overall aim of this project was to obtain microbiological and observational data to investigate exposure routes for *Salmonella* and *Campylobacter* during the handling of raw poultry in domestic kitchens.

2.9   Study objectives

The more specific objectives were to:-

Analyse and review the literature on *Salmonella* and *Campylobacter,* cross contamination and potential exposure routes.

Develop appropriate sampling, storage method to promote and maintain viability of *Salmonella* and *Campylobacter* cells.

Design, develop and optimise methods for the isolation of *Salmonella* and *Campylobacter* from the kitchen environment.

Pilot and validate cultivation methodologies and identify commonly contaminated kitchen sites and pathogen exposure routes during the preparation of a poultry-based meal in a test domestic kitchen.

Observe, record and analyse the behaviour of 70 participants preparing a poultry-based meal.

Correlate observed hygiene practices with microbial contamination of specific kitchen sites and provide data for risk assessments.

Investigate the effect of air-drying on the viability of *Salmonella* and *Campylobacter* cells, on simulated kitchen work surfaces.

Produce recommendations for future work.

Chapter 3. Design, development and validation of appropriate and sensitive microbiological methods for the isolation, enrichment and transport of *Campylobacter* and *Salmonella* samples

3.1   Introduction

Salmonella and *Campylobacter* spp. exposed to kitchen environments are subjected to a number of stresses including atmospheric oxygen concentrations, drying and cleaning chemicals. It is probable that cells, particularly *Campylobacter,* which have been reported to be sensitive to oxygen and drying (Humphrey *et al.* 1994b), are likely to be sub-lethally injured and the most sensitive isolation methods would be needed to maximise detection.

A common manifestation of sub-lethal damage to cells is a change in permeability barriers, in the cell wall and cell membrane, which makes them more sensitive to chemical agents (Ray 1979). The use of such agents is, however, a necessity during the isolation of damaged cells to prevent overgrowth of the target organisms. Numerous workers have researched methods to optimise recovery of damaged cells and the general agreement is that a delay in the addition of selective agents will promote recovery of sub-lethally damaged cells to a sound physiological condition and allow for a more rapid recovery when they are subjected to more selective media (Andrews 1986; Humphrey 2001c).

Humphrey & Cruickshank (1985) and Ray & Johnson (1984) found that damaged *Campylobacter* cells were particularly sensitive to two of the five antibiotics used in modified Exeter broth, a commonly used *Campylobacter* enrichment broth, and the delayed addition of these two antibiotics alone could improve isolation rates (Martin *et al.* 1996). During this study the effect of delaying these antibiotics (rifampicin and polymyxin) on samples derived from the kitchen was investigated as was the effect of prolonging the incubation period of broths to allow for maximum recovery of

damaged cells. Mackey & Derrick (1982) and Stephens *et al.* (1997) reported that the lag time of injured *Salmonella* cells can exceed 20 h. Given that *Campylobacter* have a slower growth rate (Solomon & Hoover 1999) it is likely that the lag time of damaged *Campylobacter* cells will greatly exceed this.

In the isolation of *Salmonella* a delay in exposure to selective agents is usually accomplished with the use of a pre-enrichment stage in a nutritious non-selective media (Ray *et al.* 1972). A number of workers have, however, reported that high levels of coliforms, present in the sample, can adversely affect recovery of *Salmonella* during this pre-enrichment state (Litchfield 1973; Oblinger & Kraft 1973; Silliker *et al.* 1964). It has been found that the presence of sulphamandelate in solid agar can prevent overgrowth of *Salmonella* during its isolation from heavily contaminated samples (Anon 2001a) and its use during the pre-enrichment stage for *Salmonella* isolation will be investigated during this study. A comparison of several different selective media for the isolation of *Salmonella*, from samples types commonly taken from kitchens, was also made in order to determine the optimum recovery method to isolate *Salmonella* from domestic kitchens.

An important aspect of this study was to develop a protocol to minimise loss of viability during transport from the kitchens to the laboratory and during overnight storage, when it was not possible to analyse samples on the same day. The use of enrichment broths as a transport medium was examined and refrigeration, which has previously been found to prolong viability of cells (Chynoweth *et al.* 1998), was utilised throughout the study.

3.1.1 Aims

Develop appropriate sampling and storage methods to promote and maintain viability of *Salmonella* and *Campylobacter* cells.

Design, develop and optimise methods for the isolation of *Salmonella* and *Campylobacter* from the kitchen environment.

3.1.2 Objectives

Develop a method to obtain cells in a similar physiological state as those contaminating kitchen surfaces.

Develop a cost effective, reliable method to maintain the temperature of samples during transport and storage.

Investigate the relationship between transport temperature, media and maintenance of viability.

Develop optimum methods for the isolation of *Salmonella* and *Campylobacter*.

3.2   Materials and method

3.2.1   Temperature regulation in a cold box

In order to maximise the recovery of target organisms and minimise overgrowth by competing organisms during transport and storage, it was decided that all samples would be transported under refrigerated conditions (above freezing but lower than 8 °C).   A number of studies (Chynoweth *et al.* 1998; Lazaro *et al.* 1999) have demonstrated that *Campylobacter* survive better at 4-5 °C than at higher temperatures, although freezing has been shown to be detrimental (Blankenship *et al.* 1983).   The cost of transporting mobile refrigerators was prohibitive and, therefore, the use of cold boxes was investigated.   A cold box (36 x 27 x 34 cm) packed with five ice packs (20 x 11 x 4 cm), additional insulation (polystyrene pieces, polystyrene boards, carrier bags and bubble wrap) and a Testostor 175 data logger (Borolabs, Berkshire, UK) was stored in a laboratory for 18 h.   The temperature of the cold box was automatically recorded, using the data logger, every six hours.   The experiment was repeated six times.

3.2.2   Isolation of *Salmonella* and *Campylobacter* from naturally contaminated samples

The bulk of this study involved the isolation of *Salmonella* and *Campylobacter* from naturally contaminated raw chicken samples and from various surfaces and materials, which may have become contaminated during meal preparation.   In order to obtain cells in a similar physiological condition, naturally contaminated samples were used as much as possible during the validation work.

3.2.2.1 Sampling methodologies used for the isolation of *Salmonella* and *Campylobacter* from raw chicken

The Food Safety and Inspection Service of the US Department of Agriculture recommends the use of whole bird rinses for the detection of *Campylobacter* and this was the method employed to obtain some of the *Campylobacter*-positive samples during the validation work (see below). The bulk of this study did however involve the use of raw chicken breasts in domestic kitchens and a rinsing method to enumerate organisms would not have been feasible. In order to assess the presence of *Salmonella* and *Campylobacter* on these samples the chicken breast skin was analysed as described below.

Chicken portions or carcases were placed in stomacher bags and shaken in 400 ml of maximum recovery diluent (MRD; CM733, Oxoid Ltd, Basingstoke) for 2 mins. *Salmonella* and *Campylobacter* were isolated from the rinse using the methods detailed below (sections 3.2.2.2 and 3.2.2.3).

Skin from two chicken breasts was removed and weighed using a PM600 balance (Mettler, Leicester). Maximum recovery diluent was added to the skin in a ratio of 10:1 and homogenised in a stomacher (Lab Blend 400, Seward Medical, London, UK) for 2 mins. The resulting 10^{-1} homogenate was examined for the presence of *Salmonella* and *Campylobacter*.

Twenty-five ml of chicken rinse or skin homogenate was added to 225ml of buffered peptone water (BPW; CM509, Oxoid Ltd.) before enrichment for *Salmonella* (see section 3.2.2.2).

For *Campylobacter* enrichment 225 ml of modified Exeter broth (Nutrient broth [25 g l^{-1}; Mast DM180, Mast Diagnostics, Bootle, Merseyside, UK], *Campylobacter* Growth Supplement [Sodium Metabisulphate, Sodium Pyruvate and Ferrous Sulphate, all at 250 mg l^{-1}; Mast SV61], Trimethoprim 10 mg l^{-1} [monotrim, Solvay Healthcare Ltd., Southhampton], Rifampicin 5 mg l^{-1} [Rifadin,

Marion Merrell, Uxbridge], Polymyxin B sulfate 2500 iu l^{-1} [P1004, Sigma, Poole], Cefoperazone 15 mg l^{-1} [C4292, Sigma], Amphotericin B 2 mg l^{-1} [Fungizone, Squibb, Hounslow] and lysed defibrinated horse blood (10 ml l^{-1}; E & O Laboratories, Bonnybridge, Scotland) was added to 25 g of chicken rinse or chicken skin homogenate in a 250 ml container, ensuring minimal head space.

3.2.2.2   Isolation of *Salmonella* by enrichment

For the isolation of *Salmonella* from food the International Standards Organisation recommends pre-enrichment in BPW followed by enrichment in Rappaport-Vassiliadis soya peptone broth [RVS] and cystine selenite broth [CSB]) and then subculture onto modified Brilliant Green agar [mBGA] and one other selective plating media (Anon 1998a). Due to time constraints only RVS and xylose lysine desoxycholate agar (XLD) were used to routinely isolate *Salmonella* from chicken carcases in this study using the methods described below.

Samples in BPW were incubated at 37 °C for 24 h. A 100 µl aliquot was then sub-cultured into 10 ml RVS (CM866, Oxoid Ltd.) before incubation at 41.5 °C for 18-24 h. Subcultures (10µl) were streaked for single colonies on to XLD (CM469, Oxoid Ltd.) and incubated at 37 °C for 18-24 h.

Presumptive *Salmonella* were identified by colony morphology and confirmed using standard biochemical and serological techniques (Anon 1995b).

3.2.2.3   Isolation of *Campylobacter* by enrichment

The method described is an adaptation of that used by the PHLS (Anon 2002c). It has been found to improve the isolation rate of *Campylobacter* from chickens compared to the ISO- recommended methods which utilise either Park and Sanders or Preston enrichment media (Humphrey 1995b; F. Jorgensen personal communication, 9th April 2003).

After the addition of modified Exeter broth samples were incubated at 37 °C for 48 h. Ten µl aliquots were then streaked for single colonies on to charcoal cefoperazone desoxycholate agar (CCDA, CM739, SR155, Oxoid Ltd.), which was incubated under micro-aerobic conditions at 37 °C for 48 h. Micro-aerobic conditions were generated as described below (section 3.2.2.5). Presumptive *Campylobacter* were identified by colony morphology and confirmed by oxidase activity and cell morphology using phase contrast microscopy with a 100 x objective (Leitz, Wetzlar, Germany). Growth on blood agar (BA) at 20 °C for 48 h in an aerobic atmosphere was used to discriminate between *Campylobacter* spp. and *Arcobacter* spp..

3.2.2.4   Enumeration of *Campylobacter* present in naturally contaminated chicken samples

Campylobacter present on the skin of the chicken breasts were enumerated using an MPN technique (Anon 1995b). Three 1 ml aliquots of the neat, 10^{-1}, 10^{-2} and 10^{-3} homogenates (see section 3.2.2.1) were each cultured in 30 ml modified Exeter broth and enriched for *Campylobacter* as described above (section 3.2.2.3). The number of *Campylobacter*-positive broths was recorded and the MPN calculated from the probability of finding growth after successive dilutions.

3.2.2.5   Generation of a micro-aerobic atmosphere

Micro-aerobic conditions were generated by the gas replacement method. A partial vacuum of 500 mm Hg in a 10 l jar was replaced with a mixture of CO_2, H_2 and N_2 resulting in a gas concentration in the jar of approximately 5% O_2, 5% CO_2 and 5% H_2 in a balance of nitrogen (Bolton *et al.* 1992).

3.2.3 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Campylobacter*

3.2.3.1   Recovery of *Campylobacter* cells damaged by refrigeration

Any *Campylobacter* strains isolated from foods, such as raw poultry, during this study were likely to have been exposed to refrigeration temperatures. Raw chicken was used directly from the chiller cabinet for the food preparation sessions and, as discussed earlier, all samples were transported from domestic kitchens to the laboratory at temperatures of 1 - 8 °C. In this experiment the effect of prolonged storage at refrigeration temperatures on the survival of *Campylobacter* was examined.

Campylobacter present in four chicken skin homogenates were enumerated using an MPN method (section 3.2.2.4). Homogenates were then stored at 4 °C (± 1 °C) for one week to generate *Campylobacter* cells damaged by prolonged refrigeration. Ten ml aliquots of homogenate were dispensed into each of 10 30 ml sterile universals. This was repeated for 1 ml (n=10) and 0.1 ml aliquots (n=10). Modified Exeter broth was added to each universal to a level which ensured minimal head space. The dilution of the modified Exeter broth, associated with the different sample volumes, was not thought to affect the performance of the broth. Five of the universals from each batch were incubated directly and five were stored at 4 °C for 18 h before incubation. All broths were incubated at 37 °C for 120 h. After 48 h and 120 h incubation 10 µl was sub-cultured onto CCDA to obtain single colonies. Inoculated plates were incubated micro-aerobically at 37 °C for 48 h before examination. Based on the number of positive broths per sample the MPN was calculated (Anon 1995b). This experiment was repeated using four separate chicken skin homogenates.

3.2.3.2 Storage and recovery of *Campylobacter* cells damaged following air drying on a surface

It is important that any cells used to validate the microbiological methods used for this study are in a similar physiological condition as those which will be isolated from kitchens. Air drying is a common environmental stress which *Campylobacter* cells contaminating a kitchen are likely to be exposed to and was, therefore, chosen as a means to validate the use of transport media and the transport temperature of samples.

The number of *Campylobacter* present in naturally contaminated chicken rinse was determined (see section 3.2.2.4) and 100µl added to 50 5 x 5 cm squares, marked out on a sheet of Formica. To produce even coverage a cotton tipped swab, pre-moistened in MRD, was used to spread the rinse across the Formica square. It is possible that the swab removed a small proportion of the inoculum but, since each square was subjected to the same treatment, the effect of this would have been minimal. The inoculum was left to dry for 30 mins at 21 °C ± 1 °C. To recover the organisms, a cotton tipped swab, pre-moistened in MRD, was used to swab each inoculated square and a second dry swab was used to remove any remaining sample, the two swabs were placed in one universal. Ten swab samples (each sample consisting of two swabs) were enriched directly for *Campylobacter* with no refrigeration step. Broths were incubated at 37 °C for 96 h, and sub-cultured (10 µl) onto CCDA at 48 h, 72 h and 96 h. Inoculated plates were incubated micro-aerobically at 37 °C for 48 h. Modified Exeter broth was added to further 20 swab samples before storage at 1 °C (n=10) and 4 °C (n=10) for 18 h and 10 further swab samples were stored at 4 °C and 10 at 1 °C for 18 h before the addition of broth. After storage all broths were enriched for *Campylobacter* as described above.

Using the method described above each Formica square was inoculated with approximately three *Campylobacter* cells. Due to the rapid decrease in the viability of *Campylobacter* cells dried on surfaces (Doyle & Roman 1982a) the

majority of swabs were *Campylobacter*-negative and, in order to obtain more meaningful results, this experiment was repeated a further two times using artificially inoculated chicken rinse. *Campylobacter coli* strain 15N (originally isolated from the neck skin of a chicken) was streaked on to BA and incubated in a micro-aerobic atmosphere at 37 °C for 48 h before storage at 4 °C for 24 h to stress cells. Colonies were suspended in 9 ml MRD to an optical density (OD) of 0.1 at 600 nm before 1 ml was added to 250 ml chicken carcase rinse (final concentration ~ 6 x 10^4 cfu ml^{-1}). *Campylobacter* cells from both the initial inoculum and the inoculated rinse were enumerated according to the method by Miles and Misra (Miles & Misra 1938). Inocula were serially diluted to 10^{-5} in MRD before 20 µl aliquots of each dilution were dropped on to BA and CCDA respectively. Plates were incubated in a micro-aerobic atmosphere for 48 h at 37 °C before colonies were counted.

To determine the number of *Campylobacter* present in the inoculated chicken rinse 500 µl was spread onto the surface of two CCDA plates, which were incubated under appropriate conditions. *Campylobacter* colonies were counted and the number per ml of rinse calculated.

3.2.3.3   Effect of polymyxin and rifampicin on the recovery of *Campylobacter*

A number of studies have utilised the ability of some species of *Campylobacter* to grow at 43 °C (Agulla *et al.* 1987; Doyle & Roman 1982b) to enhance selectivity. Other workers have demonstrated, however, that this technique may prevent the growth of sub-lethally damaged cells and suggest that they may need at least 2 h growth at a lower temperature whilst they repair damage to outer membranes, cytoplasmic membranes and / or nucleic acid (Humphrey 1986a; Mason *et al.* 1996). During this study broths were incubated at 37 °C to allow for maximal recovery of damaged cells. Unfortunately this temperature also promotes the growth of numerous competing organisms and the presence of selective agents is needed to suppress their growth. Modified Exeter broth contains a number of antibiotics but only two, rifampicin and polymyxin, have been found to adversely affect recovery of damaged cells (Humphrey 1995a).

In this experiment the effect of polymyxin and rifampicin on undamaged *Campylobacter jejuni* WK3A and *C. coli* 2604 was examined. The two strains were streaked onto BA and incubated at 37 °C for 16 h under appropriate conditions before colonies were suspended in 9ml MRD to an OD of 0.2 at 600 nm. Aliquots (200 μl) were added to 800 μl of NB containing aerotolerant supplement (0.2% ferrous sulphate, sodium pyruvate and sodium metabisulphate) to create an inoculum. *Campylobacter* numbers were determined using direct plating and an MPN technique. For direct plating two 20 μl aliquots of inoculum were added to 2 x 5 ml MRD and serially diluting (in MRD) to 10^{-3}. Three 20 μl drops of dilutions -1, -2 and -3 were dropped onto BA which were incubated at 37 °C for up to 48 h under appropriate conditions before colonies were enumerated.

Enumeration, using an MPN, method was carried out by diluting the inoculum to 10^{-9} in MRD and adding 100 μl of dilutions 10^{-6} to 10^{-9} to 9 x 7ml bijoux. Modified Exeter broth containing no antibiotics was added to six bijoux, broth lacking rifampicin and polymyxin was added to three more and complete broth was added to another three. A sufficient volume of broth was added to allow only minimum head space in each bijoux. The broths were all incubated at 37 °C for 120h. After six hours incubation rifampicin and polymyxin (5 μg / ml and 2.5 iu / ml respectively) were added to three of the bijoux containing modified Exeter broth lacking these antibiotics and incubated for a further 112 h. Aliquots (10 μl) of each broth was streaked onto CCDA plates after 48 and 120 h. Plates were examined for the presence of *Campylobacter* after 48 h incubation and the MPN calculated (Anon 1995b).

3.2.3.4 Effects of delaying the addition of rifampicin and polymyxin to modified Exeter broth on *Campylobacter* recovery from a heavily contaminated sample

Martin *et al.* (1996) found that the delayed addition of the antibiotics rifampicin and polymyxin to modified Exeter broth resulted in an improved isolation rate of *Campylobacter* from river water but a decrease in the recovery rate from chicken samples. Based on this work the delayed addition of rifampicin and polymyxin by

6 h was investigated and since the delayed addition of antibiotics at such an interval could be inconvenient, depending on their arrival time and the length of the working day, the effect of delaying the antibiotics by 24 h was also examined.

A dishcloth (used in a domestic kitchen for one week) was homogenised with 25 g of *Salmonella* and *Campylobacter*-positive chicken skin in 250 ml BPW for two mins. One ml of homogenate was added to each of 60 30 ml universals before 29 ml of modified Exeter broth lacking rifampicin and polymyxin, at a temperature of 20 °C, was added to 30 samples. The same batch of broth, held at 6 °C, was added to the remaining 30 samples. All broths were incubated at 37 °C for 120 h. After 0, 6 and 24 h incubation the antibiotics rifampicin and polymyxin (5 µg / ml and 2.5 iu /ml) were added to ten broths from each of the initial broth temperature groups. Broths (10 µl) were sub-cultured onto CCDA after 48 and 120 h incubation. Plates were incubated as previously described. This experiment was carried out on the same day.

3.2.4 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Salmonella*

3.2.4.1 The effect of sulphamandelate and type of plating media on the isolation of *Salmonella* from a heavily contaminated sample type

In this experiment the media involved in all of the different stages (pre-enrichment, enrichment and plating media) of *Salmonella* isolation were examined.

Artificially contaminated samples were analysed due to the low prevalence of *Salmonella* on retail chicken carcases (Anon 2001e; Jorgensen *et al.* (2002).

Due to the large number of competing organisms which may be present on some kitchen samples (Scott *et al.* 1982; Speirs *et al.* 1995) the use of sulphamandelate to recover *Salmonella* from a heavily contaminated sample type was also investigated. Sulphamandelate has been found to promote the recovery of *Salmonella* from other

heavily contaminated sample types (sewage and sewage sludge) when incorporated into brilliant green agar (Anon 2001a) and its ability to improve recovery when added to a pre-enrichment broth was examined.

Salmonella Enteritidis PT4 (strain I) was inoculated into nutrient broth (NB) and incubated at 37 °C for 18 h. This strain was chosen as it has previously been found to be sensitive to a number of environmental stresses, including air drying (Humphrey *et al.* 1998), and would, therefore, represent some of the more sensitive *Salmonella* isolates which may contaminate a kitchen. The culture was then standardised to 0.2 at 600 nm before being diluted to 10^{-4} and stored at 4 °C for 72 h. One ml (containing ~2 x 10^4 cfu) was added to 300 ml of a heavily contaminated sample type, generated by homogenising a third of a dishcloth, previously used in a domestic kitchen for one week, in 30 ml of chicken quarter rinse and 270 ml of BPW for two minutes. One hundred μl of inoculated homogenate was added to ten 29 ml volumes of BPW and ten 29 ml volumes of BPW with sulphamandelate (sodium sulphacetamide [1mg / ml], sodium mandelate [0.25 mg / ml], SR87, Oxoid Ltd.). Broths were incubated for 24 h at 37 °C before subculture into RVS (100 μl), CSB (1000 μl; CM699, L121, Oxoid Ltd.) and Diassalm plates (200 μl; LAB 537, LAB M, Bury). The RVS broths were incubated at 41.5 °C for 24 h and the CSB at 37 °C for 24 h before 10 μl was streaked on to XLD, mBGA (CM329 Oxoid Ltd.) and mannitol lysine crystal violet brilliant green agar (MLCB; CM783, Oxoid Ltd.). All plates were incubated at 37 °C for 24 h. Presumptive *Salmonella* positive Diassalm plates were sub-cultured from the edge of the black area, on to XLD and incubated as previously described. *Salmonella*-negative Diassalm plates were incubated for a further 24 h at 37 °C. The number of *Salmonella* positive plates was recorded. This experiment was repeated three times.

3.2.4.2 Recovery of *Salmonella* after 18 h storage at 4 °C in various diluents

In order to determine the most appropriate transport/storage diluent for potentially damaged *Salmonella,* cells were air dried, to mimic conditions they may be

exposed to in the kitchen, and stored for 18 h under different conditions using the following method.

A *Salmonella* strain isolated from a chicken carcase was incubated in NB at 37 °C for 18 h. The culture was then standardised to 0.2 at 600 nm before being diluted to 10^{-4} and stored at 4 °C for 72 h. One ml (containing ~2 x 10^4 cfu) was added to 200 ml of chicken rinse (section 3.2.2.1). One hundred μl of inoculated chicken rinse (containing ~ 10 *Salmonella*) was added to 40 5 x 5 cm^2 Formica squares and spread using a swab pre-moistened in MRD. It is possible that the swab removed a small proportion of the inoculum. This may have reduced the overall number of positive samples obtained but since each square was subjected to the same treatment this would not have effected any conclusions. After one hours drying at 21 ±1 °C squares were swabbed using two swabs, the first pre-moistened in MRD followed by a second dry swab. Twenty pairs of swabs were placed into universals containing 20 ml BPW, 10 into universals containing 15 ml MRD and 10 into universals containing no media. The ten pairs of swabs in BPW were then enriched for *Salmonella* as previously described (3.2.2.2) and the remaining swabs were stored at 4 °C for 18 h. Fifteen ml of double strength BPW was added to swabs stored in MRD and 20 ml of BPW was added to swabs stored in no diluent. All swab samples were then enriched for *Salmonella* as previously described. This experiment was repeated three times.

3.2.4.3 Storage of dishcloths at 4 °C in various diluents

Due to the large numbers of competing organisms present on dishcloths (Wilson *et al.* 1998) care must be taken to transport samples in a manner which will not promote the growth of these bacteria and lead to overgrowth of the target organism, in this case *Salmonella*. In this experiment the use of BPW, MRD as transport media was investigated as was the use of no diluent.

Salmonella Enteritidis PT4 (strain I; see section 3.2.4.1) was inoculated into nutrient broth (NB) and incubated at 37 °C for 18 h. The culture was then standardised to 0.2 at 600 nm before being diluted to 10^{-8} in 18 ml MRD. One ml

of the inoculum was spread evenly across the surface of two BA plates, which were incubated at 37 °C for 24 h before *Salmonella* colonies were enumerated. The remaining inoculum was stored at 4 °C for 18 h. A dishcloth, which had previously been used in a domestic kitchen for one week, was then homogenised in 250 ml MRD for two mins. The used dishcloth was then removed and a dishcloth (unused), which had previously been cut into 18 equal pieces, was then added to the homogenate and homogenised for a further two minutes. Each piece of dishcloth was removed, added to a separate 250 ml pot, and one ml of refrigerated inocula was added to each piece. Maximum recovery diluent (125 ml) was added to six pieces of dishcloth, BPW (125 ml) to six pieces and no media was added to the remaining six pieces. The dishcloth pieces were then stored at 5 °C ± 1 °C and removed for sampling immediately (T0), and after 24 and 48 h storage.

On each sampling day two pieces of dishcloth stored in MRD and two stored in BPW were homogenised for two mins. Maximum recovery diluent (125 ml) was added to a further two dishcloth pieces, which were not stored in a diluent, before they were also homogenised for 2 mins. One ml of each of the homogenates was then removed and diluted to 10^{-8} in 9 ml MRD. One ml of each dilution was added to four petri dishes. Fifteen ml aliquots of molten plate count agar (PCA; CM325, Oxoid Ltd) and violet red bile glucose agar (VRBGA; CM485, Oxoid Ltd) maintained at 45 – 48 °C were added to duplicate plates. Each plate was mixed and allowed to set. Plates poured with VRBGA were overlaid by a further 10 ml of molten VRBGA before incubation at 37 °C for 24 h. PCA plates were incubated at 30 °C for 72 h. Aerobic colony counts were obtained from PCA and presumptive *Enterobacteriaceae* counts from VRBGA. Plates containing 30-300 colonies per plate were counted using a colony counter (SC5, Stuart Scientific, Staffordshire, UK). Double strength BPW (125 ml) was added to the MRD homogenates and BPW (125 ml) to the BPW homogenates to provide 250 ml of pre-enrichment broth. Enrichment for *Salmonella* was then carried out using the methods earlier (3.2.2.2). This experiment was repeated on a separate day using three sample replicates.

3.2.5 Statistical analysis

Statistical analysis was carried out in Microsoft Excel '97 using a t test on two samples, assuming equal variance. This test allows actual differences between the two means to be compared in relation to variation in the data (Deacon 2003).

3.3   Results

3.3.1 Temperature regulation of cold boxes

Cold boxes packed with five ice packs and additional insulation maintained temperatures of between 1 and 8 °C (Table 3-1) for up to 18 h in the laboratory. The use of additional ice packs was prohibitive due to the lack of space in the cold box and the increase in weight. The overall change in temperature was small (mean 1.2 °C, maximum 2.1 °C). Generally the temperature increased during the 18 h storage period although a decrease in temperature was recorded during the first 12 h for 2 / 6 cold boxes.

Table 3-1 The temperature and temperature changes inside a cold box with 5 ice packs and additional insulation.

| | Temperature (°C) | | | | |
	0 h	6 h	12 h	18 h	Overall change in temperature (°C)
Box 1	2.8	2.6	2.7	3.5	+ 0.7
Box 2	4.9	5.8	6.2	6.5	+ 1.6
Box 3	4.1	3.9	4	4.4	+ 0.5
Box 4	0.6	1.8	2.3	2.7	+ 2.1
Box 5	3.0	3.2	3.5	4.0	+ 1
Box 6	2.8	3.1	3.4	3.9	+ 1.1
Average	3.0	3.4	3.7	4.2	+ 1.2

3.3.2 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Campylobacter*

3.3.2.1 Recovery of *Campylobacter* cells damaged by refrigeration

To assess the effect of prolonged refrigeration on *Campylobacter*, cells in four chicken breast skin homogenates were enumerated (using an MPN method) before and after storage at 4 °C ($\pm$ 1 °C) for one week. The number of *Campylobacter* in the chicken skin homogenates ranged from 0.4 - 200 cfu ml^{-1} before storage (Table 3-2). After storage a decline in the number of *Campylobacter* in chicken homogenates B-D was detected, but this difference was not significant ($P = 0.328$). Unfortunately, because of the large number of cells present, the number of *Campylobacter* present in chicken skin homogenate A could not be calculated.

Table 3-2 Number of *Campylobacter* present on chicken breast skin homogenate before and after storage at 4 °C for one week.

| Homogenate | cfu ml^{-1} homogenate | |
	Before storage	After storage
A	200	> 16[a]
B	2.3	1.3
C	2.3	1.1
D	0.4	0.2
Average[b]	1.7	0.9

[a] upper limit of detection, [b] homogenates B-D only

To investigate the effect of refrigerating chicken rinses in modified Exeter broth prior to incubation, a range of dilutions were examined to calculate the MPN ml^{-1} of homogenate and enable differences before and after storage to be detected. Again the number of *Campylobacter* in chicken skin homogenate A could not be determined as the large number of bacteria present exceeded the upper limit of detection.

Campylobacter numbers in chicken rinses B-D were lower (Table 3-3). There was no significant difference in the number of *Campylobacter* in broths incubated directly and those stored for 18 h in modified Exeter broth before incubation ($P = 0.52$) indicating that modified Exeter broth could be a suitable storage medium (Table 3-3). The number of *Campylobacter*-positive broths did not increase after 120 h incubation in modified Exeter broth (data not shown).

Table 3-3 The effect of overnight storage in modified Exeter broth on the recovery of *Campylobacter* from chicken rinses.

Homogenate	No. of viable *Campylobacter* (cfu ml^{-1}) after 48 h incubation at 37 °C	
	No Storage	18 h storage at 4 °C
A	>16	16
B	1.3	0.8
C	1.1	1.3
D	0.2	1.3

3.3.2.2 Storage and recovery of *Campylobacter* cells damaged following air drying on a surface

Droplets of a chicken rinse that were naturally contaminated with *Campylobacter* were placed onto Formica squares and allowed to air dry. Each square was inoculated with approximately three *Campylobacter* cells (S.D. 0.05) suspended in a 20 µl volume. After 30 mins air-drying at 21 °C (± 1 °C) each Formica square was sampled and the swabs either incubated directly in modified Exeter broth or stored at 4 or 1 °C (temperatures which could be maintained in a cold box) either with or without modified Exeter broth. *Campylobacter* were recovered from only 5 / 50 swabs. Three of the swabs had been incubated directly, one had been stored for 12 h in modified Exeter broth at 4 °C and one in modified Exeter broth at 1 °C (data not shown). Due to the low number of positive samples it was not possible to determine the most appropriate storage conditions.

Because of the above findings, in further experiments, Formica squares were contaminated with chicken rinse that had been inoculated with high numbers of *Campylobacter* (4.3 $\log_{10}$ cfu per Formica square [S.D.0.36]). After 30 mins drying *Campylobacter* was recovered from an average of 60% of the squares from swabs incubated directly.

Storage of swabs at 4 °C and 1 °C in modified Exeter broth for 18 h before enrichment was found to be as effective as when swabs were enriched directly in modified Exeter broth ($P = 0.5$; Table 3-4). When no broth was added to swabs during storage at 4 °C and 1 °C the recovery of *Campylobacter* was significantly affected ($P = 0.01$ and $P = 0.04$ respectively).

Table 3-4 The effect of storage conditions on the recovery of *Campylobacter* cells damaged by surface drying in chicken skin homogenate.

	Difference between the number of positive swabs after 18 h storage and those incubated directly[a]			
Storage temperature	4 °C	4 °C	1 °C	1 °C
Modified Exeter broth	Absent	Present	Absent	Present
A	-9	-3	-9	0
B	-3	-1	-3	-1
C	-1	0	-2	+1
D	-5	0	-5	-3
Average	-4.5	-1	-4.75	-3

[a] calculated from the number of positive broths after 48 h incubation in modified Exeter broth at 37 °C

All broths were incubated for 120 h at 37 °C. In one experiment four swabs, which were not positive at 48 h, were positive after 120 h. Two of these swabs had been stored at 4 °C without broth being added and two had been stored at 4 °C in modified Exeter broth (data not shown).

3.3.2.3   Effect of polymyxin and rifampicin on the recovery of *Campylobacter*

The number of two *Campylobacter spp.* (*C. jejuni* WK3A, HS13, phage type [PT] 1, isolated from a work surface and *C. coli* 2604, HS59, PT 44; isolated from a chicken breast) present in pre-prepared inocula were enumerated by direct plating on to blood agar and by enrichment in modified Exeter broth lacking different antibiotics. Recovery of cells was greater when inoculated directly onto blood agar than when enriched in broths ($P = 0.04$) and the log reduction was calculated from the blood agar count. The addition of antibiotics to the broths after 0 or 6 h had no effect on the recovery of *C. jejuni* strain WK3A (data not shown) but the addition of rifampicin (5 μg ml^{-1}) and polymyxin (2.5 iu ml^{-1}) at 0 h or 6 h significantly reduced the recovery of the *C. coli* strain 2604 ($P = 0.0002$; Figure 3-1).

Figure 3-1 Recovery of *C. coli* (2604) from modified Exeter broth lacking different antibiotics.

3.3.2.4   Effects of delaying the addition of rifampicin and polymyxin to modified Exeter broth on *Campylobacter* recovery from a heavily contaminated sample

Results indicated that all samples were contaminated with *Campylobacter* from naturally contaminated chicken skin. A large number of competing bacteria was present in each chicken skin / dishcloth homogenate ($\sim$2.4 x 10^8). Delaying the addition of rifampicin (5 μg ml^{-1}) and polymyxin B (2.5 iu ml^{-1}) by 6 h after inoculation of the broth with this homogenate and incubation at 37 °C was found to increase the isolation rate of *Campylobacter* from this naturally heavily contaminated sample after 48 h incubation (Table 3-5). Delaying the addition of rifampicin and polymyxin by 24 h, however, resulted in a decrease in the recovery of *Campylobacter* (Table 3-5).

Table 3-5 The effect of incubation time and the delayed addition of rifampicin and polymyxin B on the recovery of naturally occurring *Campylobacter* from a heavily contaminated sample.

Broth temperature[a]	Incubation duration	Number of positive samples after the addition of rifampicin (5 μg ml^{-1}) and polymyxin (2.5 iu ml^{-1}) @		
		0 h[b]	6 h	24 h
6 °C	48 h	2 / 10	7 / 10	3 / 10
6 °C	120 h	8 / 10	8 / 10	5 / 10
20 °C	48 h	1 / 10	2 / 10	1 / 10
20 °C	120 h	9 / 10	8 / 10	7 / 10

[a] at time of addition, [b] after incubation

All broths were sub-cultured at 48 and 120 h. More broths were *Campylobacter*-positive after the prolonged incubation period (Table 3-5). Recovery of *Campylobacter* after 48 h incubation appeared greater when the broth temperature, on addition, was 6 °C than when at 20 °C (Table 3-5). After 120 h incubation this difference was no longer seen.

3.3.3 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Salmonella*

3.3.3.1   The effect of sulphamandelate and type of plating media on isolation of *Salmonella* from a heavily contaminated sample type

Recovery of *Salmonella* Enteritis PT4 strain I (see section 3.2.4.1) from a carcass rinse homogenised with a dishcloth was investigated using different enrichment techniques.

The addition of sulphamandelate to the pre-enrichment media was found to significantly increase isolation rates of *Salmonella* subbed onto MLCB plates after enrichment in RVS ($P = 0.001$) and CSB ($P = < .0001$; Table 3-6). The recovery rate from XLD and mBGA plates subbed from CSB was also improved ($P = 0.001$ and $P = < 0.0001$ respectively; Table 3-6).   Although the addition of sulphamandelate appeared to improve isolation rates from mBGA subbed from RVS the difference was insignificant ($P = 0.07$; Table 3-6). The presence of sulphamandelate in BPW did not significantly improve the isolation rate when RVS was subbed onto XLD ($P = 0.3$) and although lower levels of background flora were observed on Diassalm plates (data not shown) when sulphamandelate was present in the BPW the isolation rate did not significantly improve ($P = 0.7$; Table 3-6).

Table 3-6 Recovery of *Salmonella* from a heavily contaminated sample type using different enrichment methodology.

| | No. of samples positive[a] | | | | | | |
| | RVS | | | CSB | | | Diassalm |
	XLD	MBGA	MLCB	XLD	MBGA	MLCB	XLD[b]
BPW	24	1	11	2	0	0	23
BPW + sulphamandelate	28	16	29	27	16	30	25

[a] total of 3 experiments, [b] Presumptive *Salmonella*-Positive Diassalm plates were streaked onto XLD for confirmation

When no sulphamandelate was present in the BPW *Salmonella* was recovered from a significantly higher number of XLD plates subbed from RVS and Diassalm than from CSB ($P = 0.01$ and 0.006 respectively).   Significantly more MLCB plates were also *Salmonella*-positive when subbed from RVS than from CSB, in the absence of sulphamandelate ($P = 0.001$; Table 3-6).

Recovery of *Salmonella* on mBGA after enrichment in BPW and RVS, in the absence of sulphamandelate (Table 3-6) was significantly lower than from XLD ($P = 0.01$) and MLCB ($P = 0.01$).   There was no significant difference in the isolation rates of XLD and MLCB subbed from RVS in the absence of sulphamandelate ($P = 0.3$ and 0.1 respectively; Table 3-6) but a higher percentage of colonies were selected as presumptive *Salmonella* from MLCB, which were later found not to be *Salmonella*, than from XLD (data not shown).   When sulphamandelate was added to the BPW there was no difference between the plating media ($P = 0.1$).   Isolation rates of *Salmonella* from CSB when sulphamandelate was present in the BPW were significantly lower using mBGA as a plating medium rather than XLD ($P = 0.01$) or MLCB ($P = <0.001$).

3.3.3.2   Recovery of *Salmonella* after 18 h storage at 4 °C in various diluents

Recovery of *Salmonella* from a Formica surface was greater when swabs were stored at 4 °C for 18 h than when they were directly enriched for *Salmonella* (Table 3-7). The highest rates of recovery were achieved when swabs were stored with no diluent or in MRD (Table 3-7).

Table 3-7 Recovery rates of *Salmonella* after drying on a Formica surface and after storage in different media at 4 °C.

	Number of positive samples (n=10)			
		Storage diluent		
Experiment	Direct enrichment	None	BPW	MRD
1	6	9	3	8
2	0	1	4	3
3	1	4	2	2
Total	7	14	9	14

3.3.3.3 Storage of dishcloths at 4 °C in various diluents

Dishcloths (n=5) were stored for 48 h in BPW, MRD or no media. They were contaminated with an average of 5.2 $\log_{10}$ *Enterobacteriaceae* (standard error [SE] 5.0) and an average of 5.6 $\log_{10}$ ACC (SE 5.4; Table 3-8). Numbers of both *Enterobacteriaceae* and ACC were found to increase during the storage period but due to the high level of variability no statistical differences between the different storage media could be detected (Table 3-8).

Table 3-8 Changes in numbers of *Enterobacteriaceae* and aerobic colony count on dishcloths after 48 h storage at 4 °C in different diluents.

	Enterobacteriaceae ($\log_{10}$ increase)			TVC ($\log_{10}$ increase)		
Diluent	BPW	MRD	No diluent	BPW	MRD	No diluent
24 h storage	6.5	6.4	6.0	6.8	7.0	6.7
	(SE 6.5)	(SE 6.4)	(SE 6.0)	(SE 6.8)	(SE 7.0)	(SE 6.6)
48 h storage	7.0	7.0	5.3	8.1	7.7	6.7
	(SE 7.0)	(SE 7.0)	(SE 3.4)	(SE 8.1)	(SE 7.7)	(SE 6.7)

Salmonella was inoculated onto the dishcloths at T_0 and could be recovered from all dishcloths (5/5) stored in MRD for up to 2 days (data not shown). When dishcloths were stored in BPW *Salmonella* could be isolated from 4 / 5 on day one and from 3 / 5 on day two. *Salmonella* was isolated from only two of the four dishcloths examined after one and two days storage when no media was present.

3.4 Discussion

3.4.1 Temperature regulation of cold boxes

The ability of a cold box and ice packs to regulate the temperature of a cold box in a range of 0 – 8 °C was investigated. Results demonstrated that a satisfactory temperature range could be maintained during transport and overnight storage, when it was not possible to analyse samples on the same day.

3.4.2

3.4.3 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Campylobacter*

3.4.3.1 Recovery of *Campylobacter* cells damaged by refrigeration

Chilling is a common stress encountered by *Campylobacter* isolated from chicken breasts and, in this study, from contaminated samples transported and stored at <8 °C. The storage of chicken homogenate, in MRD, for 1 week at 4 °C appeared to reduce the number of viable cells. Similar results have been reported by Chynoweth *et al.* (1998), Moore & Madden (2001) and Oosterom *et al.* (1983). Lee *et al.* (1998), however, reported an increase in the viable count of *C .jejuni* present on chicken breasts, during one weeks storage at 4 °C. There was no evidence of such a phenomenon during this experiment but the results reported by Lee *et al.* (1998) may, however, represent a recovery of viability rather than growth, the minimum temperature growth temperature for *Campylobacter* is widely reported to be above 28 °C (Humphrey 1992; Skirrow 1994; Solomon & Hoover 1999).

This study differed from other published studies in terms of the nature of the storage medium and the presence of competing microflora. The survival of *Campylobacter* in chicken skin homogenate was examined due to the homogenous nature of the

medium. Chynoweth *et al.* (1998) used a sterile chicken mince model but reported variability within replicates. Chicken skin contains many microenvironments within the folds of the skin and the feather follicles, which could affect the survival of *Campylobacter*. For the purposes of this study a model was required which was repeatable and did not contain a large a degree of inherent variation, the chicken skin homogenate model provided this. The chicken skin homogenate model also utilised the natural microflora present on the chicken skin thus any *Campylobacter* naturally present would have been subjected to similar pressures as the strains present on the chicken breasts during the food preparation.

Earlier work demonstrated that the reduction of recoverable *Campylobacter* cells by refrigeration was consistent. In a second experiment the effect of refrigerating cells in modified Exeter broth was determined. Modified Exeter broth (with its full complement of antibiotics) has been shown to affect the recovery of cold-damaged *Campylobacter* (Humphrey 1986b). There was no difference between the cells stored at 4 °C for an additional 18 h in modified Exeter broth and those incubated directly, indicating that modified Exeter broth could be used as a storage medium for cold-damaged *Campylobacter*.

3.4.3.2   Storage and recovery of *Campylobacter* cells damaged following air drying on a surface

Campylobacter spp. contaminating domestic kitchens are likely to be exposed to a number of environmental stresses including sub-ambient temperatures and drying on surfaces. *Campylobacter* has been reported to be sensitive to drying with cells becoming non-viable soon after the suspending media appeared dry (Humphrey *et al.* 1994b; Oosterom *et al.* 1983) and this work confirmed these previous findings. A preliminary study using naturally contaminated *Campylobacter* samples indicated that tolerance to air-drying was poor (*Campylobacter* was isolated from only 5% of the swabs). Due to the low number of recoverable *Campylobacter* cells after the drying period no differences between storage conditions could be detected. Such a poor level of recovery was in part due to the relatively low initial numbers of *Campylobacter* present in the inoculum but also because of the rapid decline in viability after drying.

Due to the sensitivity of *Campylobacter* to drying and limits of detection, this work needed to be repeated using a sample artificially inoculated with high numbers of cells. After only 30 mins of drying, the Formica squares still appeared moist, and *Campylobacter* could be recovered from 60% of inoculated squares, which given the fragility of the organism under conditions of laboratory desiccation is acceptable for the later work to be performed.

The swabs used to recover *Campylobacter* from the Formica surface were stored at 4 or 1 °C with or without modified Exeter broth in order to determine optimum transport conditions for the cells. The recovery of *Campylobacter* was greatly improved when swabs were stored in modified Exeter broth, which contains oxygen quenching agents, sodium pyruvate, sodium metabisulphite and ferrous sulphate added in the form of FBP and 5% lysed horse blood. *Campylobacter* are sensitive to atmospheric oxygen concentrations so a reduced oxygen concentration and reduced oxygen radicals would promote the survival of *Campylobacter* spp. Perhaps the most important aspect of using a broth as a transport medium is the prevention of further desiccation stress for the cells associated with the swab.

Only enrichment media were examined for sample transport during this study. Large numbers of samples were taken from kitchens for *Campylobacter* enrichment later in this study and it would not have been feasible to change the transport media for enrichment media on arrival at the laboratory. Humphrey *et al.* (1994b), however investigated survival of *C. jejuni* in a number of different diluents and found greater isolation rates from swabs of contaminated surfaces stored in selective media than those stored in other media before culture.

There appeared to be no difference in recovery of *Campylobacter* from swabs stored at 1 °C and those stored at 4 °C demonstrating that a certain degree of temperature fluctuation in this range (a range achievable using a cold box) would not be detrimental.

Broths were incubated for up to 120 h because *Campylobacter* are known to generally grow more slowly than other enteric flora, particularly when damaged (Solomon &

Hoover 1999). During one experiment the increased incubation period led to the isolation of *Campylobacter* from swabs which were previously culture negative. This led to the belief that a prolonged incubation period would maximise the chances of recovering sub-lethally damaged cells and was the recommended protocol for all kitchen samples.

3.4.3.3   Effect of polymyxin and rifampicin on the recovery of *Campylobacter*

In this experiment the effect of rifampicin and polymyxin on the recovery of undamaged *Campylobacter* cells was examined. Results showed that rifampicin and polymyxin had no effect on the recovery of *C. jejuni* strain WK3A. They did, however, inhibit the recovery of *C. coli* strain 2604. It is not possible to determine, from these results, if this difference is related to the characteristics of the species or of the strains examined. It would not be feasible to totally exclude polymyxin or rifampicin from modified Exeter broth used during this study. High levels of *Enterobacteriaceae* have been reported in domestic kitchens (Scott *et al.* 1982; Speirs *et al.* 1995) and without antibiotics, these organisms could prevent the isolation of *Campylobacter*.

Recovery of cells was much greater from blood agar than from the enrichment broth even when no selective agents were present. The cells may be better able to replicate when attached to the agar surface or the nutritionally complex enrichment broth could be reducing the recovery of the cells. Gomez *et al.* (1973) found that resuscitation of heat-damaged *S.* Typhimurium cells was greater in a minimal, defined medium than a nutritionally complex media and the *Campylobacter* cells damaged by air surface drying may be displaying a similar phenomenon.

Blood agar is only useful when *Campylobacter* are present in pure culture and would be impractical to use in the isolation of *Campylobacter* from the kitchen environment due to the low numbers present and the high levels of competing organisms.

3.4.3.4 Effects of delaying the addition of rifampicin and polymyxin in Modified Exeter broth on *Campylobacter* recovery from a heavily contaminated sample

Delaying the addition of rifampicin and polymyxin by 6 h after incubation achieved maximal recovery of *Campylobacter* from the heavily contaminated samples examined. This delayed addition gives any sub-lethally damaged *Campylobacter* time to recover yet prevents over-growth by competing micro-flora.

Heavily contaminated samples were used in order to test the effect of delayed addition in the most extreme case. Delaying addition of antibiotics by 24 h resulted in a decrease in the recovery of *Campylobacter*, probably due to over growth by competing micro-flora. Martin *et al.* (1996) found similar results using river water and chicken samples. A greater number of broths were *Campylobacter*-positive at 120 h than at 48 h, again indicating the presence of an extended lag phase as sub-lethally damaged cells recovered.

After 48 h incubation, a greater number of broths were *Campylobacter*-positive when the broth was added at a temperature of 6 °C than at 20 °C. The colder broth would have delayed the growth of competing organisms, which may have otherwise delayed or even prevented the recovery of the *Campylobacter* spp. Although *C. Jejuni* and *C. coli* cannot grow at temperatures below 30 °C (Anon 1995a) the gradual warming of the media will give the cells a greater length of time to recover before having to compete with contaminating organisms.

3.4.4

3.4.5 Selection of the most appropriate / sensitive microbiological methods for isolation and survival of *Salmonella*

3.4.5.1   The effect of sulphamandelate and type of plating media on the isolation of *Salmonella* from a heavily contaminated sample type

An investigation into the addition of sulphamandelate into pre-enrichment media (BPW) in conjunction with standard isolation methodologies found that overall the addition of sulphamandelate improved the isolation rate of *Salmonella* from both RVS and CSB, although this was dependent on the plating medium used. Sulphamandelate is a supplement containing sodium sulphacetamide and sodium mandelate, normally added to brilliant green agar to improve isolation of *Salmonella* from sewage and sewage sludge due to a greater inhibition of *Escherichia coli* and *Proteus* and a restriction of the growth of *Pseudomonas* spp. (Anon 2001a).   The sulphamandelate would, therefore, inhibit the growth of these competing organisms in the pre-enrichment broth. Competing micro-flora has been shown to one of the most important factors in the isolation of *Salmonella* (Arroyo & Arroyo 1995) and by inhibiting the growth of such organisms the sensitivity of the detection method can be improved.   Indeed van Schothorst & Renaud (1985) found that the addition of malachite green to BPW (the pre-enrichment broth) could improve the isolation of *Salmonella* from heavily contaminated samples due to its ability to limit the growth of Gram-positive bacteria. As with other selective agents sulphamandelate had been shown to affect the growth of some *Salmonella*. Jones *et al.* (1984) found that sodium sulphacetamide and sodium mandelate (the constituents of sulphamandelate) resulted in a reduction of the colony size of some *Salmonella* on brilliant green agar. There was no evidence, however, that the presence of sulphamandelate in the pre-enrichment broth inhibited the recovery of *Salmonella*.

Sulphamandelate in BPW did not improve the isolation rate from Diassalm or XLD subbed from RVS, which were already high. This suggests that these media are

perhaps more selective than the others examined. Diassalm is a semi-solid media rather than an enrichment broth and as well as selective agents (malachite green oxalate, magnesium chloride and novobiocin) it also utilises the ability of *Salmonella* to move through this highly selective motility medium.

RVS also appears to have a greater inhibitory effect on competing micro-flora than CSB. Munoz *et al.* (1987) and Harvey & Price (1981) have also reported a greater isolation rate from RVS than CSB and Morinigo *et al.* (1993) reported that enrichment media containing selenite were less inhibitory to Gram-positive organisms than those containing malachite green (e.g. RVS). When sulphamandelate was present in the BPW the difference in the isolation rates of the three enrichment media was minimal indicating that the inhibitory effect of sulphamandelate in the pre-enrichment broth (BPW) is sufficient to reduce the number of competing micro-flora entering the enrichment broths and promote recovery of *Salmonella*.

Although Diassalm performed well, only RVS was available pre-prepared by the media department. Due to the large number of samples involved in this project and the consequent time constraints pre-enrichment of samples in BPW supplemented with sulphamandelate followed by enrichment in RVS was chosen for use in this project.

Recovery of Salmonella on mBGA was poor compared to XLD and MLCB. All of the plating media contained selective agents to prevent overgrowth. Both mBGA and MLCB utilise brilliant green, a triphenylmethane dye, to reduce competitors, whilst XLD utilises the inhibitory effects of sodium deoxycholate. As already discussed the presence of selective agents are important to prevent overgrowth of *Salmonella* by competing micro-flora. Levels of brilliant green are higher in MLCB than in mBGA and this may account for the higher isolation rate of *Salmonella* on this medium. *Salmonella* colonies on XLD and MLCB were also easier to visualise than on mBGA and this may also have led to a lower isolation rate of *Salmonella* on mBGA. Although XLD and MLCB both performed well there were fewer false positives using XLD and this plating media was selected for use in the project.

3.4.5.2 Recovery of *Salmonella* after 18 h storage at 4 °C in various diluents

Salmonella were recovered from only 37% of the swabs examined indicating that one hour of drying was sufficient to damage the *Salmonella* cells present to such an extent that they were unrecoverable using these methods, or that the they were unable to survive the drying process.

Although there was no statistical difference, the total number of *Salmonella*-positive swabs was greater when they were stored overnight at 4 °C than when directly incubated. It is likely that at least a proportion of the cells recovered were sub-lethally damaged and that the delay before incubation may promote the recovery of *Salmonella,* possibly because the storage time allowed for the gradual re-hydration of the dried *Salmonella* cells. Mattick *et al.* (2001) reported that gradual re-hydration greatly improved the isolation rate of cells damaged by low water activity and high temperatures. Other workers have found that storage of swine faeces at 4 °C did not result in a decrease in the isolation rate of *Salmonella* (O' Carroll *et al.* 1999).

There was no statistical difference in the recovery rate of the swabs stored in the different diluents. Storage in BPW may, however, prove problematic if the temperature of the cold box were to rise above the predicted temperature if, for example, the cold box was delayed during transport. Buffered peptone water is a nutrient rich growth medium and any competing organisms present on samples could increase during transport, if the temperature were to rise, whilst conditions were still sub-optimal for the growth of *Salmonella*. The growth of competing organisms could affect the recovery of *Salmonella* so for the purposes of this project all swab samples were transported in 10 ml MRD.

3.4.5.3 Storage of dishcloths at 4 °C in various diluents

Dishcloths stored in BPW or MRD appeared to show a greater increase in *Enterobacteriaceae* and aerobic colony counts than those stored without diluent. Due

to high levels of variability, however, the differences were not significant. Dishcloths from domestic kitchens were used as the inocula and this may have been one factor involved in the high level of variability. The number of bacteria present on the dishcloths at T_0 varied greatly and also the amount of organic material present on the dishcloths was likely to have varied. Only presumptive *Enterobacteriaceae* were identified and it is likely that different species of bacteria were present on the dishcloths for each of the experiments, possibly affecting the results. Despite the problems of variability it appears that when cloths were stored without diluent the number of bacteria present on the cloth during storage dropped, possibly due to desiccation stress. This was reflected in the isolation of *Salmonella* from the dishcloths when only 2 / 4 examined after one and two days storage were *Salmonella*-positive. The isolation rate of *Salmonella* from dishcloths stored in BPW decreased from 4 / 5 to 3 /5 between days one and two. It is possible that the increased levels of competing bacteria affected the isolation of the *Salmonella*. There was, however, no decrease in the isolation rate of *Salmonella* from dishcloths stored in MRD and this was the storage diluent chosen to transport dishcloths from the kitchens to the laboratory.

Chapter 4. Pilot work for determining exposure routes during food handling, in a test domestic kitchen, using observation and microbiological assessment

4.1 Introduction

In recent years there has been considerable attention drawn to the increasing numbers of food poisoning cases (23,000 confirmed cases of *Salmonella* and over 58,000 cases of *Campylobacter* in 1998; Anon 2000b) the majority of which are believed to originate in the domestic kitchen (Griffith *et al.* 1994). The sporadic nature of many of these cases means, however, that it is often hard to identify the source and exposure route. More than one control point may be involved and few participants are able to accurately recall hygienic practices (Jay *et al.* 1999).

Numerous studies have determined that the spread of both *Salmonella* and *Campylobacter* can be facilitated by poor hygiene practices and observational studies have demonstrated that such practices are relatively common. Cross contamination incidences were identified as a contributing factor in 25% of foodborne outbreaks in England and Wales between 1993 and 1998 (Tirado & Schmidt 2000) and it is likely to be important in sporadic cases of food poisoning originating in the home.

Raw poultry is an important source of *Salmonella* and *Campylobacter* and is commonly linked to food poisoning incidences (Bryan & Doyle 1995; Hopkins & Scott 1983; Kapperud *et al.* 1992). Large numbers of *Campylobacter*, particularly, have been isolated from carcases and a recent study by Jorgensen *et al.* (2002) found that about 30% of carcases were contaminated with more than $\log_{10}5$ cfu. Given the apparent ease at which *Campylobacter* can be transferred from carcasses to kitchen surfaces (Cogan *et al.* 2000; de Boer & Hahne 1990) these bacteria represent a significant risk.

Unwashed or inadequately washed hands contaminated by raw chicken are believed to be a significant factor in the transfer of organism within the kitchen with

approximately half of consumers failing to wash their hands after handling raw meat or its packaging (Jay *et al.* 1999; Worsfold & Griffith 1997a). Scott & Bloomfield (1990) found that after contact with contaminated surfaces significant numbers could be transferred to fingers and Chen *et al.* (2001) demonstrated transfer of bacteria from hands to other kitchen surfaces. Other commonly observed behaviour, which has been found to cause cross contamination includes the use of chopping boards for raw meat and then ready to eat vegetables and the inappropriate use of wiping cloths (Jay *et al.* 1999; Worsfold & Griffith 1997a). Given the large number of such unhygienic practices which occur in domestic kitchens daily and given the low infectious dose of *Campylobacter* and *Salmonella* is it likely that cross contamination incidents as a contributing factor in food poisoning cases is severely under estimated.

The improved isolation methodologies for *Salmonella* and *Campylobacter* discussed in Chapter three will be used to determine sites of contamination, based on exposure routes. Participants will prepare a chicken salad, whilst under observation, in a test domestic kitchen before potentially contaminated sites are sampled. Unlike other studies, which have sampled base line contamination in kitchens, this study will sample potentially contaminated sites soon after the contamination incidents have occurred and, therefore, gain a realistic insight into which hygiene errors are likely to lead to contamination, allowing minimal time for target organisms to become damaged. The use of a test kitchen, which has been cleaned and disinfected, means that all sources of potential contamination can be identified and enables a more accurate determination of exposure assessment. In this study the raw ingredients used to prepare the chicken salad are the only source of pathogens and, given the low rates of contamination on salad vegetables, raw poultry is likely to be the only significant source.

Groups, which may be particularly at risk from low numbers of contaminating organisms, include the elderly and very young. In order to determine cross contamination rates during the preparation of meals for these vulnerable groups the hygienic practices of the elderly and mothers, who prepare food for their young children, will be examined. The hygienic practises of single young men, a group which have been found to suffer a high proportion of intestinal disease (Skirrow 1987), will also be studied.

4.1.1 Aims

Pilot and validate cultivation methodologies and identify commonly contaminated kitchen sites and pathogen exposure routes during the preparation of a poultry-based meal in a test domestic kitchen.

4.1.2 Objectives

Analyse raw ingredients and meals for *Salmonella*, *Campylobacter*, *Enterobacteriaceae* and aerobic colony counts.

Examine the food handling practices of 30 participants (consisting of single young men, mothers with young children and retired participants) in a test domestic kitchen to determine pathogen exposure routes and the most appropriate sampling sites.

Analyse selected sites/materials in the kitchen for the presence of *Salmonella*, *Campylobacter* and *Enterobacteriaceae* using previously validated protocols.

Compare contamination rates, after food preparation, of single young men, mothers with young children and retired participants.

4.2   Materials and method

4.2.1   Design of the test kitchen

In order to record the behaviour of participants and the microbial contamination involved in preparing a chicken salad, a domestic kitchen was recreated to mimic a domestic one. The kitchen consisted of 6 wall cupboards, 6 floor cupboards and 3 work surfaces (each of which was divided into two areas when sampled). The kitchen units, sink, taps, fridge and oven were of a design commonly seen in the domestic kitchen and the kitchen was equipped with a range of sanitation and disinfection products. The test kitchen was thoroughly cleaned and disinfected, before each food preparation session, using a previously validated protocol (Griffith *et al.* 2002), to ensure that all surfaces were free from contaminants.

4.2.2   Rationale for choice of poultry-based meal

Given the relatively high levels of *Salmonella* and *Campylobacter* isolated from poultry (Jorgensen *et al.* 2002) and its associated with foodborne disease (Tirado & Schmidt 2000) a poultry-based meal was selected for preparation in this study. The chicken salad recipe chosen (Figure 4-1) was relatively straightforward and could be completed within a short space of time. Importantly the chicken salad recipe also provided opportunities for the handling of raw and ready to eat foods and allowed cross contamination of not only the kitchen but also the prepared meal to be investigated.

Figure 4-1   The chicken and pasta salad recipe participants were asked to prepare

Ingredients

1 chicken breast (with skin)

15 ml / 1 tbsp. vegetable oil

50g / 2oz Fusilli pasta shapes salt and pepper

¼ Iceberg lettuce, chopped

2 tomatoes, chopped

2 spring onions, sliced

2-3 slices of cooked ham

10ml / ½ tbsp. olive oil

1 0ml / ½ tbsp. pesto

chopped mixed herbs

Method

1. Cook the pasta in boiling salted water for 8 to 10 minutes (or according to packet instructions)

2. Remove skin from chicken breast

3. Chop chicken into suitably sized pieces and shallow fry using 1 tbsp. vegetable oil

4. Drain the pasta, cool and place into a mixing bowl

5. Cut the slices of ham into strips

6. Prepare all salad vegetables

7. Meanwhile gently heat the olive oil and pesto in a small pan

8. Add cooked chicken pieces, sliced ham and salad vegetables to the pasta and mix well

9.Remove the pesto mixture from the heat and pour over the salad ingredients, chicken ham and pasta and season to taste using chopped mixed herbs and any additional salt and pepper

10. Serve one portion of the salad and keep the remaining salad for a meal `the following day'

4.2.3 Profile of recruited participants[1]

Participants were identified by a recruiting agency (Beaufort research, 2 Museum Place, Cardiff, CF10 3BG) using a specific questionnaire. Based on the responses to the questionnaire, the recruitment agency selected three groups of participants: mothers with young children (< 10 years), single young men (18 – 28 years) and a post retirement group (>60-75 years). These participants all regularly cooked at least one meal a day and had no food hygiene qualifications. All participants were asked to prepare and store a chicken salad (Figure 4-1) and serve a pre-prepared salad, brought as a convenience meal. After preparing the chicken salad, participants were asked to clean the kitchen as they would do in their own home. During the meal preparation participants' behaviour was recorded, onto video, using two cameras. Possible contamination events, and the method used to clean any possibly contaminated items were recorded using two checklists (Appendix A). Participants were given a £15 supermarket gift voucher on completion of the practical.

4.2.4 Sampling of raw materials and selected materials / areas and salads after the food preparation session

Two 25 g aliquots of the majority of raw ingredients, and salads were weighed into separate 250 ml containers before being transported and stored as described below (section 4.2.5, Figure 4-2).

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[1] The recording of participant's behaviour, and the taking and transport of samples was carried out by E. Redmond using the methods described in sections 4.2.3, 4.2.4, and 4.2.5.

Figure 4-2 Methods to recover *Salmonella* and *Campylobacter* from raw materials and to enumerate ACC's and *Enterobacteriaceae*

```
┌─────────────────────────────────┐
│ 2 x 25 g of raw material, prepared salad, │
│ and served convenience salad were added │
│ to 250 ml containers │
└─────────────────────────────────┘
```

```
┌──────────────────────────────┐   ┌──────────────────────────────┐
│ 25 g added to 225 ml of BPW  │   │ 25 g added to 225 ml of modified Exeter │
│ (with 0.05% sodium thiosulphate) │   │ broth (with 0.05% sodium thiosulphate) │
│ Homogenised for 2 mins       │   │ leaving minimum head space, addition of │
│ Homogenate diluted, in MRD, to │   │ rifampicin and polymyxin was delayed │
│ appropriate dilution         │   │ until 6 h after incubation │
└──────────────────────────────┘   └──────────────────────────────┘
```

```
┌────────────┐ ┌────────────┐ ┌────────────┐ ┌────────────┐
│ 1ml of each │ │ 1ml of each │ │ BPW incubated │ │ Incubated for 120 h at │
│ dilution added to │ │ dilution added to │ │ for 24 h at │ │ 37 °C, sub-cultured │
│ each of 2 petri │ │ each of 2 petri │ │ 37 °C │ │ onto CCDA at 48 and │
│ dishes. Pour plates │ │ dishes. Pour │ │ │ │ 120 h. Plates │
│ were prepared │ │ plates prepared │ └────────────┘ │ incubated │
│ using PCA and │ │ using VRGBA, │ │ microaerobically at 37 │
│ incubated at 30 °C │ │ and incubated at │ ┌────────────┐ │ °C for 48 h. │
│ for 72 h. │ │ 37 °C for 24 h. │ │ 0.1 ml inoculated │ └────────────┘
└────────────┘ └────────────┘ │ into 10ml RVS, │
                                │ incubated for 24 │
                                │ h at 41.5 °C │
                                └────────────┘

                                ┌────────────┐
                                │ Sub-cultured (10 │
                                │ µl) onto XLD │
                                │ and incubated at │
                                │ 37 °C for 24 h │
                                └────────────┘
```

Note:- The dashed line separates methods carried out before transport and storage

Pasta, salt, pepper and olive oil were not analysed microbiologically and the dried herbs were only examined on one occasion. These ingredients were not expected to be a probable source of high numbers of bacteria due to their inherent low water activities. Although *Salmonella* had been isolated from dried herbs (Bocckemuhl & Wohlers 1984) because of the very small quantities used during meal preparation they were unlikely to be a source of contamination unless growth occurred on the food.

Swabs of kitchen surfaces and samples of materials were taken after preparation of the chicken salad and cleaning, by the participant. Surfaces and / or materials which were directly contaminated from chicken or packaging or indirectly contaminated by hands contaminated from the raw chicken up to three actions later were sampled. Samples taken included swabs of hob controls, fridge handles and work surfaces. A maximum of 31 samples were analysed for each practical.

Cotton tipped swabs, pre-moistened in MRD containing 0.05% sodium thiosulphate, were used to swab surfaces. Each swab site was divided into two areas. The first area was swabbed for *Enterobacteriaceae* including *Salmonella* and the second for *Campylobacter*. The swabs were placed into either 15 ml of MRD containing 0.05% sodium thiosulphate for enumeration of *Enterobacteriaceae* and *Salmonella* enrichment or 20 ml of modified Exeter broth, lacking polymyxin and rifampicin, containing 0.05% sodium thiosulphate for enrichment of *Campylobacter* (Figure 4-3). Swab samples were transported and stored as described below (section 4.2.5).

Tea and hand towels were shaken for 2 minutes in 400 or 500 ml of MRD respectively. One hundred and twenty five ml of rinse was added to each of two 250 ml containers. Double strength modified Exeter broth, lacking rifampicin and polymyxin, containing 0.05% sodium thiosulphate (125 ml) was added to one of the aliquots before transportation and storage (section 4.2.5, Figure 4-4).

Figure 4-3 Methods to recover *Salmonella* and *Campylobacter* from swabs

```
                        ┌─────────────────────┐
                        │ Swabs of surfaces (x2) │
                        └─────────────────────┘
                          ╱                  ╲
                        ╱                      ╲
```

┌───────────────────────────┐   ┌───────────────────────────┐
│ One swab added to a universal │   │ One swab added to a   │
│ containing modified Exeter broth │ │ universal containing 15   │
│ with 0.05% sodium thiosulphate, │   │ ml MRD with 0.05%   │
│ leaving minimal head space,   │   │ sodium thiosulphate   │
│ addition of rifampicin and   │   └───────────────────────────┘
│ polymyxin was delayed until 6 h │
│ after incubation   │
└───────────────────────────┘

- -

┌───────────────────────────┐   ┌───────────────────────────┐
│ Broths were incubated for 120 h at │ │ 10 ml of double strength (DS) │
│ 37 °C and 10 µl sub-cultured CCDA │ │ BPW, containing DS   │
│ after 48 and 120 h. Inoculated plates │ │ sulphamandelate was added before │
│ were incubated microaerobically at │ │ incubation at 37 °C for 24 h │
│ 37 °C for 48 h.   │   └───────────────────────────┘
└───────────────────────────┘

                                      ┌───────────────────────────┐
                                      │ 0.1 ml added to 10 ml RVS, and │
                                      │ incubated at 41.5 °C for 24 h │
                                      └───────────────────────────┘

                                      ┌───────────────────────────┐
                                      │ 10 µ sub-cultured onto XLD and │
                                      │ incubated at 37 °C for 24 h │
                                      └───────────────────────────┘

Note:- the dashed line separates methods carried out before transport and storage

Figure 4-4 Methods to recover *Salmonella* and *Campylobacter* from tea towels and hand towels

```
┌─────────────────────────────┐        ┌─────────────────────────────┐
│ Tea towel                   │        │ Hand towel                  │
│ Homogenised in 400 ml MRD   │        │ Homogenised in 500 ml MRD   │
│ with 0.05% sodium           │        │ with 0.05% sodium           │
│ thiosulphate                │        │ thiosulphate                │
└─────────────────────────────┘        └─────────────────────────────┘
                │                                    │
                └───────────┐            ┌───────────┘
                            ▼            ▼
                ┌─────────────────────────────┐
                │ 125 ml of homogenate added  │
                │ into each of 2x 250 ml      │
                │ containers                  │
                └─────────────────────────────┘
                   │                      │
     ┌─────────────┘                      │
     ▼                                    │
┌─────────────────────────────┐          │
│ DS modified Exeter broth,   │          │
│ with 0.05% sodium           │          │
│ thiosulphate, added to one  │          │
│ container leaving minimum   │          │
│ head space, addition of     │          │
│ rifampicin and polymyxin    │          │
│ was delayed until 6 h after │          │
│ incubation                  │          │
└─────────────────────────────┘          │
          │                               │
```

```
          │                               ▼
          │            ┌─────────────────────────────┐
          │            │ 125 ml of DS BPW added to   │
          │            │ one container before        │
          │            │ incubation at 37 °C for 24 h│
          ▼            └─────────────────────────────┘
┌─────────────────────────────┐          │
│ Broths incubated for 120 h  │          ▼
│ at 37 °C, and sub cultured  │  ┌─────────────────────────────┐
│ (10 µl) onto CCDA at 48 and │  │ 0.1 ml inoculated into 10 ml│
│ 120h. Plates were incubated │  │ RVS, before incubation at   │
│ microaerobically at 37 °C   │  │ 41.5 °C for 24 h            │
│ for 48 h                    │  └─────────────────────────────┘
└─────────────────────────────┘          │
                                          ▼
                               ┌─────────────────────────────┐
                               │ RVS sub-cultured onto XLD   │
                               │ before incubation at 37 °C  │
                               │ for 24 h                    │
                               └─────────────────────────────┘
```

Note:- the dashed line separates methods carried out before transport and storage

Dishcloths were cut into two. One half was added to MRD (100 ml) containing 0.05% sodium thiosulphate and the other to a 250 ml container full of modified Exeter broth, lacking rifampicin and polymyxin, but containing 0.05% sodium thiosulphate. Samples were then transported and stored as described below (section 4.2.5, Figure 4-5).

Figure 4-5 Methods to recover *Salmonella* and *Campylobacter* from dishcloths

Note:- the dashed line separates methods carried out before transport and storage

4.2.5 Transport and storage of samples

Samples were transported in cold boxes (36 x 27 x 34 cm) with added polystyrene for insulation and up to six ice packs (20 x 11 x 4 cm). The temperature of the cold box during transit was recorded at regular interval using a Testostor 175 data logger (Borolabs, Berkshire). Samples, which could not be analysed on the same day, were stored for no longer than 20 h, under the conditions described above.

4.2.6 Enumeration of *Enterobacteriaceae* and ACCs on raw materials and selected samples (*Enterobacteriaceae* only)

Twenty-five gram samples of the raw ingredients and salads were added to 225 ml of BPW containing sulphamandelate and homogenised for two minutes (Figure 4-2). The homogenate was serially diluted to 10^{-7} in 9 ml of MRD. One ml of each dilution was added to four petri dishes. Fifteen ml aliquots of molten PCA and VRBGA maintained at 45 – 48 °C were added to duplicate plates. Each plate was mixed and allowed to set. Plates poured with VRBGA were overlaid by a further 10 ml of molten VRBGA before incubation at 37 °C for 24 h. PCA plates were incubated at 30 °C for 72 h. Aerobic colony counts were obtained from PCA and presumptive *Enterobacteriaceae* counts from VRBGA. Plates containing 30-300 colonies per plate were counted using a colony counter.

Swabs of areas likely to be highly contaminated, taken after the meal preparation of 20 participants, were also enumerated for presumptive *Enterobacteriaceae*. Swabs transported in MRD were mixed for 1 min using a vortex mixer (Jencons Miximatic, Jencons PLS, Leighton Buzzard) before two one ml aliquots were removed and pour plated with VRBGA (see above). Plates were incubated and enumerated as previously described. The remaining swab diluent was enriched for *Salmonella* as described below (4.2.7).

4.2.7 Enrichment and identification of presumptive *Salmonella*

Homogenate from the raw materials or salads (244 ml, see above) was incubated for 24 h at 37 °C before 100 µl was removed and used to inoculate 9 ml RVS broths. Broths were incubated for 24 h at 41.5 °C before subculture (10 µl) on to XLD plates to obtain single colonies. Plates were incubated at 37 °C for 24 h before being examined for *Salmonella*.

Double strength BPW containing double strength sulphamandelate was added to swabs in MRD (10 ml), dishcloths (100 ml), tea towels (100 ml) and hand towels (125 ml) before incubation at 37 °C for 24 h. Enrichment was then carried out for *Salmonella* as described above.

Identification of *Salmonella* was based initially on colony morphology and confirmed using standard biochemical and serological techniques (Jorgensen *et al.* 2002).

4.2.8 Enrichment and identification of presumptive *Campylobacter*

Twenty-five grams of raw material or salad was added to 225 ml of modified Exeter broth in 250 ml containers. Broths were incubated at 37 °C for 120 h. After 6 h incubation rifampicin and polymyxin (5 µg / ml and 2.5 iu / ml respectively) were added. After 48 and 120 h incubation 10 µl was sub-cultured on to CCDA, to obtain discrete colonies. Inoculated plates were incubated under micro-aerobic conditions for 48 h being examined for *Campylobacter*. Presumptive *Campylobacter* isolates were confirmed using growth on blood agar in aerobic and micro-aerobic atmospheres at 37 °C after 48 h, oxidase activity and cell morphology observed by phase contrast microscopy (Bolton *et al.* 1992).

Swabs, dishcloth, tea towel and hand towel samples were all transported in modified Exeter broth as described in sections 4.2.5. These samples were incubated at 37 °C for up to 120 h. After 6 h incubation rifampicin and polymyxin (5 µg / ml and 2.5 iu /

ml respectively) were added. Ten µl was removed and streaked on to CCDA after 48 and 120 h. Plates were incubated and examined as described above.

4.2.9 Storage of isolates

Isolates were sub-cultured no more than five times, to avoid changes in strain characteristics, and stored on cryobeads (CRYO/M MAST Diagnostics) at –40°C. Strains were recovered by streaking a bead onto blood agar before incubation under appropriate conditions.

4.2.10 Typing of *Campylobacter* isolates

The *Campylobacter* Reference Unit (CRU) at the Central Public Health Laboratory (CPHL) speciated, serotyped and phage typed the *Campylobacter* isolates. Selected isolates were identified to species level using standard phenotypic tests (Bolton *et al.* 1992) and further characterised using an adaptation of the Penner serotyping scheme (Frost *et al.* 1998). Phage typing (Frost *et al.* 1999) was carried out on isolates for more detailed characterisation.

4.2.11 Statistical analysis

Statistical analysis was carried out in Microsoft Excel '97 using a *t* test on two samples, assuming equal variance.

4.3 Results

4.3.1 *Enterobacteriaceae* and aerobic colony counts from salad ingredients and salads

The number of *Enterobacteriaceae* and aerobic colony count (ACC) present on the fresh ingredients used for the food preparation sessions, the ready meals (after serving) and the completed homemade salad were determined for each of the 30 food preparation sessions. *Enterobacteriaceae* and ACC on the dried herbs from one food preparation session were also calculated. Pasta, salt, pepper and olive oil were not analysed microbiologically.

The range of dilutions selected, as appropriate, for each given sample was occasionally unable to give an accurate number due to an unexpectedly high result. On such occasions the maximum number of colonies which could be enumerated were calculated (300 per plate; Anon 1995b) and this figure was used to calculate averages. It is likely that the average values in these samples are underestimates of the true figure but the effect is likely to be minimal. Sample types with values above the detection limit included 3 / 30 spring onion samples (*Enterobacteriaceae* and ACC), 2 / 30 ham samples (ACC only) and 2 / 30 raw chicken breast samples (ACC only).

The lower limit of detection was 0.4 cfu per gram of sample. To obtain averages when no bacteria were detected (i.e.< 0.4) a value of 0.2 cfu per gram, mid way between the possible values (0 and 0.4 cfu g^{-1}) was given. The *Enterobacteriaceae* counts for a number of raw materials sampled were below the lower limit; 2 / 30 lettuce, 7 / 30 tomatoes, 23 / 30 ham, 27 / 30, Pesto, 11 / 26 convenience meals and 2 / 30 homemade salads. Aerobic colony counts were occasionally below the detection level (1 / 30 ham, 1 / 30 pesto, 1 / 30 convenience meal).

Enterobacteriaceae and ACCs per gram of ingredients are given in Figure 4-6.

Figure 4-6 Mean bacterial counts per gram of ingredient.

Raw chicken breasts and spring onions were both contaminated with large numbers of bacteria (Figure 4-6) and *Enterobacteriaceae* were isolated from all of the 30 samples analysed. The average weight of the two chicken breasts was 310 g and, given that the average number of *Enterobacteriaceae* and total aerobic colony counts (ACCs) were 2.1 X 10^4 and 6.6 x 10^5 cfu g^{-1} respectively (Figure 4-6), the average pair of chicken breasts were contaminated with approximately 6.5 x 10^6 *Enterobacteriaceae* and 2.0 x 10^8 ACCs. On spring onions the average number of *Enterobacteriaceae* was 2.7 x 10^4 cfu g^{-1} and the ACC was 2.11 x 10^5 cfu g^{-1} (Figure 4-6).

The average number of *Enterobacteriaceae* and ACCs were relatively low for the convenience meals (40 and 6.8 x 10^4 cfu g^{-1} respectively), ham (2.1 and 4.3 x 10^5 cfu g^{-1}) and pesto (0.6 and 21 cfu g^{-1}). A minority of convenience meals (2 / 30) and ham

samples (2 / 30) were, however, contaminated with significantly higher ACCs than other samples with counts exceeding 3.2×10^5 cfu g^{-1} ($P < 0.0001$).

Counts from chicken salads prepared in the test kitchen were higher than for ready-meals bought pre-prepared from the supermarket. This difference was significant for *Enterobacteriaceae* ($P = 0.02$) but not ACCs ($P = 0.45$)

Dried herbs were examined on one occasion and counts were low (*Enterobacteriaceae* < 0.4 cfu g^{-1} and ACC = 2 cfu g^{-1}).

4.3.2 Profile of recruited participants

Thirty participants, consisting of 10 men and women aged >60 – 75 years, 10 mothers with children (< 10 years old) and 10 single young males (aged 18 –28 years) were recruited to prepare a chicken salad. Older participants (>60-75yrs) and young children (whose mothers prepared the salad) represent groups of the population, which may be more susceptible to pathogenic bacteria (Farthing 2000). Single young males have been found to frequently consume high risk food and implement inappropriate food handling practices (Klontz *et al.* 1995).

4.3.3 Prevalence of *Salmonella* and *Campylobacter* from raw chicken

Eighty percent (24 / 30) of chicken breasts were contaminated with *Campylobacter spp.* and six percent (2 / 30) with *Salmonella spp.* Six of the 10 chicken breasts handled by participants aged 60- 75 years were *Campylobacter*-positive as were 9 / 10 chicken breasts handled by mothers with young children and single young men. Two of the chicken breasts were contaminated with both *Salmonella* and *Campylobacter*, they were both handled by mothers with young children.

4.3.4 Prevalence of *Salmonella* and *Campylobacter* from salad ingredients

All fresh salad ingredients from the 30 food preparation sessions were enriched for *Salmonella* and *Campylobacter*. Dried herbs were only enriched after one food preparation session. None of the ingredients (apart from the raw chicken) were contaminated with *Salmonella* or *Campylobacter*.

4.3.5 Prevalence of pathogens in prepared salads, convenience salads and areas / materials in the kitchen

In total, 56 salads were analysed (30 homemade and 26 convenience salads). All participants prepared a chicken salad but four did not serve the convenience salad, despite instructions to do so. Four of the 56 salads analysed were *Campylobacter*-positive, three were homemade and one was a convenience salad. Contamination of salads by older participants (aged 60-75 years) appeared greater than in the other groups examined, with two of the six participants (33%) who handled a *Campylobacter*-positive chicken contaminating a salad compared to 1 / 9 (11%) mothers with young children and 1 / 9 (11%) single young males. The difference in the isolation rate of *Campylobacter* from salads prepared / served by each of the groups was, however, not significant ($P = 0.78$) because of the small numbers of people involved.

Possible routes of contamination were observed for all of the homemade salads but no exposure route was observed for the convenience meal (Table 4-1). All of the *Campylobacter* isolated from the salads and the raw chickens used during the corresponding food preparation session were *C. jejuni* and had the same sero /phage type (Table 4-2).

Table 4-1 *Campylobacter***-positive salads and their suspected routes of contamination.**

Participant	Salad	Suspected route of cross contamination
23[a] (Female, 60-75 years)	Homemade chicken salad	The same knife and chopping board (inadequately washed[b] and no drying) were used to prepare the raw chicken and the salad vegetables and / or the chicken salad and ingredients were touched with unclean / potentially contaminated hands.
29 (Male, 60-75 years)	Homemade chicken salad	The same knife (unwashed) and chopping board (inadequately washed and no drying) was used for raw chicken and then the salad vegetables and / or the chicken salad and ingredients were touched with unclean / potentially contaminated hands.
18 (Mother with young child)	Convenience meal	No observed possible route of contamination
28 (Single young male)	Homemade chicken salad	The same chopping board (sprayed with sanitiser and wiped with paper towel, but unwashed) was used for preparation of raw chicken and then salad ingredients and / or the chicken salad and ingredients were touched with unclean / potentially contaminated hands

[a] arbitrary number assigned to each participant, [b] inadequately washed – no detergent, no hot water, no physical action

Table 4-2 *Campylobacter* **subtypes isolated from salads and the subtypes isolated from the raw chicken.**

Participant no.	Type of salad	Species Sero/phage type	
		Salad	Raw chicken
23[a]	Homemade salad	*C. jejuni* HS13 / 1	*C. jejuni* HS13 / 1
29	Homemade salad	*C. jejuni* HS13 / 1	*C. jejuni* HS13 / 1
18	Convenience salad	*C. jejuni* Untypable / 1	*C. jejuni* Untypable / 1
28	Homemade salad	*C. jejuni* HS13 / 1	*C. jejuni* HS13/1

[a] arbitrary number assigned to each participant

Potentially contaminated dishcloths, tea towels and hand towels were taken after each of the 30 meal preparation sessions and potentially contaminated areas (determined by observation) were swabbed. All samples were analysed for the presence of *Salmonella* and *Campylobacter*. Samples from 20 meal preparation sessions were also examined for numbers of *Enterobacteriaceae*. Aerobic colony counts were not calculated due to the chance of external contamination by, for example, air borne fungal spores. The average number of samples taken per participant, including the salads and ingredients, was 30 and ranged from 27 to 36. Commonly sampled areas included work surfaces, chopping boards, cupboard door handles, bin lids, knifes, tap handle, dishcloths, hand towels and tea towels, as well as the ingredients and salads.

Three of the 41 dishcloths sampled (participants had access to more than one dishcloth) became contaminated with *Campylobacter* during separate food preparation sessions, one dishcloth became contaminated after a participant aged 60-

75 had prepared a chicken salad and one after the food preparation session of a mother with a young child. One participant (aged 60-75) contaminated a dishcloth, a work-surface (1 / 180 sampled; six work surface samples were taken per participant) and a hand towel (1 / 29). One / 30 tea towels sampled became contaminated with *Campylobacter* during a food preparation session by a single young male.

It appears that older participants have contaminated more areas / materials of the kitchen than the other groups. Two of the six participants aged 60-75 years who handled a *Campylobacter*-positive chicken contaminated at least one areas / cloth in the kitchen compared to 1 / 9 (11%) mothers with young children and 1 / 9 (11%) single young males. These participants were not the same as those which contaminated the salad they prepared. Participants from any of the three group were not significantly ($P = 0.78$) more likely to contaminated an area / material in the kitchen.

Possible contamination routes were observed for all items (Table 4-3) but typing results (Table 4-4) could not confirm that the strains originated from the raw chicken.

On two occasions (participants 1 and 9) the *Campylobacter* strain isolated from the raw chicken could not be recovered for typing and on two occasions (participants 13 and 19) the sero/phage types isolated from the kitchen and from the raw chicken were not the same.

Although *Campylobacter* was only isolated from a small percentage of dishcloths, tea towels and hand towels *Enterobacteriaceae* were isolated from the majority (Figure 4-7).

Table 4-3 *Campylobacter*-**positive locations and the suspected route of cross contamination.**

Participant	*Campylobacter* +ve location	Suspected route of cross contamination
1[a] (Male 60-75 years)	Work surface[b]	Contaminated directly from raw chicken and raw chicken packaging and / or indirectly from contaminated utensils (not cleaned)
	Dishcloth[b]	Placed on contaminated work surface at the end of food preparation session
	Hand towel[b]	Wiped with contaminated inadequately washed hands and used to wipe contaminated work surface
19 (Female 60-75 years)	Dishcloth	Contaminated with raw chicken, used during washing up and wiping of surfaces
9 (Mother with young child)	Dishcloth	Used to wipe work surface contaminated indirectly with utensils used to prepare raw chicken
13 (Single young male)	Tea towel	Used to wipe work surface where raw chicken had been prepared. Handled throughout the food preparation session

[a] arbitrary number assigned to each participant, [b] participant no. 1 contaminated three items

Table 4-4 *Campylobacter* subtypes isolated from the areas/ items and salads contaminated during the preparation of a chicken salad and the subtypes isolated from the raw chicken.

Participant no.	*Campylobacter*-positive area / material	Species Sero/phage type Area / material	Raw chicken
1[a]	Dishcloth	*C. jejuni* HS 13 / 67	NK[b]
1	Worksurface 3B	*C. jejuni* HS13 / 1	NK
1	Hand towel	*C. jejuni* HS13 / 40	NK
19	Dishcloth 2	*C. jejuni* Hs13/ 67	*C. jejuni* Untypable / 1
9	Dishcloth	NK	NK
13	Tea towel	*C. jejuni* HS 50 / 64	*C. jejuni* HS50 / 1

[a] arbitrary number assigned to each participant, [b] Not known

Figure 4-7 Number of *Enterobacteriaceae* isolated from cloth in the test kitchen.

Participants had access to unlimited dishcloths and each one they used was numbered consecutively. One participant used four dishcloths (the majority [15] used one). The limit of detection on these samples was 230 cfu per dishcloth, 400 cfu per tea towel and 500 cfu per hand towel. The majority of tea towels (10 / 20) were contaminated with more than 5 $\log_{10}$ cfu *Enterobacteriaceae* and the majority of hand towel (12 / 19) and dishcloth samples (16 / 30) were contaminated with more than 2 $\log_{10}$ cfu per samples (Figure 4-7). When participants used more than one dishcloth, contamination rates of dishcloths 2, 3 and 4 were not significantly different than those from dishcloth 1.

Enterobacteriaceae were also isolated from 7 % of samples taken from other areas / materials in the kitchen although usually in much lower numbers, contaminated either directly or indirectly from the raw chicken or its packaging (Figure 4-8).

Figure 4-8 Number of *Enterobacteriaceae* isolated from surfaces in the test kitchen.

All of the samples were taken after the food preparation sessions and the chopping boards and knifes had all been washed by the participants. Although no *Enterobacteriaceae* were isolated from the majority of samples it can be seen that some of the samples remained contaminated after cleaning.

If more than one chopping board or work surface was sampled during a meal preparation, the sample site contaminated with the highest number of *Enterobacteriaceae* has been given, and thus the worst-case scenario is represented. The limit of detection was 10 cfu for these samples.

4.4 Discussion

4.4.1 *Enterobacteriaceae* and aerobic colony counts from salad ingredients and salads

Raw chicken breasts and spring onions were both contaminated with large numbers of *Enterobacteriaceae* and ACCs. In the case of chicken the majority of contamination is likely to be from intestinal contents, derived from the chicken itself or from other, previously processed, carcasses. It is this which is partly responsible for the high levels of *Campylobacter* on poultry products (Rivoal *et al.* 1999). Numerous studies have tried to address this problem by preventing contamination, by *Campylobacter*, at the farm (Humphrey *et al.* 1993; Van de Giessen *et al.* 1996; Van de Giessen *et al.* 1998), and at the processing plant (Jones *et al.* 1991b; Mead *et al.* 1995). As yet home hygiene still provides the best critical control point in the transfer of this organism from the raw food product into a prepared meal.

Despite the fact that *Enterobacteriaceae* are commonly associated with faecal contamination, the high numbers of *Enterobacteriaceae* present on the spring onions may have originated from a different source. *Enterobacteriaceae* have also been shown to colonise the environment (Cox *et al.* 1988) and may also be a result of decaying vegetation (Anon 2001a). The process of growing (including fertilising), harvesting, handling and storing vegetables can all contribute to the level of contamination by *Enterobacteriaceae* and ACCs. Fresh fruit and vegetables naturally carry a surface flora of micro-organisms consisting of soil saphrophytes, air borne fungal spores and, possibly, plant parasites (Anon 1995b), which will contribute to the ACC and this count is, therefore, not a measure of external contamination.

Bacterial counts for ham and pesto (before cooking) were low with levels of *Enterobacteriaceae* usually < 0.4 cfu g^{-1}. These samples both undergo heat treatment during processing, which would account for the low number of bacteria. On two occasions, aerobic colony counts for ham were significantly higher than on other

samples ($P < 0.0001$). These samples, which were brought pre-wrapped and sealed, may have become contaminated during sampling and / or transport. Two convenience meal samples were also significantly more contaminated than the other 24 examined ($P < 0.0001$). It is possible that the participants contaminated the convenience meal during serving, although handling was minimal. It was not always possible to purchase the same type of convenience salad and it is possible that the higher contamination levels in these salads was due to differences in the original ingredients.

Convenience meals had a lower *Enterobacteriaceae* count than the homemade chicken salads. The convenience salads were commercially produced and the hygiene practices are likely to be of a higher standard than those of the participants in the test kitchen. The ready-made salads were not always identical to the salad prepared in the test kitchen so it is possible that the different ingredients contributed to the different levels of contamination.

4.4.2 Prevalence of *Salmonella* and *Campylobacter* from raw chicken

The isolation rate of *Campylobacter* from the raw chicken breasts was 80%, similar to those found by Jorgensen *et al.* (2002) and Kramer *et al.* (2000) who found that 76 and 83% of chickens were *Campylobacter*-positive respectively. These isolation rates were, however, higher than those recently reported by the Food Standards Agency (FSA), who found that 46% of the chickens they examined were *Campylobacter*-positive (Anon 2001e).

Six percent of raw chicken breast used during this study were contaminated with *Salmonella*, the same contamination rate recently reported to have been isolated from chicken carcases by the FSA (Anon 2001e). The isolation rates are, however, lower than those found by Jorgensen *et al.* (2002) who isolated *Salmonella* from 21% of chicken carcases.

Differences in the isolation rates of *Salmonella* and *Campylobacter* between this study and that by the FSA and Jorgensen *et al.* (2002) may be due to a difference in sampling technique. Twenty-five grams of the chicken breasts were examined during

this study, whilst carcass rinses and / or neck skin were examined in the others. Another difference may be due to the sampling times of the experiments, see Chapter five, section 5.4.2.

4.4.3   Prevalence of *Salmonella* and *Campylobacter* from ingredients

Salmonella or *Campylobacter* were not isolated from any of the other raw ingredients used to prepare the chicken salad, although other workers have isolated these bacteria from vegetables.   Lettuce (3.1%) and spring onions (2.5%) were amongst the vegetables found to be *Campylobacter*-positive by Park & Sanders (1992) and Kumar *et al.* (2001) isolated *Campylobacter* from 3.6% of raw vegetables examined. Isolation rates of *Salmonella* from fresh vegetables have been reported to be between 8 and 22% (Rude *et al.* 2001; Tamminga *et al.* 1978) and   6.7% from dried herbs (Bocckemuhl & Wohlers 1984).

The occurrence of *Salmonella* and *Campylobacter* in vegetables is of concern for public health.   A high proportion of consumers do not wash vegetables before consumption (Redmond *et al.* 2001) and in the case of ready to eat vegetables the pathogens are unlikely to be processed further before ingestion. Inadequate storage of salads and salad vegetables may exacerbate the problem, particularly in the case of *Salmonella* where rapid growth at 20 °C, on fruit for example, has been demonstrated (Bradford *et al.* 1996).

4.4.4 Prevalence of pathogens in prepared salads, convenience salads and areas / materials in the kitchen

Three of the 30 prepared salads (10%) and 1 / 26 of the convenience salads (4%) were *Campylobacter*-positive. No one group of participants were significantly more likely to contaminate the salad they prepared (*P*= 0.78), although the presence of *Campylobacter* in the salads prepared by the older participants (aged 60 –75 years) did appear more common. Although 30 participants were asked to prepare a chicken salad only 24 were given a chicken breast contaminated with *Campylobacter*

(microbiological analysis of the chicken breasts was not carried out until after the food preparation session). A further study, using larger sample sizes may find significant differences between the groups. Redmond *et al.* (2001) assessed the hygiene of the same three groups of participants, using a risk based scoring system, whilst the chicken salad was prepared and found that, older participants (aged 60 –75 years) also had the highest average risk scores and, therefore, the worst food hygiene behaviour. Given that 33% of the homemade salads prepared by the older participants were *Campylobacter*-positive and the elevated susceptibility of this group to food poisoning organisms (Farthing 2000) a food safety campaign targeted at this group of participants may be beneficial.

The presence of *Campylobacter* in the convenience salad was unexpected. The participant may have contaminated the salad whilst serving it, although this required minimal handling and there was no observed route of contamination, or it may already have been contaminated. Typing of the isolate demonstrated that the raw chicken was probably the source of the contamination because isolates from both the convenience meal and the raw chicken prepared in the test kitchen were *C. jejuni*, untypable by serotyping and phage type (PT) 1. Unfortunately PT1 is a common isolate from chicken and human sources; (Kramer *et al.* 2000) and since both strains were not typable by serotyping it cannot be certain that the two isolates are the same. Genotyping of the two isolates either by pulse field gel electrophoresis or *Fla* typing (Wassenaar & Newell 2000) would provide further information but such methods were not available during this project.

Typing of *Campylobacter* strains from the raw chicken and the three homemade *Campylobacter*-positive salads confirmed that the isolates were likely to have originated from the raw chicken. All of the strains isolated from the homemade salads and the raw chicken used to prepare them were *C. jejuni*, serotype HS 13, PT 1. In a recent survey (Kramer *et al.* 2000), PT 1 was the most commonly isolated phage type from raw chicken. In contrast serotype HS13 was only isolated from 1.6% of the samples in their study. The raw chicken breasts used for the food preparation session were all brought from a local supermarket, probably originating from a restricted geographical area, within a relatively small time frame (three months). This may have reduced the diversity of *Campylobacter* subtypes isolated and explain why HS13

was isolated from such a high proportion of samples. In contrast Kramer *et al.* (2000) purchased samples from a variety of retail outlets. It is also possible that serotype HS13 has enhanced attachment and survival properties compared to other serotypes which may have facilitated its ability to cross contaminate or survive after the cross contamination event has occurred.

The presence of *Campylobacter* in three homemade salads could be traced back to a number of hygiene errors involving raw chicken. All three participants used the same unwashed or inadequately washed chopping board for the preparation of raw chicken and then the salad ingredients. One of the participants used the same inadequately washed knife for the preparation of the salad ingredients after raw chicken and one participant handled the salad with potentially contaminated hands, unwashed after handling the raw chicken.

The transferral of *Campylobacter* from raw chicken to chopping boards and knifes used during its preparation is well documented. de Boer & Hahne (1990) showed that *C. jejuni* was easily transferred from raw chicken products to cutting boards and raw vegetables placed on dishes that had previously contained raw chicken products. Zhao *et al.* (1998) found that slicing vegetables on a chopping board, contaminated from raw chicken previously inoculated with an indicator organism, could transfer large populations of bacteria to the vegetables. All of the participants who contaminated their salads washed the chopping board between the raw chicken and ready to eat vegetables but none of them did so adequately (i.e they may not have used a detergent, hot water, a physical action or rinsing). Chopping boards were sampled after each food preparation session and, although none were positive for *Salmonella* or *Campylobacter, Enterobacteriaceae* were isolated from 3 / 62 chopping boards examined despite cleaning (albeit inadequately) by the participants. One chopping board was contaminated with between 10-100 *Enterobacteriaceae* and two with > 100. Tebbutt (1999), who visited busy hotel kitchens, reported that the cleaning of chopping boards was often inadequate and found that 42% were contaminated with more than 10^3 cfu bacteria per board. He also reported that the condition of the chopping boards affected the cleaning as boards that were heavily scored were harder to clean. The boards used in the test kitchen were all in good condition but despite this cleaning was still ineffective on three occasions.

Participants used both plastic and wooden chopping boards according to their preference. There have been conflicting reports detailing the disadvantages of using wood as chopping boards with workers reporting greater levels of recovery of *Salmonella* (Gough & Dodd 1998; Gilbert & Watson 1971) from wooden boards and enhanced survival of *C. jejuni* associated with wood (Boucher *et al.* 1998). Ak *et al.* (1994a) and Ak *et al.* (1994b), however, reported that with adequate cleaning wooden chopping boards are unlikely to increase the risk of cross contamination.

It can be seen that contaminated chopping boards pose a risk of cross contamination in the kitchen even when participants believe the boards to be cleaned. Such inadequate cleaning highlights the need to educate consumers in food hygiene. As well as adequate cleaning and replacing of heavily scored boards, the use of separate chopping boards for raw meats and ready to eat foods is recommended.

Another exposure route by *Campylobacter*, for one of the salads, may have been from hands, unwashed after handling the raw chicken. Contamination of kitchen equipment by unwashed or inadequately washed hands is a major problem in the domestic kitchen. de Boer & Hahne (1990) found that *C. jejuni* was isolated from 73% of hands after handling raw chicken and *Salmonella* from 6%. These organisms are transient on hands and are easily transferred to other areas of the kitchen including salad vegetables, wiping cloths, dishcloths and tap handles. Hand towels, tea towels and dishcloths tend to become particularly contaminated as they are often used to wipe inadequately washed or unwashed hands.

In this study *Campylobacter* was isolated from three dishcloths, a hand towel and a tea towel. One participant (aged 60-75 years) contaminated a dishcloth and a hand towel, as well as a work surface. The other cloths were contaminated during separate food preparation sessions. As with the salads participants aged 60 –75 years appeared more likely to contaminated areas / materials in the kitchen but this difference was not significant ($P = 0.78$). The high level of cross contamination does, as previously discussed, indicate the need for improved awareness of food safety in the home, particularly within this vulnerable group of the population.

The probable route of contamination for the hand towel was from inadequately washed hands. Contamination of the other items could have occurred as a result of them being used to wipe contaminated surfaces. Although routes of contamination from the raw chicken to all of the contaminated items / areas were observed, the phage type of *Campylobacter* strains isolated from the raw chicken were not the same as those isolated from the kitchen (due to a decline in viability during storage only two isolates from the raw chicken were recovered for typing). Kramer *et al.* (2000) found that almost 30% of the samples tested yielded more than one subtype and stressed the need to type more than one isolate from a sample. Due to a lack of resources it was not possible to type more than one isolate per sample but it is likely that the chicken breasts were contaminated with more than one subtype. The *Campylobacter* strain isolated from the kitchen was not necessarily present on the raw chicken in the largest numbers or the subtype, which was more likely to be isolate. It is possible that the isolation of *Campylobacter* by enrichment, may have affected which *Campylobacter* strains were isolated. Baylis *et al.* (2000) found that not all *Campylobacter* strains were recoverable in *Campylobacter* enrichment broth and, although modified Exeter broth was used to recover isolates in this study, it is still possible that this may have biased which strains were isolated.

The most heavily contaminated sites in the kitchen were dishcloths, tea towels and hand towels, the majority of which were contaminated with > 100 *Enterobacteriaceae*. Despite the fact that participants had access to as many dishcloths as they needed, 45% only used one cloth. Although some participants did use more than one cloth, this did not result in a significant drop in contamination, presumably because the participants did not designate specific uses for each cloth and allowed all the cloths to come into contact with contaminated areas of the kitchen. The cloths used in the kitchens all tended to have multiple uses including the wiping of work surfaces (a contributing factor in the contamination of all the cloths in this study), the wiping of inadequately washed or unwashed hands after the handling of raw chicken (contributing to the contamination of the hand towel), and cleaning or drying of potentially contaminated utensils. The multiple use of cloths means that not only are they frequently exposed to contaminating bacteria but that they may also act a vectors transferring potentially harmful bacteria from one area of the kitchen to the other. Indeed Scott & Bloomfield (1990) found that cloths contaminated with even

low numbers of bacteria could transfer sufficient to contact surfaces (such as a work surface or hand) to represent a potential infection hazard. It is unlikely that the three dishcloths, hand towel, tea towel and work surface were the only items contaminated during each meal preparation session but the levels of moisture in the cloths are likely to have enhanced the survival of *Campylobacter* and increased the isolation rate. Work-surfaces were frequently contaminated with raw chicken and /or raw chicken packaging (Redmond *et al.* 2001) but *Campylobacter* was only isolated on one occasion, presumably because of the drying stresses associated with such an environment (Chapter six) and the measures taken by the participants at the end of the food preparation session.

The moist environment that cloths provide has been shown to promote the survival and even growth of some organisms (Scott & Bloomfield 1990) and in the case of *Campylobacter*, which is regarded as being sensitive to drying, a moist environment may be particularly important for its prolonged survival. Other workers have reported high levels of contamination on dishcloths and wiping cloths from the domestic kitchen but few have isolated *Campylobacter* from kitchens, which have not recently been used to prepare raw poultry. Rusin *et al.* (1998) reported that the highest concentrations of bacteria were found on sites which provided moist environments and / or were frequently touched, dishcloths fall into both of these categories. Other studies Scott *et al.* (1982) have reported highest levels of microbial contamination from wet sites around the kitchen including dishcloths and cleaning cloths. Thorough drying of the cloths and/ or towels is likely reduce levels of contaminating bacteria and prevent their transfer around the kitchen but failing this Scott (1984) recommended a decontamination procedure to prevent the spread of pathogens by cloths.

Bin lids, tap handles and fridge handles were commonly sampled after the food preparation sessions as they were areas commonly touched immediately after participants had handled the raw chicken or raw chicken packaging. None were positive for *Salmonella* or *Campylobacter* and despite the high levels of *Enterobacteriaceae* isolated from the two raw chicken breasts (6.5×10^6 cfu) very few were transferred to the bin lids or the tap handles and on the majority of occasions no *Enterobacteriaceae* were isolated. Chen *et al.* (2001) demonstrated a transfer rate of

8.7% from chicken, artificially inoculated with *Enterobacter aerogenes* B199A to hands and a mean transfer rate of 0.16% from hands to tap handles, although a high degree of variability was also recorded. If the transfer rates in the test kitchen matched those found by Chen *et al.* (2001) it would be expected that 5×10^5 *Enterobacteriaceae* would be transferred to the hands and 9.1×10^2 cfu would be subsequently transferred to the tap handles. The hands of participants were not sampled during the food preparation sessions since this may have affected how they completed the chicken salad. Results, therefore, cannot be compared, but the levels of *Enterobacteriaceae* contaminating the test kitchen appear to have been lower than those suggested. A number of factors may have been involved in the different levels of contamination. Chen *et al.* (2001) used chicken breasts artificially contaminated with *E. aerogenes* B199A rather than naturally contaminated chicken breasts and the attachment of this organism to the chicken breasts differ from the *Enterobacteriaceae* that naturally contaminate raw chicken breasts. Also Chen *et al.* (2001) sampled tap handles soon after the contamination event. Samples taken from the test kitchen were not taken until after the food preparation session and after cleaning of the kitchen by participants. Although participants did not attempt to clean the tap handles they were handled after the contamination event and the *Enterobacteriaceae* may have been wiped away. It is possible that between the contamination of the taps and the sampling time a proportion of the *Enterobacteriaceae* may have died.

Although the rates of contamination were lower than those predicted by Chen *et al.* (2001) the rates of contamination by *Campylobacter* were high. Of the 24 participants who handled a *Campylobacter*-positive chicken three contaminated the homemade salads, one contaminated a convenience meal and four contaminated areas/materials in the kitchen. Overall cross contamination events occurred after the food preparation sessions of 29% of the participants who handled a *Campylobacter*-positive chicken. Cross contamination by *Salmonella* was not detected, probably due to the low number of positive- chicken breasts. Only two of the thirty chicken breasts examined were *Salmonella*-positive and workers have shown that numbers of *Salmonella* cells contaminating raw chicken are low compared to contamination levels by *Campylobacter* (Berrang *et al.* 2001; Dufrenne *et al.* 2001).

Chapter 5. Exposure routes of *Salmonella* and *Campylobacter* during meal preparations in domestic kitchens; assessed by observational and microbiological analysis

5.1 Introduction

Cross contamination in the domestic kitchen is thought to be a major contributing factor in sporadic food poisoning cases, occurring directly from the raw product onto the ready to eat food or indirectly from a previously contaminated area, material or hand.

Contaminated foodstuffs are likely to be one of the more common ways in which pathogenic bacteria are transferred into the domestic home and de Wit *et al.* (1979) found that cross contamination occurred in a high proportion of kitchens where contaminated chicken carcases were handled with organisms still isolated after cleaning. A more recent study by Gorman *et al.* (2002) who used naturally contaminated chicken carcases similarly reported high levels of cross contamination.

Once contamination of a site has occurred numerous workers have demonstrated that organisms contaminating areas or materials in the kitchen have the ability to survive for long periods (Bradford *et al.* 1996; Humphrey *et al.* 1994a; Scott & Bloomfield 1990) and Scott *et al.* (1982) found that the majority of homes sampled were contaminated with potentially pathogenic organisms. The largest concentrations of potentially pathogenic organisms have been isolated from the kitchens and bathrooms of domestic homes, with wet sites such as the sponge/dishcloth, the kitchen, drain area, the bath sink area and the kitchen tap handle frequently contaminated with *Enterobacteriaceae* (Scott *et al.* 1982; Speirs *et al.* 1995). *Campylobacter* and *Salmonella* have been isolated relatively infrequently from households, where there is no infection and where some time has elapsed since the preparation of a meal. When these organisms have been isolated they have similarly been isolated from moist sites within the kitchen (Josephson *et al.* 1997; Scott *et al.* 1982) which enhances their

survival and which would, if conditions were appropriate, allow for multiplication of *Salmonella.*

Isolation of these organisms from the household environment appears to be much more common when there is a case of infection. Wilson *et al.* (1998) reported that significantly more *Salmonella* were isolated from the homes where there had recently been a case of *Salmonella* than from homes of controls. Schutze *et al.* (1999) inspected the homes of patients, younger than four years, infected with *Salmonella* and isolated the bacterium from 38% of the homes investigated. Contaminated sites included dirt surrounding the front door, the vacuum cleaner and a refrigerator. It is not possible to assess whether these contaminated sites are a result of contamination from the infected patient, have become contaminated from a secondary source or whether they were the source of the initial infection. Oosterom *et al.* (1984) also isolated *Campylobacter* from 7.5% of lavatory bowls from the homes of infected individuals and from 0.9% of kitchen surfaces. Although this degree of contamination represents poor hygiene practices within the home it is not representative of the level of contamination in the domestic homes of the general population. The isolation of *Salmonella* and *Campylobacter* from domestic kitchens is relatively uncommon, prompting Speirs *et al.* (1995) to state that domestic food poisoning outbreaks are probably associated with specific incidents and practices rather than their being continually present as large populations of bacteria.

Work in Chapter four provided information on sites commonly contaminated during the preparation of the chicken salad and in this chapter the sampling and observational techniques refined in Chapter four are used to confirm exposure routes after participants untrained in food safety, prepare a chicken salad. The work carried out in this chapter differs from that in Chapter four in that participants' food preparation behaviour is examined in their own kitchens. In this way, cross contamination could be examined and incorporate not only hygienic practices of the participants during one meal preparation session but also the hygiene level of the kitchen. By visiting the domestic kitchen, variation in kitchen cleanliness (Worsfold & Griffith 1997b) and differences in the kitchen layout, affecting the ability of participants to separate raw and ready to eat foods, and the condition and type of construction materials, which may determine the effectiveness of cleaning, can be examined.

5.1.1 Aims

Observe, record and analyse the behaviour of 70 participants preparing a poultry-based meal.

Correlate observed hygiene practices with microbial contamination of specific kitchen sites and provide data for risk assessments.

5.1.2 Objectives

Recruit participants from a range of social classes and ages to reflect the population of Exeter .

Observe, record and analyse the food handling actions of 70 participants during the preparation of a chicken salad in the domestic home.

Sample selected sites in each kitchen for *Salmonella* and *Campylobacter* using pre-defined methods, based on previously identified exposure pathways.

Determine the effect of *Campylobacter* numbers on raw poultry on cross contamination during the meal preparation.

Confirm main exposure routes for use in risk assessment models.

Visit the homes of consumers who have recently suffered a case of food poisoning and compare their food preparation practices to consumers who haven't recently suffered food poisoning.

5.2 Materials and Method

5.2.1 Profile of recruited participants

The aim of this study was to recruit participants from a range of social classes and ages to reflect the population of Exeter, i.e equal proportions of each group (Anon 1991).

Beaufort recruiting agency identified one hundred and four respondents, equally recruiting participants from social classes ABC1 and C2DE and participants between the ages of 18-34, 35-54 and 55-75. Social classes were determined from the occupation of the chief wage earner of the household, retired people were graded according to their grade before retirement. Participants in group A represent approximately 3% of the total population and includes professional people, very senior managers or top civil servants. Participants from group B (~14% of the total population) included middle management executives in large organisations, principal officers in local government and civil service and top management of owners of small business concerns, educational and service establishments. Group C1 participants (~26% of the total population) include junior management, owners of small establishments, and all others in non-manual positions. All skilled manual workers, and those manual workers with responsibility for other people were designated group C2 (~25% of the total population), semi-skilled and unskilled apprentices and trainees to skilled workers were designated group D (~19% of the total population) and those participants entirely dependent on the state long term or casual workers and those with out a regular income were designated group E (~13% of the population).

Nicolaas (1995) found that 80% of women prepared every meal compared to only 22% of men. In order to reflect what happens in the population, women were recruited at a ratio of 8:2. Of the 104 respondents recruited 70 participated in the preparation of the chicken salad. All participants regularly cooked at least one meal a day and had no hygiene qualifications. Participants were asked to prepare the same chicken salad prepared in the test kitchen (Figure 4-1). All of the ingredients and the recipe for the chicken salad were provided at least 15 h before the practical to

determine how each of the participants stored the raw ingredients and to allow them to become familiar with the recipe. Participants used their own cooking and cleaning equipment. Throughout the food preparation session participants' behaviour was recorded using two checklists (Appendix A). Before any samples were taken participants were asked to clean the kitchen, as they would normally do. On completion of the practical participants were given a £15 supermarket gift voucher. Participants behaviour was scored, based on the checklists, using a risk based scoring system (Appendix B, Griffith *et al.* 1999, Redmond *et al.* 2001). The scoring system used enabled quantitative assessment of food preparation practices with demerit scores given for specific food handling malpractices. Scores were given on a logarithmic scale with a higher score given to practices, which have been shown to constitute a higher risk. High risk actions, which could lead to a high probability of a microbial hazard were given a score of 1000. Such actions included the failure to wash a knife or chopping board between the preparation of raw chicken and salad vegetables. Medium risk actions, which in isolation were unlikely to lead to a microbial hazard, were given a score of 100. The washing of raw chicken and the inadequate washing of hands after handling the raw chicken were put in this category. Malpractices, which were considered low risk, included the failure to wash salad vegetables or to preheat the frying pan. These actions were given a score of 10.

Attempts were also made to recruit participants who had recently suffered a case of food poisoning. Due to patient confidentiality it was not possible to receive details of food poisoning cases and contact had to be made via environmental health officers. Officers from Mid Devon and Exeter City councils sent letters (Figure 5-1) to patients, who had suffered a case of sporadic food poisoning in the last six months, requesting volunteers to prepare the chicken salad as described above. The response rate from participants suffering a case of sporadic food poisoning was poor and only three participants agreed to take part in the study. The low response rate meant that it was not possible to analyse the food preparation of sporadic cases.

Figure 5-1 The letter used to recruit consumers who had recently suffered a case of sporadic food poisoning

Dear

I am asking for your help with some work for the Food Standards Agency. I work for a research unit in Exeter and am carrying out a study to determine how different people prepare food, in this case a chicken salad. It is a large study covering South Wales and the West and involves members of the public some of whom, such as your-self, may have recently suffered food poisoning. All participants are treated exactly the same.

Would you be willing to help us?

The main food handler of the house will be visited at a mutually convenient time and would be asked to prepare a meal, with the ingredients provided, and to answer a series of general questions about the foods you usually purchase and how you cook and store them in your kitchen. Some samples will be taken for analysis.

A £15 gift voucher is given to all participants for their time

If you are able to help please phone Mrs Jenny Slader 01392 402967 and an appointment will be made to visit you.

I hope you will be able to help us with this study. **All participants and information are strictly confidential.**

If you would like to discuss the study before you decide to take part, or have any questions please, do not hesitate to contact me.

Yours sincerely

Jenny Slader
Clinical Scientist A
Food Microbiology Research Unit, Exeter PHLS

5.2.2 Sampling of raw materials and selected materials and salads after the food preparation session

All of the salads prepared by the participants were removed from the kitchens in a sealed plastic container. The raw chicken skin, removed by the participants, was collected in a 250 ml container for analysis at the laboratory.

Swabs of surfaces / areas contaminated directly or indirectly with raw chicken or raw chicken packaging were taken up to three actions after the initial contamination event using cotton swabs. Smaller areas such as hob controls, fridge handles tap handles were sampled using cotton tipped swabs pre-moistened in MRD containing 0.05% sodium thiosulphate. For larger areas, such as kitchen surfaces and chopping boards, absorbent cotton wool swabs (~7 cm^3) pre–moistened in MRD containing 0.05% sodium thiosulphate were used. Each swab site was divided into two areas. The first area was swabbed for *Salmonella* and swabs were placed in BPW containing sulphamandelate (20 ml or 200 ml). Swabs used to sample the second area, for *Campylobacter*, were placed in MRD containing 0.05% sodium thiosulphate (15 ml or 125 ml).

Tea towels and hand towels were shaken for 2 mins in 400 and 500 ml MRD respectively. One hundred and twenty five ml of rinse was then added to each of two 250 ml containers. Dishcloths were transported in sealed stomacher bags.

5.2.3 Transport of samples

All samples, except the salad, were transported from the domestic homes to the laboratory in an insulated cold bag (45 cm x 25 x 25 cm) containing two ice packs. The average journey time was 25 mins.

5.2.4   Enumeration of *Campylobacter* from chicken skin

A 1:10 suspension of the chicken skin from the two raw chicken breasts was made in MRD and homogenised for two minutes. Enumeration of the homogenate was carried out using the MPN technique (Anon 1995b), as described earlier (Chapter three, section 3.2.2.4).

5.2.5   Enrichment and identification of *Salmonella*

Two hundred and twenty five ml of BPW containing sulphamandelate was added to 25 g of prepared chicken salad and enriched for *Salmonella* as described in Chapter four, section 4.2.7. The swabs (already in BPW with sulphamandelate) were enriched for *Salmonella* using the same method.

One hundred and twenty five ml of DS BPW containing DS sulphamandelate was added to the chicken skin homogenate (125 ml) and the tea towel and hand towel rinses (125 ml) before enrichment using the methods described earlier. Half of the dishcloth was enriched for *Salmonella* after the addition of 225 ml BPW containing sulphamandelate.

Presumptive *Salmonella* isolates were confirmed using standard biochemical and serological techniques (Jorgensen *et al.* 2002).

5.2.6   Enrichment and identification of *Campylobacter*

Twenty five grams of chicken salad was added to 225 ml of modified Exeter broth and enriched for *Campylobacter* as described in Chapter four, 4.2.8.

Equal volume of double strength modified Exeter broth was added to swabs in MRD, containing 0.05% sodium thiosulphate, (15 ml or 125 ml), to the chicken skin

homogenate (125 ml) and to the tea towel and hand towel rinses (125 ml) and enriched for *Campylobacter* as previously described.

The dishcloth was cut into two and one half enriched for *Campylobacter* after the addition of 225 ml modified Exeter broth (Chapter four, 4.2.8).

Presumptive *Campylobacter* isolates were confirmed using standard methodology (Bolton *et al.* 1992) and speciated, serotyped and phage typed by the CRU.

5.2.7 Statistical analysis

A multivariable analysis, used to examine several variables measured in the same experiment, was carried by F. Walburt (statistical unit, CPHL) to determine if sex, age, social class, hygiene score or the number of *Campylobacter* per gram were related to kitchen contamination. Other statistical analysis was carried out in Microsoft Excel '97 using a *t* test on two samples, assuming equal variance.

5.3   Results

5.3.1   Participants

The homes of 70 participants, in the Exeter area, were visited between May 2000 and July 2001. Forty-six percent were between the ages of 18-34, 24% between 35-54 and 30% between 55-65. Eleven percent were in social class AB, 41 % in social class C1, 20 % in social class C2 and 27% in social class DE. Social classes A, B and C1 represent non-manual workers whilst social classes C2 and D represent manual workers and class E represents people dependent on the state long term, casual workers and those without a regular income. Fifty-four women carried out the practical work compared to 16 men (a ratio of 8:2).

5.3.2   Contamination levels on chicken breasts

Sixty-nine (99%) of the 70 chicken breasts were contaminated with *Campylobacter*. The average number of *Campylobacter* present on skin taken from the pairs of raw chicken breasts was 4.4 $\log_{10}$, with the geometric mean being 3.4 $\log_{10}$. Numbers ranged from <111 (the lower limit of enumeration) to 5.4 $\log_{10}$ cfu on the chicken breast skin (Figure 5-2). The average weight of the skin from two chicken breasts was 45 g. Five of the 70 chicken breasts (7%) used during this study were contaminated with *Salmonella*, all isolates were serotyped as *Salmonella* Enteritidis.

Figure 5-2 Number of *Campylobacter* present on each chicken breast.

5.3.3   Contaminated salads and probable route of contamination

Two of the 69 participants (3%) that handled a *Campylobacter*-positive chicken contaminated the salad during preparation. One participant contaminated a salad from inadequately washed hands and a second contaminated a salad and a dishcloth by a series of hygiene errors, including handling salad vegetables with hands inadequately washed hands after handling raw chicken (Table 5-1).

Table 5-1 Areas / items and salads contaminated with *Campylobacter* in domestic homes and possible routes of contamination.

Contaminated area	Suspected route of cross contamination
Salad[a]	Raw chicken dripped across salad vegetables on draining board
	Water droplets, from inadequately washed[b] hands, flicked over salad vegetables
	Salad vegetables handled with inadequately washed hands that had been contaminated by raw chicken
	Used the same knife for raw chicken and then the salad vegetables after inadequate washing
Salad[c]	Ready to eat ingredients contaminated by hands which had previously touched contaminated equipment / part of kitchen
Dishcloth[a]	Raw chicken dripped across dishcloth
	Droplets flicked from inadequately washed hands over dishcloth
	Used to wipe down surfaces, previously contaminated with raw chicken
Dishcloth[c]	No observed route of cross contamination, possibly already present on cloth, which was screwed up and visibly soiled. The dishcloth was used to wipe down surfaces using a degreaser (surfaces were not observed to be contaminated)
Dishcloth	Used to wipe down contaminated work surface (with detergent) and contaminated utensils (with no detergent)
Dishcloth[d]	Used to wash contaminated chopping board (with detergent)
Tea towel	Used to wipe unwashed and/or inadequately washed hands contaminated by raw chicken
Hand towel[d]	Used to wipe inadequately washed hands contaminated by raw chicken
	Used to dry inadequately washed knife
Chopping board	A contaminated utensil, used to prepare raw chicken, was placed on the chopping board at the end of the meal preparation session
Work surface	Contaminated directly with raw chicken and indirectly with contaminated utensils, was wiped down with hot (50°C) water containing an antibacterial washing up liquid

[a] isolated from the home of the same participant, [b] inadequately washed – no detergent, no hot water, no physical action, no rinsing (Griffith *et al.* 1999) [c] isolated only after 120 h incubation, [d] isolated from the home of the same participant

5.3.4 Contaminated sites, materials and probable routes of contamination

A total of 609 samples from the 70 domestic homes visited were analysed for the presence of *Salmonella* and *Campylobacter*, with an average of nine samples taken per kitchen. The prepared salad was sampled on every occasion. Other commonly sampled areas included tap handles (sampled from 100% of domestic homes), hob controls (100%), dishcloths (84%), tea towels (33%), hand towels (23%) and cupboard / drawer handles (44%). Ten samples (1.6%) were contaminated with *Campylobacter* (Table 5-1) and two (0.3%) with *Salmonella* (Table 5-2). Seven of the 69 participants (10%) that handled a *Campylobacter*-positive chicken contaminated areas or materials, within the kitchen, with the bacterium (Table 5-1). Two participants contaminated two items in the kitchen. Contaminated items included dishcloths (4), one tea towel, one hand towel, a chopping board and a work surface. Possible routes of contamination were observed on the majority of occasions (Table 5-1). One dishcloth was *Campylobacter*-positive despite no observed route of cross contamination.

Salmonella (serotype Enteritidis) was isolated from items from two of the 70 homes (3%, Table 5-2). No *Salmonella* was isolated from the raw chicken breasts used during the food preparation, although the participants did make a number of hygiene errors which could have led to the contamination of these items (Table 5-2).

Table 5-2 Areas / items contaminated by *Salmonella* in domestic homes and possible routes of contamination.

Contaminated area	Suspected route of cross contamination
Vegetable oil container	Handled with unwashed hands contaminated by raw chicken
Dishcloth	Used to wipe down work surfaces contaminated with raw chicken, used with an antibacterial cleaner

5.3.5   Unhygienic actions which did not lead to contamination

The majority of participants (87%) made at least one hygiene error which could have potentially led, either directly or indirectly, to the contamination of the salad (Table 5-3).   Ninety-six percent of participants failed to carry out even basic hygiene procedures such as adequate washing and drying of hands immediately after handling raw chicken and 47% then went on to handle the salad vegetables or ham. Fourteen percent of participants used an inadequately washed or unwashed knife to prepare the salad vegetables or ham, which had previously been used to cut up the raw chicken and 9% used an inadequately washed or unwashed chopping board.

Table 5-3 Behaviour of participants who contaminated an area or material of their kitchen with *Campylobacter* or *Salmonella*.

Action	All participants
Failure to adequately wash and dry hands immediately after handling raw chicken	96%
Failure to use separate knifes or use adequately washed knifes between raw chicken and then salad vegetables or ham	14%
Failure to adequately wash chopping board between raw chicken and salad or ham	9%
Salad vegetables or ham touched by hands not adequately washed after handling raw chicken	47%
Contamination of salad vegetables or ham from raw chicken or raw chicken packaging	7%
Touched contaminated equipment then touched vegetables / ham	80%
Failure to heat chicken pieces adequately	3%
Potential for contamination of end product[a]	87%

[a] Calculated by adding together any hygiene errors which may have led to the contamination of the end product

5.3.6 Sub-typing of *Campylobacter* isolates from raw chicken and contaminated sites, materials or salads

Campylobacter isolated from the raw chicken breasts used for food preparation and areas/ materials from the kitchen and salads were sub typed using sero- and phage typing (Table 5-4). Although some of the *Campylobacter* isolates from the kitchen area, material or salad shared the same phage type as those isolated from the raw chicken none shared the same serotype.

Table 5-4 *Campylobacter* subtypes isolated from the areas/ items and salads contaminated during the preparation of a chicken salad and the subtypes isolated from the raw chicken.

Contaminated area / item	Isolate from contaminated area / item Species; Sero/phage type	Isolate from raw chicken Species; Sero/phage type
Dishcloth[a]	*C. jejuni*; UT[b]/ 1	*C. jejuni*; HS12 / 44
Dishcloth	*C. jejuni*; UT / 44	*C. jejuni*; HS37 / 44
Dishcloth	*C. jejuni*; UT / 20	Not typed
Dishcloth[c]	*C. jejuni*; HS22 / 1	*C. jejuni*; HS60 / 1
Tea towel	*C. jejuni*; HS31 / 1	*C. jejuni*; HS13 / 1
Hand towel[c]	*C. jejuni*; UT / RDNC[d]	*C. jejuni*; HS60 / 1
Salad[a]	*C. jejuni*; UT / 1	*C. jejuni*; HS12 / 44
Salad	*C. coli*; HS56 / 2	*C. jejuni*; UT / 1
Chopping board	*C. jejuni*; UT / 1	*C. jejuni*; UT /RDNC
Work surface	*C. jejuni*; HS27 / 1	*C. jejuni*; HS60 / 1

[a] isolated from the home of the same participant, [b] Untypable, [c] isolated from the home of the same participant, [d] reacts with phage but not in a recognised pattern

5.3.7 The relationship between contaminated kitchens, the number of *Campylobacter* contaminating chicken breasts and hygiene scores

There was no significant difference in the hygiene scores between males and females ($P = 0.426$), participants of different ages ($P = 0.130$) or participants of social groups ABC1 or C2DE ($P = 0.085$; Table 5-5). Participants who contaminated their kitchen had slightly higher scores than those who did not (5148 vs 4078; Table 5-6, Table 5-7) but this difference was not statically significant ($P = 0.775$). Gender, age and social class of participants were not found to significantly increase the chances of isolating *Campylobacter* from the kitchen ($P = 0.222, 0.700, 1.460$ respectively).

Table 5-5 Summary of risk scores from participants preparing the chicken salad in their domestic homes.

Participants	Average score	Standard deviation	Minimum score	Maximum score
Male	4505	2605	160	8780
Female	3958	2045	210	8740
18-34 years	3861	2112	280	7550
35-54 years	3587	2152	160	7760
55-75 years	4913	2161	1520	8780
ABC1	3636	2065	160	7440
C2DE	4546	2215	540	8780

Table 5-6 Details of participants who contaminated their kitchens with *Campylobacter*, their hygiene score and the number of *Campylobacter* present on the raw chicken.

Contaminated Area/material/salad	Participant details			Hygiene score	Number of *Campylobacter* on chicken ($\log_{10}$)
	Gender	Age	Social class		
Dishcloth[a]	Female	55-75	D	8740	NT[b]
Dishcloth	Female	35-54	C1	210	NT
Dishcloth	Female	18-34	C1	6780	3.4
Dishcloth	Male	55-75	C1	3450	4.9
Tea towel	Female	18-34	D	6580	NT
Hand towel	Male	55-75	C1	3450	4.9
Salad[a]	Female	55-75	D	8740	NT
Salad	Male	18-34	C1	3580	3.8
Chopping board	Female	55-75	C1	7440	NT
Work-surface	Female	55-75	C1	4760	5.2

[a] isolated from the home of the same participant, [b] NT - Not tested

Table 5-7 Details of participants who contaminated their kitchens with _Salmonella_ and their hygiene score.

Contaminated Area/material/salad	Participant details			
	Gender	Age	Social class	Hygiene score
Vegetable oil Container	Female	35-54	C2	5460
Dishcloth	Female	18-34	C2	2590

All of the chicken breasts used when a cross contamination event occurred were _Campylobacter_-positive. _Campylobacter_ in 4 breasts were enumerated by MPN (Table 5-6). Participants who prepared chicken breasts contaminated with a high level of _Campylobacter_ were more likely to contaminate their kitchen than those who did not (Figure 5-2, Table 5-6 [$P = 0.05$]). The odds ratio for the number of _Campylobacter_ (per gram of chicken breast) demonstrated that for each additional organism the kitchen is 1.001 times more likely to be contaminated. In this study the probability of a participant contaminating a kitchen, with _Campylobacter,_ was 14%. These results indicate that if the average number of _Campylobacter_ (2.5×10^4 at the time of the study) increased by 1 the probability of a participant contaminating a kitchen would increase to 14.014% (1 x 1.001 x 14), if it increased by 2 the probability of contaminating the kitchen would increase to 14.028% (1 x 1.001 x 14.014).

5.4   Discussion

5.4.1 Participants

Participants between the ages 35–54 were under recruited by 9% showing the difficulties of recruiting people from this age group, possibly due to constraints of time due to work or children. In contrast, participants between the ages 18 – 34 were over recruited by 13%. This group included a high percentage of mothers with young children who may have been more interested in home hygiene.

The lack of recruitment of participants recently suffering from sporadic food poisoning may have been, in part, a reflection of the recruitment technique used, although due to patient confidentiality means of contact were limited. Participants may also have been unwilling to take part because of the implication that the food poisoning they suffered may have been a result of their kitchen hygiene.

5.4.2 Contamination levels on raw chicken

Ninety-nine percent of raw chicken breasts were contaminated with *Campylobacter*. This figure is higher than that found in studies by Jorgensen *et al.* (2002) and Kramer *et al.* (2000), discussed in Chapter four, section 4.2.8 and higher than the 80% isolation rate found on chicken breasts used in the test kitchen (4.2.8). It is possible that the change in isolation rates is due to a difference in the time period over which the chicken breasts were sampled. The chickens used in Chapter four were examined in December 1999 to February 2000, where as the majority of chicken breasts used during this present part of the study were examined between July and November 2000. This difference may be a result of changes at the farms producing the birds, changes at the factory or due to the difference in changes in the sampling times. Hanninen *et al.* (2000) found that the peak level of *Campylobacter*-positive retail chicken pieces, in Finland, was between July and August each year. Workers in

Holland (Jacobs-Reitsma *et al.* 1994) and the UK (Wallace *et al.* 1997) have reported increased carriage rates of *Campylobacter* in broilers during the summer months, a result which is likely to be reflected in the number of *Campylobacter*-positive carcases at retail outlets.

The skin from the raw chicken breasts was removed and *Campylobacter* enumerated. The number of *Campylobacter* per gram of chicken breast skin was much higher in this study than that found in America by Berrang *et al.* (2001), (615 cfu compared to 11 cfu per gram of chicken breast skin), and also higher than those found by Dufrenne *et al.* (2001) in the Netherlands. These authors found that that 18% of *Campylobacter*-positive chicken samples were contaminated with >5,500 of this pathogen whilst in this study the figure was 28%. It is possible that the difference is due to the way the poultry was processed. Given the variability of the recovery rate of *Campylobacter* from different enrichment media (Baylis *et al.* 2000) it is also possible that these differences were due to the different media used in the two studies

Berrang *et al.* (2001) found that the numbers present on the skin of chicken breasts were similar to those isolated from the breast meat, with no skin. They theorised that the processing steps involved in producing the chicken breasts compromised the skin by exposing meat edges to skin surfaces and allowing movement of water and other fluids from the skin to the meat. During the preparation of the chicken salad, participants were asked to remove the skin from the breasts. Given the above results it can be assumed that this did not greatly decrease the number of *Campylobacter* present on the chicken breast meat itself.

The isolation rate of *Salmonella*, from the raw chicken breasts prepared in domestic kitchens, was 7%, similar to the 6% contamination rate discussed in Chapter four.

All *Salmonella* isolated were serotype *S.* Enteritidis. This result is not unusual, Jorgensen *et al.* (2002) found that *S.* Enteritidis was amongst the most prevalent serotype isolated from chicken carcases.

5.4.3 Contaminated salads and probable routes of contamination

The most probable route of contamination for one of the *Campylobacter*-positive chicken salads was from the handling of the ready to eat ingredients with hands contaminated indirectly from raw chicken. *Campylobacter* was isolated from the salad only after 120 h incubation indicating that only low levels of contamination were present in the salad, consistent with indirect contamination and/or that the *Campylobacter* cells were stressed with a prolonged lag time.

Inadequate hand washing was observed to be a major problem during the food preparation sessions. Ninety-six percent of the participants visited, and 100% of those from whose homes *Salmonella* or *Campylobacter* were isolated, failed to carry out adequate hand washing and drying immediately after handling raw chicken. Other studies have shown equally poor hand washing practices. Doyle *et al.* (2000) found that only 40 to 60% of adults consistently wash their hands when appropriate and when they do, some do little more than rinse their hands under the tap. Jay *et al.* (1999) listed inadequate hand washing as one of the most common unhygienic practices in domestic homes, 47% of the persons observed did not wash their hands after handling raw meat. Inadequate or no hand washing after handling raw chicken could lead to the transferral of pathogens to other kitchen surfaces, utensil or (in perhaps the worst case) to the ready to eat foods, as described above.

The handling of the salad vegetables with inadequately washed hands was believed to be a contributing factor in the contamination of a second salad, although a number of other hygiene errors were involved, which also resulted in the contamination of a dishcloth. Not only did the participant fail to adequately wash her hands, she also flicked off excess moisture, generating contaminated droplets which could have contaminated a large area of the kitchen. Indeed, Humphrey *et al.* (1994a) demonstrated that aerosols generated by the beating of contaminated eggs led to the isolation of *Salmonella* over 40 cm away. Other factors involved included the rinsing of chicken under the tap and its consequent transfer across the kitchen. The washing of poultry has been linked to cross contamination in the kitchen (Worsfold & Griffith 1997b; Worsfold & Griffith 1998) and although the running water may remove some

of the bacteria present on the chicken it also provides a medium in which the bacteria can be easily transferred to other areas of the kitchen. In this case the participant carried the dripping chicken over the salad vegetables drying on the draining board. The participant also used the same knife to prepare first the chicken and then the salad vegetables with inadequate washing in between.

5.4.4 Contaminated sites, materials and probable routes of contamination

After the preparation of the chicken salad a number of samples were taken. The choice of samples to take was made on the basis of observations made throughout the preparation of the chicken salad and from results obtained from the pilot study (Chapter four). Tap handles were sampled on every occasion. The majority of participants (90%) contaminated the taps during the meal preparation with hands contaminated with raw chicken when they turned on the taps to wash their hands. None of the tap handles sampled during these food preparation studies were contaminated with either *Salmonella* or *Campylobacter,* however. Other studies have demonstrated that tap handles have the potential to become contaminated (Chen *et al.* 2001; de Wit *et al.* 1979; Kassa *et al.* 2001). Rusin *et al.* (1998) and Chen *et al.* (2001) demonstrated that hands could become re-contaminated after washing, when the contaminated taps were turned off.

Dishcloths were sampled on 84% of the food preparation sessions. Seven percent of cloths were *Campylobacter*-positive and 2% were *Salmonella*-positive. Throughout this study and in the pilot study (Chapter four), dishcloths were seen to have multiple uses and were often used to wipe work surfaces contaminated with raw chicken. No *Salmonella* was isolated from the raw chicken breasts used in the session from which the *Salmonella*-positive dishcloth was taken. It is possible that the chicken breasts were positive for *Salmonella* but that it was not detected or that *Salmonella* was already present on the cloth. Studies on the number of *Salmonella* present on contaminated carcases have given variable results. Kotula & Davies (1999) isolated an average of $3.8 \log_{10}$ *Salmonella* per gram of chicken breast skin and Bailey *et al.* (2000) isolated the same number from whole carcases. A more recent report by

Dufrenne *et al.* (2001) found that 89% of *Salmonella*-positive chickens were contaminated with < 10 *Salmonella* per carcase. In this experiment only chicken breasts were examined and the limit for detection was approximately 4 cells per breast. If *Salmonella* were present in the low numbers found by Dufrenne *et al.* (2001) it is unlikely that all of the *Salmonella*-positive chicken breasts were identified. It was not possible to increase the detection level as the remaining skin-homogenate was used for the isolation of *Campylobacter*.

Other studies have found that dishcloths in domestic homes can be contaminated with *Salmonella*. Wilson *et al.* (1998) found that 7% of dishcloths, from homes where a member of the family had suffered a sporadic case of *Salmonella* were *Salmonella*-positive, compared with 1.5% of dishcloths from control homes. It is, therefore, possible that the *Salmonella* was present in the dishcloth before the observed meal preparation. Other studies (Scott *et al.* 1982; Speirs *et al.* 1995) have isolated high numbers of bacteria, including *Enterobacteriaceae,* from cloths. The moist environment has been found to promote the survival of this group of bacteria which includes *Salmonella* spp. Scott & Bloomfield (1990) found that *Salmonella* could be recovered from the cloths at least 48 h after inoculation indicating that contamination of the dishcloth could have occurred days before the food preparation session. Dishcloths can be particularly problematic in the domestic kitchen, acting not only as reservoirs but also vectors of cross contamination.

On three of the four occasions when a *Campylobacter*-positive dishcloth was found, there were obvious actions that could have led to contamination of the cloth. On one occasion, when the route of contamination was less clear, the cloth was used to wipe a work surface. No contamination of this surface was observed although the area was used to prepare the raw chicken on a chopping board. It is possible that the dishcloth was already contaminated with *Campylobacter*. These bacteria are alleged to be sensitive to a wide range of environmental factors including sensitivity to drying, oxygen concentrations above 5%, osmotic stress and well as exposure to a variety of chemical rinses and disinfectants (Humphrey 1995a; Solomon & Hoover 1999) and have rarely been isolated from kitchens where raw chicken has not just been prepared although Josephson *et al.* (1997) isolated *Campylobacter* from the sink area of two houses. The cloth was soiled and screwed up before the start of the food preparation.

The organic matter present on the cloth could have acted as a buffer or organic matrix for the *Campylobacter* and by screwing up the cloth it was not able to dry out, which may have enhanced the survival of *Campylobacter*. Throughout this study, isolation methods have been used to enhance recovery of *Campylobacter* by prolonging the incubation period of enrichment broths (Chapter three, Humphrey *et al.* 2001a). *Campylobacter* was only isolated from this dishcloth after 120 h enrichment indicating that *Campylobacter* would not have been recovered using standard recovery protocol involving 48 h incubation.

A chopping board and a work surface also became contaminated during the preparation of the chicken salad. A contaminated utensil was placed on the chopping board, which was not cleaned after the practical, and raw chicken meat was visible. This, and the short time between contamination and sampling, promoted the recovery of *Campylobacter*.

During the preparation of a separate salad *Campylobacter* was isolated from a work surface previously contaminated with raw chicken but which had been washed using hot soapy water. This type of cleaning was common during the food preparation sessions but not effective (Cogan *et al.* 2000; Scott & Bloomfield 1990). Indeed, Cogan *et al.* (2000) found that not only was the use of water and detergent ineffective but that the cloth could then further spread pathogens around the kitchen.

Campylobacter spp. were isolated from one hand towel and one tea towel, both of which probably became contaminated when they were used to dry unwashed or inadequately washed hands after handling raw chicken. Transfer rates of *Campylobacter* from raw chicken to hands are high (de Boer & Hahne 1990) and are then easily transferred from hands onto towels used to wipe contaminated hands (Chapter four). The towels would have maintained a degree of moisture during the cooking session, promoting the survival of *Campylobacter* spp., which might otherwise have been unable to survive.

A vegetable oil container, handled with hands contaminated with raw chicken was found to *Salmonella*-positive. The chicken breast used during this food preparation session, however, was *Salmonella*-negative. As discussed earlier, it is possible that

Salmonella may have been present on the chicken but in numbers below the limit of detection. The vegetable oil container was delivered to the house 15 h before the practical and reportedly remained in the delivery bag, indicating that it was not contaminated prior to the practical. The participant could have contaminated their hands from an area of the kitchen previously contaminated with *Salmonella* and indirectly contaminated the vegetable oil container. A number of studies have shown that *Salmonella* is able to survive for prolonged periods in the kitchen environment (Josephson *et al.* 1997; Scott & Bloomfield 1990; Wilson *et al.* 1998).

5.4.5 Unhygienic actions which did not lead to cross contamination

The majority of participants made hygiene errors, which could have led to the contamination of the salad they prepared. Almost half of participants touched the ready to eat salad ingredients with hands not adequately washed after touching the raw chicken and 80% touched potentially contaminated equipment and then touched the salad ingredients. Such unhygienic behaviour represents a potential health risk. If *Salmonella* were transferred to a prepared salad, which was then inadequately stored, there would be the potential for even small numbers of the organisms to multiply and constitute an infective dose. Unlike *Salmonella, Campylobacter* is unable to grow at temperatures of less than 30 °C and it is, therefore, unlikely to multiply in salads left at room temperature in this country. Given the large number of *Campylobacter* cells present on the raw chicken, the large number of opportunities for cross contamination and the low numbers of cells needed to cause an infection (Robinson 1981) the risk of infection with *Campylobacter* via this route could still be high.

Other opportunities for indirect contamination of the salad included the use of contaminated chopping boards to prepare the ready to eat ingredients. Nine percent of participants used a potentially contaminated chopping board (unwashed or inadequately washed after being used for preparation of the raw chicken) to prepare the salad vegetables and/or ham. None of the salads prepared on these boards were contaminated with *Salmonella* or *Campylobacter* despite work confirming that this is a high risk action (see Chapter four, section 4.4.4). It is probable that a small degree

of cross contamination did occur but was not detected, the limit of detection being 27 cells per salad.

5.4.6 Subtyping of *Campylobacter* isolates from raw chicken, and from contaminated areas, items and salads

Subtypes were determined by combining the results of sero- and phage typing. Using this method a high level of discrimination was achieved (Frost *et al.* 1999). Despite the connections made between the contaminated items and the raw chicken by observational analysis, none of the isolates from the raw chicken had the same subtype as those isolated from the contaminated items. A similar phenomenon was reported in Chapter four. Due to limited resources only one isolate per sample could be typed. It is probable that the chicken breasts were contaminated with *Campylobacter* serotypes which were not identified on the raw chicken but which were transferred to the contaminated items during the preparation of the meal (see Chapter four, section 4.4.4).

One of the participants contaminated both their dishcloth and salad with *Campylobacter*. They carried out a number of hygiene errors, as discussed earlier. The *Campylobacter* subtype isolated from the dishcloth and salad had the same serotype, indicating that they originated from the same source although the subtype isolated from the chicken was not the same.

Campylobacter coli was isolated from the salad of one of the participants but not from the raw chicken used to prepare the salad. Other workers have described isolation rates of *C. coli* from 6.6% of chicken portions (Kramer *et al.* 2000) and have described the isolation of more than one *Campylobacter* species from 30% of meat samples analysed. If *C. coli* was present on the chicken it is possible that the *C. jejuni* was better able to grow in the enrichment broth and out competed the *C. coli*. Indeed it was not until 120 h incubation, that *Campylobacter* was isolated from the salad, indicating a prolonged lag phase, possibly because the broth did not optimise the growth of this organism. Baylis *et al.* (2000) reported five *Campylobacter* strains that were unable to grow in one of the broths they examined although could not determine if this was species or strain dependant. The long period of enrichment needed to

isolate the *Campylobacter* in the salad could also have been due to a low inoculum, consistent with indirect contamination and/or because the *Campylobacter* cells were sub-lethally injured and required a prolonged incubation. Humphrey *et al.* (2001a) described how cells, damaged by the extra-intestinal environment are unable to grow under culture conditions shown to be suitable for un-damaged cells. Cells contaminating the salad, indirectly, could have been subjected to a range of stresses including exposure and drying. Other workers (Kumar *et al.* 2001; Park & Sanders 1992) have isolated *C. jejuni* from salad vegetables and, although unlikely, it is possible that this could have been the source of the *C. coli,* under correct conditions (i.e low temperatures and high humidity) *Campylobacter* have been found to survive several days (Bracewell *et al.* 1985).

Fifty percent of *Campylobacter* subtypes isolated from the areas/material in the kitchen were non-typable using serotyping compared to only 20% of the isolates from chicken. It is possible that the subtypes isolated from the kitchen are better able to survive the stresses of the external environment but are less able to compete with other *Campylobacter* subtypes in the enrichment broths. If this is the case the enrichment broths would pre-select for certain *Campylobacter* subtypes which would be more likely to be typed. The serotyping scheme used in this study was developed in Canada in the early 1980's and, despite the efforts of the CRU to increase the number of typable strains, the geographic and chronological differences in the strains means that at present a percentage will be untypable. Untypable isolates in their study represented 19% of those tested (Frost *et al.* 1998).

At least five of the serotypes isolated during this study have been previously been associated with human infection (Kramer *et al.* 2000) indicating that these isolates would probably be capable of causing disease in humans. These serotypes included HS22 isolated from a dishcloth and two samples of chicken breast and HS37 and HS13, also isolated from chicken breasts.

Three of the 10 isolates typed from the raw chicken were serotype HS60. At present this subtype has not been associated with human illness, although the number of papers detailing strains typing using this system is limited.

The majority (55%) of the *Campylobacter* strains isolated during this study were phage type 1. Workers (Frost *et al.* 1999; Kramer *et al.* 2000) analysing isolates from human and chicken samples have similarly found that the majority of isolates belong to this phage type. One of the 10 isolates from the kitchen and three from the raw chicken belonged to phage type 44, previously isolated from 8.3% of chicken samples and 3% of human isolates (Kramer *et al.* 2000). In agreement with the results of the serotyping the phage typing indicates that isolates present on raw chicken and those isolated from the kitchen environment are also capable of causing human disease.

5.4.7 Hygiene scores and *Campylobacter* numbers on chicken breasts

The hygiene risk scores were derived from the scoring of malpractices carried out during the preparation of the chicken salad. A high risk action, which carried a high probability of causing a microbial hazard, was given a higher risk score than a medium risk action which, in isolation, would be unlikely to lead to a microbial hazard. A high hygiene risk score indicates, therefore, that a number of high risk actions were preformed during the preparation of the chicken salad or that a greater number of medium risk actions were carried out.

The greater hygiene risk scores of the participants did not, however, significantly correlate with contamination of the kitchen with *Salmonella* or *Campylobacter*. This may be because the risk scores used addressed a broader range of issues than those factors that are likely to influence cross contamination of pathogens e.g. heating of chicken pieces. It is likely that more cross contamination did occur as a consequence of the errors but because of the poor survival characteristics of *Campylobacter* not all events were detected. Factors affecting the survival of *Campylobacter* are, therefore, likely to be as significant as the contamination event its self. Such factors include the ability of each strain to survive drying, the environment it is placed in (a dishcloth may be more favourable than a work surface), as well as the number of *Campylobacter* present. It is also possible that just one hygiene error is sufficient to result in a cross contamination incident and although the hygiene score helps to compare the performance of participants even a low score can result in cross contamination.

The gender, social class or age of the participants who contaminated their kitchen was not significantly different to those who did not nor was the hygiene score significantly different between the groups. Although Griffith (2001) also found no correlation between hygiene practices and socio-economic class or age a number of other studies have demonstrated that men and young adults carry out more risky behaviour and score lower on food safety knowledge than other groups. One such study was carried out by Meer & Misner (2000) who found that females demonstrated a higher knowledge of food safety, food safety practices and food preparation and handling than the males. Their study was carried out in Arizona and it is possible that there would also be a difference in behaviour between participants (they also found that the food safety score of whites was significantly higher than that of hispanics). All participants from this study were from a range of socio-economic backgrounds. Participants surveyed by Meer & Misner (2000) were recruited during their entry to a food and education nutrition education program, a program developed for low income individuals. Shiferaw *et al.* (2000) found that young adults were less likely to wash their hands after handling raw chicken than older adults and men were less likely to wash their hands than women. These two studies were based on a questionnaire whilst this study was based on observations and involved many more parameters. Jay *et al.* (1999) found that there was a significant difference between the observed food handling and hygiene practices and those reported by the participants in a questionnaire. Some groups of participants may have had a greater knowledge of food safety (which could have been identified using a questionnaire) but failed to implement it. Participants' behaviour may have been affected because of an observer either because of a wish to impress or because of nervousness. Under such conditions it appears likely that participants would have prepared the meal with more care than they may otherwise do, utilising all the food handling knowledge available to them. Such behaviour could indicate that the 14% cross contamination event seen during this study is an under-estimate or that poor food handling practices become a habit which are not easily changed despite the knowledge that they may not be correct.

If educational material is to be successfully delivered it is important to determine which hygiene procedures are known, but commonly not carried out and which hygiene messages the public are not receiving. For example Jay *et al.* (1999) reported

that 82% of participants, polled during a phone survey, indicated that hand washing was important but 47% of participants failed to wash their hands after handling raw meat when observed.

The number of *Campylobacter* on the chicken breasts was found to be the only significant factor involved in the detection of cross contamination events in the domestic kitchens. High levels of *Campylobacter* on the chicken breasts would increase the number of organisms transferred during each hygiene error and enhance the survival of *Campylobacter* drying on surfaces (Coates *et al.* 1987). de Wit *et al.* (1979), however, found no significant difference in the percentage of sites contaminated and the contamination of the broilers but he was only looking at contamination levels differing by only one log, whereas in this study the numbers varied by up to three logs. He also used *Escherichia coli* K12, an organism with very different survival characteristics to *Campylobacter*.

Chapter 6. Survival of *Salmonella* and *Campylobacter* on a commonly used kitchen surface

6.1   Introduction

Campylobacter spp. are present in high numbers on the majority of chicken breasts purchased from retail outlets, (average 2.2 $\log_{10}$ cfu per chicken breast skin; Chapter five) and there is a high probability that surfaces in the kitchen where poultry is prepared will become contaminated (Chapters four and five).

In contrast, only a minority of chicken breasts are contaminated with *Salmonella* (6-7 %; Chapters four and five) and this, combined with the lower transfer rates of *Salmonella* (de Boer & Hahne 1990), means that cross contamination by *Salmonella* is likely to be less common than by *Campylobacter*.

The ability of organisms to cause disease is not, however, entirely dependant on their ability to cross contaminate but also on their ability to survive, or even multiply, on the surface onto which they are transferred. Any contamination incident in which the survival of the organism is prolonged, either due to external factors or due to the nature of the organism, is likely to be potentially much more serious than an incident in which the cells are rapidly killed.

A number of conditions have been found to affect the survival of both organisms once a contamination incident has occurred including the suspending media. Some media appear to promote the survival of contaminating organism and Coates *et al.* (1987) found that chicken liquor and blood had a protective effect on *Campylobacter* as did beef serum for *Salmonella*. The volume of contaminating media may also affect the organisms as may the surface onto which the organisms are transferred. Scott & Bloomfield (1990) found that microbial survival can be enhanced when the surface is wet and, therefore, the larger the volume of media and the greater the ability of the surface to retain moisture the longer the organisms are likely to survive. Dishcloths,

particularly appear to promote the survival of numerous Gram-negative organisms (Scott & Bloomfield 1990). A recent survey by Sagoo *et al.* (2002) isolated *Salmonella* and *Campylobacter* from cleaning cloths, albeit in a small number of cases, but were unable to isolate the organisms from work surfaces. Temperature has been found to influence the survival rate of organisms and Doyle & Roman (1982a) found that the greatest survival of *C. jejuni* occurred when the organism was held at 4 °C, presumably because of the drying of the suspending media was prolonged at this temperature. Conversely Doyle & Roman (1982a) and Mcdade & Hall (1964) found that *C. jejuni* and *Salmonella* Derby survives better in an environment of lower humidity and theorised that this may in part be due to a reduction in enzymatic activity at lower humidities. Soiling of a surface has also been found to promote survival of *Salmonella* (Scott & Bloomfield 1990).

Prolonged survival of organisms will increase the period in which the cells can be transferred to ready to eat foods and, therefore, increase the chances of a food poisoning incident. *Salmonella* is more tolerant to air drying than *Campylobacter* and also has the ability to multiply at room temperatures (Bradford *et al.* 1996) and, therefore, a contamination incident involving this organism is potentially more serious than one with *Campylobacter*. Differences in the ability of *Salmonella* strains to survive air drying have also been reported (Humphrey *et al.* 1998; Jorgensen *et al.* 2000) and some of these have been attributed to mutations in the *rpoS* gene, an important regulator of the general stress response of *Salmonella* cells. Differences in the ability of *Campylobacter* to survive air drying have also been reported (Doyle & Roman 1982a), but in the case of *Campylobacter* this work has been limited and the role of a global stress response is still being investigated.

Having seen the extent of to which *Campylobacter* spread during the preparation of a chicken salad (Chapters four and five) their ability to survive in the kitchen environment is investigated in this chapter. The ability of 17 *Campylobacter* and two *Salmonella* strains to survive on a Formica surface at 21 °C (room temperature) is investigated and differences in the survival of strains discussed.

6.1.1 Aims

Investigate the effect of air-drying on the viability of *Salmonella* and *Campylobacter* cells, on simulated kitchen work surfaces.

6.1.2 Objectives

Select and evaluate an appropriate model in relation to cross contamination.

Assess the ability of *Salmonella* and *Campylobacter* to withstand air drying over a 24 hour period.

Compare the survival of different strains of *Campylobacter* isolated from raw poultry and from kitchen areas / materials.

6.2   Materials and method

The method used throughout these drying experiments was adapted from that used by Humphrey *et al.* (1995) and has been proven to be able to identify differences in *Salmonella* tolerance to air drying. Nutrient broth containing FBP as a supplement was chosen as a drying menstruum. Fernandes *et al.* (1995) reported that all strains of *C. jejuni* and *C. coli* examined were highly sensitive to the bactericidal activity of human serum, thus blood was not used. Koidi & Doyle (1983) reported that survival of *C. jejuni* was promoted when no oxygen was present. The presence of the antioxidant FBP in the NB would have reduced the levels of oxygen present in the drying menstruum, promoting survival. It is not representative of the type of menstruum in which the bacteria would be suspended in the domestic kitchen, but it does provide a base line and enables differences in the ability of strains to survive to be identified. *Campylobacter* and *Salmonella* spp. contaminating the kitchen environment are likely to be suspended in chicken juice. Such a menstruum would provide a heterogeneous environment however and would add to the inherent variability of the test.

6.2.1 The ability of *Salmonella* (strains E and I) and *Campylobacter* (strains 2604 and 37N) to survive drying during a 24 h period.

The ability of two *Salmonella* Enteridis PT4 strains (E and I) and two *Campylobacter* strains (*C. jejuni* 37N and *C. coli* 2604) to survive air drying over a 24 h period was investigated. The two *Campylobacter* strains, both isolated from raw chicken, were chosen as preliminary work indicated that these two strains had very different abilities to survive air drying. The two *Salmonella* strains have also been shown to have significantly different survival characteristics. Strain E, originally isolated from a human case, has been found to be significantly more resistant to heat, hydrogen

peroxide and drying on surfaces, when in stationary phase, than strain I, originally isolated from chicken skin (Humphrey *et al.* 1998; Jorgensen *et al.* 2000).

Salmonella Strains E and I and *Campylobacter* strains 37N and 2604 were streaked onto BA and incubated at 37 °C for 16 h under appropriate conditions (*Salmonella* were incubated aerobically and *Campylobacter* microaerobically). Colonies were then suspended in 9ml MRD to an OD of 0.2 at 600 nm before 200 µl was added to 800 µl of NB containing aerotolerant supplement (0.2% ferrous sulphate, sodium pyruvate and sodium metabisulphate). Twenty µl aliquots of inoculum was added to each of 24 Formica squares (1 cm ^{2}) and left to dry at room temperature (21 °C ±1 °C) for up to 24 h. After 1, 2, 3, 4, 6, and 24 h of drying 3 squares were suspended in 3 x 1 ml aliquots of MRD and diluted to 10^{-4} before 20 µl of each dilution was dropped on to BA. Plates were incubated as previously described (see section 3.2.2) for up to 72 h before enumeration. The number of *Campylobacter* present in the initial inoculum was calculated by adding 2 x 20 µl aliquots of inoculum to 2 x 5 ml MRD which was serially diluted to 10^{-4} in MRD. Three 20 µl aliquots of each dilution were dropped onto BA which was incubated at 37 °C for up to 48 h under appropriate conditions before colonies were enumerated. Three replicates of each bacterial culture were examined.

6.2.2 Survival of 17 *Campylobacter* strains after 6 h of air drying

The ability of 17 *Campylobacter* strains to survive air drying was investigated. The strains tested comprised 14 *C.jejuni*, 2 *C. coli* and 1 unspeciated (Table 6-1).

Table 6-1 *Campylobacter* **strains used during surface survival experiments.**

Experiment no.	Isolate	Species	Sero/phage type	Site of isolation
1	13R	*C. jejuni*	HS11 / 1	Chicken
1	10R	*C. jejuni*	HS11 / 1	Chicken
1	WK3A	*C. jejuni*	HS13 / 1	Work-surface
1	18 RM	*C. jejuni*	UT[a]/ 1	Ready meal
1	23HMS	*C. jejuni*	HS13 / 1	Salad
1	2604	*C. coli*	HS59 / 44	Chicken
2	37N	*C. jejuni*	UT / 14	Chicken
2	WK3A	*C. jejuni*	HS13 / 1	Work-surface
2	17N	*C. jejuni*	HS50 / 44	Chicken
2	2604	*C. coli*	HS59 / 44	Chicken
2	2025	*C. jejuni*	UT / 1	Chopping board
2	2212	*C. jejuni*	UT / 1	Dishcloth
3	2224	*C. jejuni*	UT / 1	Dishcloth
3	2211	*C. jejuni*	UT / 1	Salad
3	2607	*C. coli*	HS56 / 2	Salad
3	2581	NT[b]	NT	Chicken
3	2604	*C. coli*	HS59 / 44	Chicken
4	5540	NT[c]	NT	Chicken
4	3351	*C. jejuni*	HS27 / 1	Work-surface
4	WK3A	*C. jejuni*	HS13 / 1	Work-surface
4	13TT	*C. jejuni*	HS50 / 64	Tea towel
4	18 RM	*C. jejuni*	UT / 1	Ready meal
4	2604	*C. coli*	HS59 / 44	Chicken
5	37N	*C. jejuni*	UT / 14	Chicken
5	2212	*C. jejuni*	UT / 1	Dishcloth
5	13R	*C. jejuni*	HS11 / 1	Chicken
5	WK3A	*C. jejuni*	HS13 / 1	Work-surface
5	2604	*C. coli*	HS59 / 44	Chicken

[a] UT-untypable, [b] NT- not typed

These strains were chosen because they had contaminated areas / materials / salads in a kitchen (strains WK3A, 2212, 18 RM, 23 HMS, 2025, 2224, 2607, 2211, 3351, 13TT; Chapters four and five), were present on chickens in large numbers but despite numerous hygiene errors were not isolated from the kitchen (strains 2604, 2581, 5540) or were phage/serotypes, isolated from raw chicken in a previous study (Jorgensen *et al.* 2002), which have caused human infection (strains 10R, 13R, 17N; Kramer *et al.* 2000). Strain 37N was untypable by serotyping (as were 26% of the *Campylobacter* isolated from humans) but had the same phage type (PT14) as strains previously isolated from humans (Kramer *et al.* 2000) and was, therefore, chosen for use in this experiment.

Each strain investigated was streaked onto BA and incubated at 37 °C for 16 h under appropriate conditions. Colonies were then suspended in 9ml MRD to an OD of 0.2 at 600 nm before 200 µl was added to 800 µl of NB containing aerotolerant supplement.   Twenty µl aliquots of inoculum were added to each of three Formica squares (1 cm ^{2}) and left to dry at 21 °C (±1 °C) for 6 h. After 6 h of drying three squares were suspended in 3 x 1 ml aliquots of MRD and diluted to 10^{-2} before 20 µl of each dilution was dropped on to BA. Plates were incubated as previously described for up to 72 h before enumeration. The number of *Campylobacter* present in the initial inoculum was calculated as described above (section 6.2.1). Three replicates of each bacterial culture were examined during each experiment and strains WK3A, 18RM, 13R, 37N, 2212 and 2604 were examined in more than one experiment. Each experiment was carried out on a separate day.
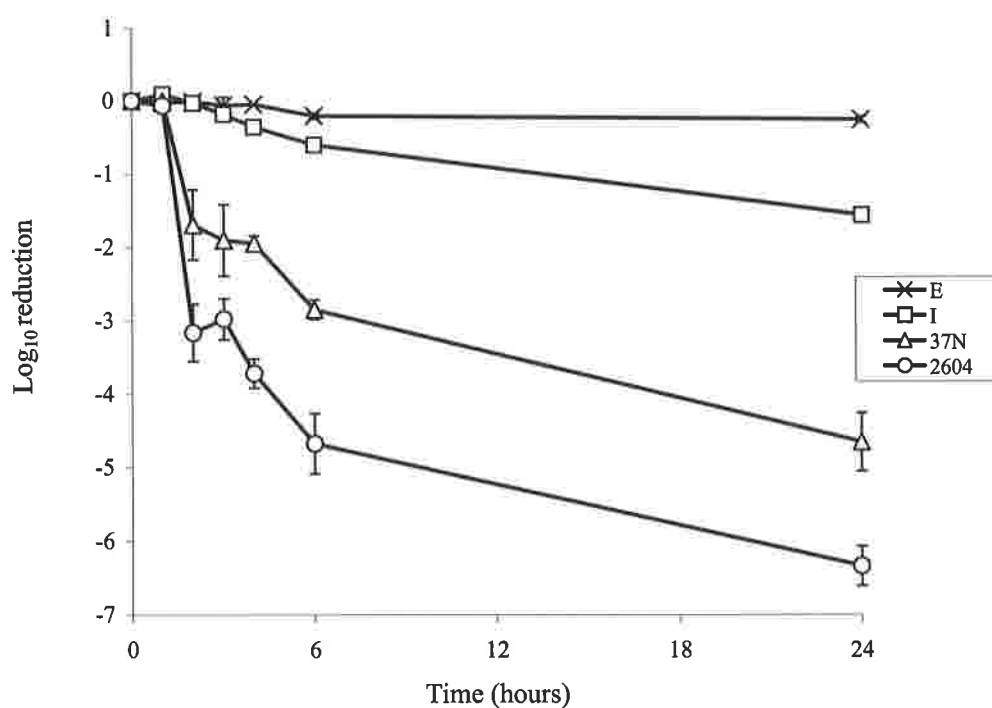
6.2.3 Statistical analysis

Analysis of variance, used to test the hypothesis that means from two or more samples are equal, and descriptive statistics, providing information about the central tendency and variability of the data, were carried out, to determine if there was a significant difference between isolates, by F. Warburt (based at the PHLS statistical unit at the Central Public Health Laboratory [CPHL]). Other statistical analysis was carried out in Microsoft Excel '97 using a *t* test on two samples, assuming equal variance.

6.3 Results

6.3.1 The ability of *Salmonella* (strains E and I) and *Campylobacter* (strains 2604 and 37N) to survive air drying during a 48 h period

The survival of two *Campylobacter* strains (*C. coli* 2604 and *C. jejuni* 37 N) and two *Salmonella* Enteritidis PT4 strains (E and I) in NB with FBP on Formica squares over a 24 h period was examined (Figure 6-1).

Figure 6-1 Log$_{10}$ reduction of *Salmonella* (strains E and I) and *Campylobacter* (strains 37N and 2604) during 24 h drying in nutrient broth (+FBP) on Formica tiles at 21 °C (n=3).

Survival of *Salmonella* and *Campylobacter* strains was high during the first hour of drying ($\log_{10}$ drops of 0.03 and 0.04 respectively) and there was no difference in their survival ($P = 0.38$).   The 20 µl drops of suspending medium still appeared wet after one hour. After two hours, a significant difference in the ability of the *Salmonella* and *Campylobacter* strains to survive drying was identifiable ($P = 0.001$).

Salmonella Enteritidis strains E and I persisted in relatively high numbers during the first 24 h of drying with a 0.27 $\log_{10}$ decrease in number of strain E and a 1.57 $\log_{10}$ decrease in strain I.   The difference in strain persistence was significant ($P = 0.0006$).

After 6 hours drying numbers of *Campylobacter* strain 37N and 2604 had dropped by 2.85 $\log_{10}$ and 4.67 $\log_{10}$ respectively and the difference in the ability of the two *Campylobacter* strains to survive drying was significant ($P = 0.015$). *Campylobacter* strains 37N and 2604 were still recoverable after 24 h with $\log_{10}$ drops of 4.65 and 6.34 respectively. The drop in cell numbers between 6 and 24 h was significantly less than the drop in numbers between 0 and 6 h for *Campylobacter* strain 37N ($P = 0.018$) and 2604 ($P = 0.005$).
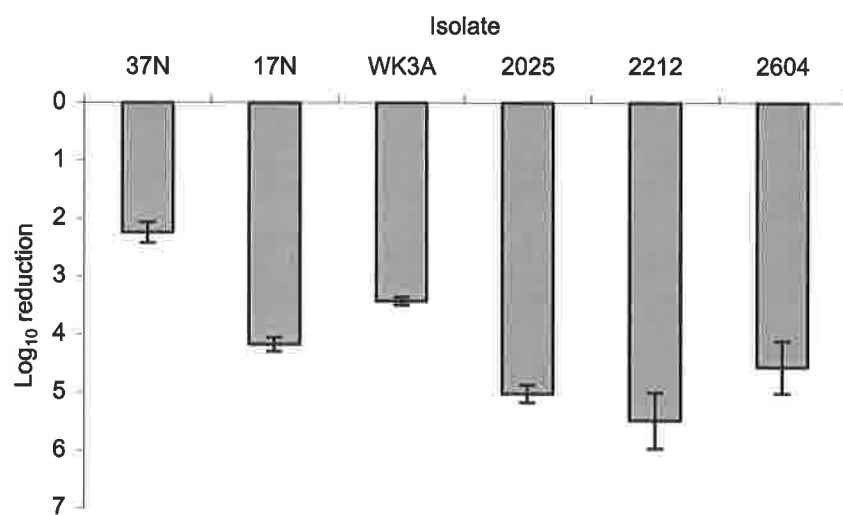
6.3.2 Survival of 17 *Campylobacter* strains after of 6 h of air drying

The ability of 17 *Campylobacter* strains to survive air drying in NB with FBP was investigated. Strains were analysed in a series of five experiments each of which was carried out on a separate day. Each strain was dried for six hours, after which time significant differences between strains could be detected (section 6.3.1; Figure 6-2-Figure 6-6).
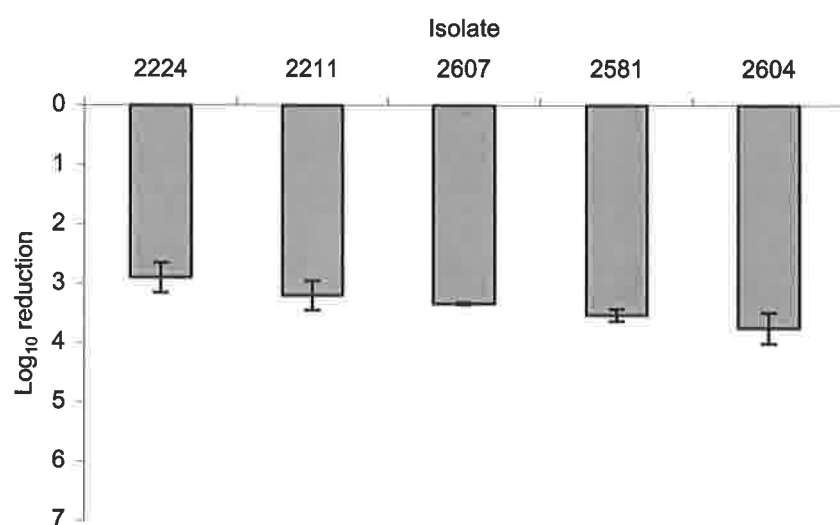
Figure 6-2 Log$_{10}$ reduction of *Campylobacter* strains after 6 h surface drying (Expt 1).
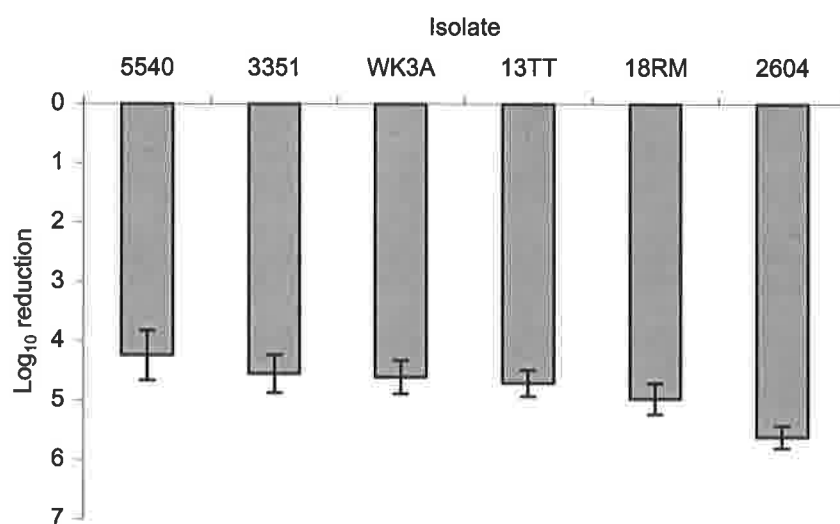

Figure 6-3 Log$_{10}$ reduction of *Campylobacter* strains after 6 h surface drying (Expt 2).

Figure 6-4 Log$_{10}$ reduction of *Campylobacter* strains after 6 h surface drying (Expt 3).
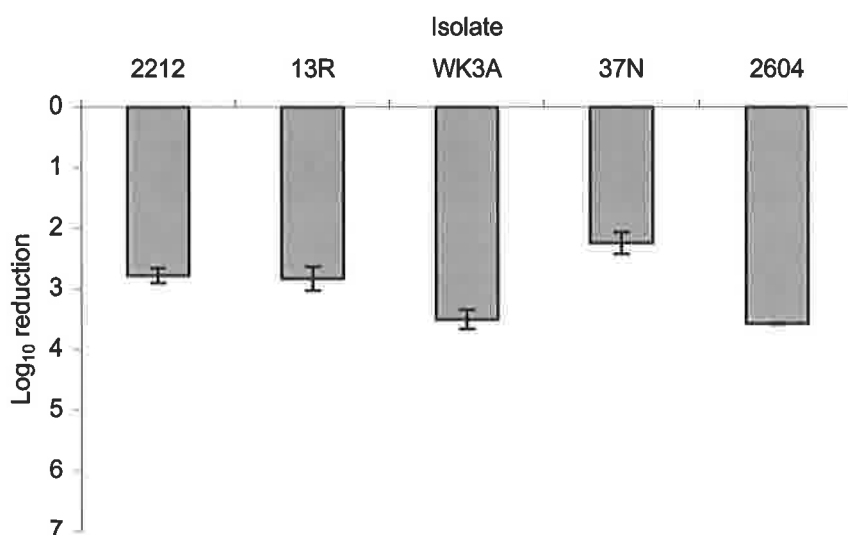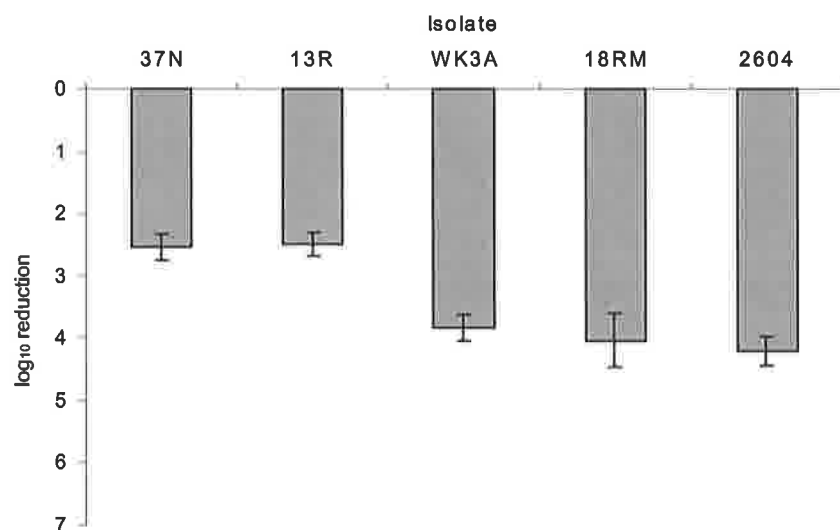

Figure 6-5 Log$_{10}$ reduction of *Campylobacter* strains after 6 h surface drying (Expt 4).

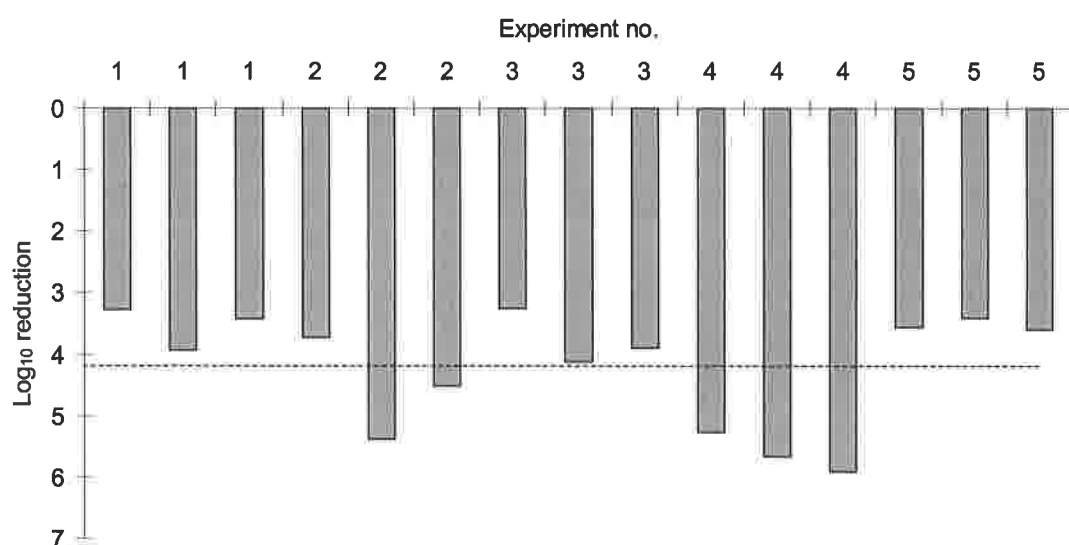**Figure 6-6 Log$_{10}$ reduction of *Campylobacter* strains after 6 h surface drying (Expt 5).**

During each analysis three replicates of each strain were examined but due to time constraints the majority of strains (11/17) were only examined once in triplicate. Five *C. jejuni* strains (WK3A, 18 RM, 13R, 37N and 2212) were examined on more than one day and *C. coli* strain 2604 was examined on the day of every experiment (Table 6-1; Figure 6-7; Figure 6-8). By examining strains, in triplicate, on more than one day apparent differences in their survival, suggested by initial replicates, could be analysed and it could be determined if these differences were real or due to inherent variation in the experiment. Variation between days was found to be significant ($P = <0.05$), therefore, only strains examined on the same day or strains, which were examined in more than one experiment, were compared, the latter being the more accurate.

Figure 6-7 Average log$_{10}$ reductions of *Campylobacter* isolates, examined on more than one day, after 6 h drying.

Figure 6-8 Log$_{10}$ reduction of *Campylobacter* strain 2604 after 6 h surface drying, examined in experiments carried out on more than one day

The dotted line represents the average log$_{10}$ drop

When strains were examined on the same day significant differences were detected. *Campylobacter jejuni* 37 N was found to survive air drying significantly better than *C. jejuni* strains 2025 and 17 N (P= 0.001 and 0.021; Figure 6-3). *Campylobacter jejuni* WK3A and *C. coli* 2604 survived significantly less well than *C. jejuni* 2212 (P= 0.012 and 0.002) in one set of experiments (Figure 6-6) and in a second *C. coli* 2604 was found to survive significantly less well than *C. jejuni* 10R (P= 0.02; Figure 6-2).

When strains were examined on more than one day differences in their ability to survive on Formica surfaces was still significant (P < 0.0001) with falls in the numbers of viable cells of between 2.49 $\log_{10}$ (*C. jejuni* 13R) to 4.20 $\log_{10}$ (*C. coli* 2604;Figure 6-7). *Campylobacter jejuni* strains 13R and 37N were significantly more resistant to air drying than *C. coli* 2604 and *C. jejuni* WK3A. There was no significant difference in the survival of *C. coli* 2604 or *C. jejuni* WK3A and *C. jejuni* 18RM. *Campylobacter jejuni* 18RM was not significantly different from any of the other strains examined, surviving moderately well with a 4.35 $\log_{10}$ drop in cell numbers.

Seven of the *Campylobacter* strains examined were isolated from raw chicken and ten were isolated from a sites / areas from within kitchens. All strains were believed to originate from chicken. There was no significant difference in the ability of *Campylobacter* strains isolated from the kitchen items/ areas or from chicken to survive on surfaces ($P = 0.21$).

6.4   Discussion

Viability of both *Salmonella* and *Campylobacter* strains remained high during the first hour of surface survival. During this time period the drop of suspending menstruum appeared wet, thus cells would not yet have been subjected to desiccation. Humphrey *et al.* (1994b) reported similar findings when *Campylobacter* spp. were dried in horse blood droplets.

After two hours of air drying the number of viable *Campylobacter* cells (strains 37N and 2604) were significantly reduced ($P = < 0.0001$) with an average drop of 2.43 $\log_{10}$ from T_0, but there was no significant difference in the number of *Salmonella* ($P = 0.22$). At this point the suspending media appeared dry and cells would have suffered desiccation. *Campylobacter* is widely reported as being sensitive. Work by Humphrey *et al.* (1994b) and Doyle & Roman (1982a) confirms these findings. Cross contamination of surfaces in the domestic kitchen is common (de Wit *et al.* 1979) but these results indicate that if the contaminated surface is allowed to dry numbers of recoverable *Campylobacter* may be significantly reduced within two hours. Humphrey *et al.* (1994b) similarly found that as soon as blood droplets dried it was not possible to isolate *Campylobacter*. Given the high levels of *Campylobacter* present on poultry, however, (Chapter five, Berrang *et al.* 2001) even a low rate of survival could represent a risk

The *Salmonella* strains examined were able to survive significantly better than the *Campylobacter spp.* after two hours of drying, and at every time point thereafter, correlating with findings by de Boer & Hahne (1990). *Salmonella* is present on chicken carcases in relatively low numbers (Bailey *et al.* 2000, Dufrenne *et al.* 2001) but it has the ability to survive long periods of air drying (Humphrey *et al.* 1995) and when transferred to a suitable food stuff may have the ability to multiply even at 20 °C (Bradford *et al.* 1996).

There was a significant difference in the ability of the two *Salmonella* strains to survive after two hours of surface drying. Other workers have previously

demonstrated that *Salmonella* Enteritidis strain E can survive significantly better than *Salmonella* Enteritidis strain I after 24 h of drying in lysed horse blood (Humphrey *et al.* 1995) and further work has demonstrated that this difference is due to a mutation in the *rpoS* gene of strain I (Humphrey *et al.* 1998; Jorgensen *et al.* 2000). The *rpoS* gene has been found to be an important regulator of other stress response genes conferring increased resistance to various environmental stresses including high temperatures, low pH, starvation conditions and drying on surfaces (Humphrey *et al.* 1995; McCann *et al.* 1991).

Viable cells of *Campylobacter* strains 2604 and 37 N decreased rapidly during the first six hours of drying but this rate of decline decreased and viable cells of both strains were still isolated after 24 hours. The decline in death rate may be due to the presence of a sub-population of cells, which are more resistant to desiccation than others.

A number of workers have reported that *Campylobacter* is able to exist in a viable but non-culturable (VBNC) state when in unfavourable conditions (Jones *et al.* 1991a; Rollins & Colwell 1986; Thomas *et al.* 2002). The significance of the presence of VBNC cells is still being debated, there have been conflicting reports as to whether such cells are capable of causing infection (Beumer *et al.* 1992; Hald *et al.* 2001 Medema *et al.* 1992; Jones *et al.* 1991a). Jones *et al.* (1991a) believed that the ability of VBNC cells to cause infection is strain-dependent and, given the high degree of variability between strains and experimental design this seem a likely explanation for the conflicting reports. None of these studies have looked at the presence of such cells on kitchen surfaces but it is seems likely that such cells may be present in the kitchen environment and that *Campylobacter* spp. may remain viable for longer than the 24 h described.

Day-to-day variability in the drying assay was significant and demonstrated the difficulties in working with this organism. Only strains examined on the same day or the average of strains examined on multiple days could be compared. It is likely that after 6 h drying the cells involved in these experiments are at the edge of their survival capabilities and that even a small fluctuation in experimental conditions would be sufficient to affect their ability to survive. Doyle & Roman (1982a), who

used a different drying protocol similarly reported inconsistencies in the ability of two *Campylobacter* strains to survive drying at 25 °C and also deduced that additional factors affected the ability of the organisms to survive drying. Wassenaar *et al.* (1998) reported evidence of genomic instability in a strain of *C. jejuni* and it is possible that such genomic instability may be an additional factor affecting the behaviour of *Campylobacter* strains examined.

The two *C. jejuni*, serotype 11 strains (10R and 13R) both appeared to survive better than the majority of other *Campylobacter* strains examined but again because of the variability inherent in this experiment and within the *Campylobacter* strains examined, more strains would need to be examined before any conclusions can be drawn. It is interesting that this serotype was commonly isolated from human faeces (Kramer *et al.* 2000). Other workers have similarly identified differences in the ability of *Campylobacter* to tolerate air drying (Doyle & Roman 1982a) and similar tolerant isolates have also been identified in other groups of bacteria, including *Salmonella* (Jorgensen *et al.* 2000). A global stress response regulator (Rpos) is believed to effect the resistance of *Salmonella* isolates to a wide range of stresses, including air drying, and when a mutation occurs isolates are less resistant to environmental stresses. Borger *et al.* (2000) have investigated stress response in *Campylobacter jejuni* but as yet have not identified a global protection system such as that seen in *Salmonella* and other Gram negative bacteria.

Campylobacter strains originally isolated from kitchen surfaces / areas of the kitchen were not more resistant than those isolated straight from the chicken portions. The ability of the strains to survive air drying was, however, only one factor and the number of cells contaminating surfaces, the type of material contaminated, the presence of cleaning chemicals and the period of time between the contamination event and sampling may also have been important.

Chapter 7. General Discussion

7.1 Discussion

The overall aim of this project was to obtain microbiological and observational data to investigate exposure routes for *Salmonella* and *Campylobacter* during the handling of raw poultry in domestic kitchens.

To date, numerous studies have examined cross contamination events but few have used naturally contaminated samples and even fewer have examined these events in domestic kitchens whilst food preparation was in practice. The use of naturally contaminated samples in the kitchen allowed more accurate assessment of cross contamination routes to be determined but meant that cells may have been present in low numbers and may have been physiologically damaged even before being subjected to the environmental stresses associated with meal preparations. The use of such samples did, however, allow a more realistic determination of contamination by *Salmonella* and *Campylobacter* during the preparation of a poultry-based meal.

Previous studies have shown that *Campylobacter* do not survive when exposed to environmental stresses such as surface drying (Doyle & Roman 1982a; Humphrey *et al.* 1994b) and prevalence studies have found that they are rarely isolated from kitchens, which have not recently been used to prepare poultry. Results from this study indicate, however, that the low isolation rates may not only be due to the sensitivity of the organisms to the environment but also due to the inability of the isolation methodologies to recovery potentially sub-lethally damaged cells. In order to accurately assess the rates of cross contamination in this study appropriate recovery techniques were designed and developed to optimise recovery of low levels of potentially damaged cells. Using these techniques isolation rates of *Campylobacter* were improved and prolonging the incubation period of the enrichment broth from 48 h to120 h alone enabled 20% more cross contamination events to be detected. Such an improvement in isolation rates indicates that the events resulting in contamination

of a kitchen area or material are likely to be more common than previously demonstrated and that *Campylobacter* cells may be more robust than otherwise thought.

Despite the use of sensitive isolation methods for the isolation of *Salmonella* this organism was only isolated from kitchens on two occasions. This was presumably due to the low prevalence of *Salmonella*-positive chicken breasts used during this study (6 - 7%) rather than a reflection of the techniques used.

Poultry was the only source of *Salmonella* and *Campylobacter* in the test kitchen and was believed to be a major source of contamination in the domestic homes examined. A high proportion of the retail chicken breasts used throughout this study were found to be *Campylobacter*-positive (80% in the test kitchen and 99% in the domestic homes) and the majority of participants handled a contaminated chicken. Given that poultry represents a large reservoir for these two major food poisoning organisms it was remarkable that in a recent report (Anon 2002d) only 57% of people questioned were concerned with the safety of raw poultry.

Given that a relatively high proportion of consumers appear to be unaware of the hazards associated with raw poultry it is perhaps not surprising that transfer of *Campylobacter* from the raw chicken to kitchen areas / materials were common and that contamination rates, by *Campylobacter*, were considerable in both the test kitchen (29%) and domestic kitchen (13%).

Contamination rates in the test kitchen may have been higher than those in the domestic kitchen due to the higher proportion of single young men and older participants (> 65 yrs) examined in the pilot study. These groups have both been shown to demonstrate a high degree of unhygienic behaviour (Chapter four; Meer & Misner 2000; Shiferaw *et al.* 2000) and may, therefore, have increased the contamination rates recorded in the test kitchen. The familiarity of participants with the location of cooking and cleaning implements in their own homes may also be involved in the different contamination rates.

Items contaminated with *Campylobacter* included the prepared salad (10% in the test kitchen, 3% in domestic homes), dishcloths, (10% and 6%), tea-towels (3% and 1%) and hand towels (3% and 1%). The contamination of salads in both the test kitchen and domestic kitchen is of greatest concern and represented a significant exposure risk to the participants. The infective dose of *Campylobacter* is low (Robinson 1981) and although the bacterium is unable to replicate at room temperature it is likely, given the high levels present on the raw chicken breasts and the high transfer rates from raw chicken to other surfaces (Chapters four and five; de Boer & Hahne 1990), that sufficient cells were transferred to cause infection in the more vulnerable groups. Work carried out in Chapter six has also demonstrated that *Campylobacter* cells have the potential to survive for extended periods of time indicating that even if the meal is not eaten immediately the contaminating cells may still remain viable and represent a risk of infection hours after the contamination event.

Contamination routes for the majority of these contaminated materials / items were determined and included the use of the same chopping board and/or knife to prepare the raw chicken and then the ready to eat vegetables or ham, with only inadequate, or no washing in between. The inadequate washing of hands after handling raw chicken and the subsequent handling of the ready to eat foodstuffs was another common route of contamination as was the drying of the potentially contaminated hands on wiping cloths. These contamination events could all be prevented if basic hygiene procedures, such as the thorough washing of hands, the use of separate chopping boards and clean knives and utensil are incorporated into meal preparations. Results from this study found, however, that the majority of participants (87%) made basic hygiene errors, which could have contributed to the contamination of the salad they prepared and 96% failed to adequately wash and dry their hands after handling raw chicken indicating that the potential for cross contamination was much higher than the incidents identified by microbiological sampling alone.

Although the exposure routes reported above could be easily avoided using adequate kitchen hygiene, the large number of participants who failed to implement these techniques highlights the practical difficulties involved in reducing cross contamination events and demonstrates the need to prioritise and target food safety messages. Adequate risk assessments cannot be made, however, unless the data

gained on the common exposure routes is combined with the ability of *Campylobacter* to persist in the environment. It has previously been thought that *Campylobacter* is sensitive to environmental stresses, but air-drying experiments carried out in Chapter six demonstrates that a proportion of cells have the ability to survive for at least 6 h after the contamination event, and may, therefore, represent a greater risk than previously thought.

The air-drying experiments demonstrated that high numbers of both *Salmonella* and *Campylobacter* were still viable after 1 hour drying. This is likely to be the time of greatest activity in the kitchen and certainly when a chicken salad was prepared in the test and domestic kitchens (see Chapters four and five, Redmond *et al.* 2001) this was the time when the majority of participants prepared the vegetables for the salad. Most of the sampling of kitchens also occurred within this one-hour window indicating that the majority of contamination events should have been detected.

Numbers of *Salmonella* remained high throughout a 24 h drying period, in contrast to the rapid decline in viability by the *Campylobacter* strains during the first two hours. Despite the rapid decline in *Campylobacter* numbers during a relatively small time span, a small proportion of cells were still viable even after 24 h, possibly representing a more resistant sub-group of the population and demonstrating that *Campylobacter* may still represent a food poisoning risk hours after the contamination incident. It is likely that the survival of *Campylobacter* may also be extended if the contamination incident occurred on a surface with a higher water content, such as a dishcloth or damp hand towel. Indeed it is such damp environments in the domestic kitchen which have yielded the greatest number of isolates (Chapters four and five; Josephson *et al.* 1997; Sagoo *et al.* 2002; Scott *et al.* 1982). Griffith *et al.* (1999) found that domestic kitchens are often used sequentially and the ability of *Salmonella* and *Campylobacter* to survive even a relatively short survival time in the domestic kitchen could have implications for consequent kitchen users who could potentially contaminate their meal, indirectly, from a previously contaminated item. Wiping / drying cloths particularly tend to have multiple uses in domestic kitchens and a contaminated cloth could, for example, potentially transfer viable cells to hands or a work surface during the preparation of a meal and may subsequently result in contamination of the meal itself.

The ability of *Salmonella* and *Campylobacter* to survive in the kitchen environment, coupled with the high contamination rates observed in this study could also have serious implications for commercial kitchens. The preparation of more than one meal in kitchens at the same time could increase the risk of contaminating ready to eat foods and the ability of both *Salmonella* and *Campylobacter* to potentially survive long periods in the kitchens could lead to the contamination of foodstuffs hours after the initial contamination event.

Although it has been shown that *Campylobacter* are able to survive in the kitchen longer than previously thought it is not known, however, if these cells have the ability to cause infection. Cells subjected to prolonged drying are likely to be sub-lethally injured and their ability to cause infection may be less than that of uninjured cells. Although there has been limited work carried out specifically on the infectivity of sub-lethally injured cells work by Jones *et al.* (1991a) suggests that the ability of VBNC cells to cause infection may be strain dependant. Given these results it appears feasible that at least some strains of sub-lethally injured *Campylobacter* cells would be capable of causing disease but further work would be needed to verify this.

Unlike *Salmonella, Campylobacter* spp. are widely reported to be unable to replicate on foods at room temperature and Oosterom (2000) believed that this, coupled with the sensitivity of the bacteria to dry conditions, meant that the infection via cross contamination would be unlikely. A number of reported outbreaks have, however, identified cross contamination as a factor (Anon 1998b; Brown *et al.* 1988; Gent *et al.* 1999; Roels *et al.* 1998) and given the high contamination rates of kitchens by *Campylobacter* seen in this study, the inadequate cleaning carried out by participants and the ability a proportion of cells to withstand drying for at least 6 h it appears likely that cross contamination may be involved in a proportion of the 60,000 reported *Campylobacter* cases occurring annually in the United Kingdom.

Given that kitchen hygiene is often seen as the last line of defence between consumers and food poisoning it is apparent that considerable work needs to be carried out to educate consumers on appropriate food handling techniques if the Food Standards Agency is to achieve its goal of reducing food poisoning by 20% by April 2006.

It appears unlikely that this reduction in food poisoning can be carried out by education alone, particularly given that the number of *Campylobacter* on the chicken breasts was found to be the only significant factor involved in the detection of cross contamination events in the domestic kitchens, with statistics demonstrating that for every additional organism the probability of contaminating the kitchen was 1.001 times greater. Although there is some evidence of a reduction in *Salmonella* contamination in UK chickens (Jorgensen *et al.* 2002) reduction in the number of *Campylobacter*-positive carcases has proven difficult. Mead *et al.* (1995), however, examined methods of reducing contamination levels of carcases at the processing plant by improving hygiene controls. By incorporating a series of improvements, including the use of chlorinated water sprays to limit microbial contamination on equipment and working surfaces, he was able to significantly reduce contamination levels on carcases but suggested that the relatively small reduction was unlikely to affect the consumer's exposure to *Campylobacter*. Given the results found in this thesis it appears likely that even a small reduction in numbers of *Campylobacter* on carcases may reduce the number of cross contamination incidents occurring in domestic kitchens on a daily basis.

Other workers have reported high levels of cross contamination at poultry processing plants and Newell *et al.* (2001) found that even *Campylobacter*-negative flocks rapidly became contaminated by various *Campylobacter* subtypes during processing. If more farmers are able to produce *Campylobacter*-negative flocks (through the use of appropriate hygiene measures, such as boot dipping (Humphrey *et al.* 1993) to reduce the chances of contamination from the external environment) it is likely that the impact of the hygiene measures suggested by Mead *et al.* (1995), to reduce cross contamination, may be greater than previously thought and is an area of work which could benefit from further research. The ability of *Campylobacter* to contaminate the abattoir environment and then to contaminate previously *Campylobacter*-negative flocks confirms its ability to survive a range of environmental conditions, including in this instance carcase chilling, and to then go on to cause a cross contamination incident. This work, along with that discussed in this thesis, confirms the need for appropriate hygiene controls throughout the food production system i.e. from "farm to fork" if food poisoning by *Salmonella* and *Campylobacter* is to be reduced and

demonstrates the need for cooperation of not only consumers and restaurateurs but of everybody involved in food production.

7.2   Concluding remarks

At least 700 million chickens are sold each year in the UK (Anon, 1995b) and based on the findings by Jorgensen *et al.* (2002), 532 million could be contaminated with *Campylobacter*.   At least 13% of the meal preparation events involving a *Campylobacter*-positive chicken resulted in cross contamination in the test kitchen and by extrapolating these results an estimated 69 million cross contamination events, involving raw poultry alone, could occur each year in the UK. It is unlikely that every cross contamination event results in a case of food poisoning but this figure still represents a substantial risk.

The inadequate washing of hands, cloths and equipment were found to be the most common errors in the kitchen, which led to a contamination incident, and the majority of contamination incidences could have easily been avoided. If the general public could be better educated, and this is correlated with a change in behaviour, it is likely the number of contamination incidences which result in infection could be greatly reduced at little cost to the consumer. Further reductions in food poisoning cases may also be made if contamination rates of raw poultry with *Campylobacter* could be reduced through appropriate hygiene controls at each of the production stages.

7.3   Future work

Useful further work would include:-

Further investigations into the hygiene practices of different groups of consumers, such as single young men, mothers with young children and a post retirement group, using larger study samples, to determine if there is a significant difference in their hygiene

The determination of transfer rates of *Campylobacter*, from chicken breasts naturally contaminated with different numbers of bacteria, during common kitchen practices to assess what level of reduction in numbers would be needed to significantly reduce cross contamination rates

Real time sampling of incidents likely to lead to contamination to determine transfer rates of organisms

To compare the ability of a greater number of sero/phage typed *Campylobacter* isolates to survive air drying to determine if some sero/phage types are more resistant to air drying than others

To compare the ability of *Campylobacter* isolates to survive on range of commonly contaminated kitchen surfaces, including dishcloths (dry and damp) and other wiping cloths

To determine if sub-lethally damaged *Campylobacter* strains are capable of causing disease

Appendix A. Checklists used to record participants behaviour and the kitchen environment during food preparation sessions in domestic homes

Observational Checklist: Chicken and Pasta Salad
EXETER HOME OBSERVATION DATA

Participant No: ....   Date: ....

Handling and Preparation

Handwashing after touching inside of raw chicken packaging (RCP)

Washes hands immediately after touching RCP ☐

Contamination of the kitchen before washing ☐

• Touches tap before washing ☐

• Touches tap after washing ☐

Contamination of kitchen items within kitchen ☐

Adequacy of washing / drying hands

• Washes adequately ☐

• Washes Inadequately ☐

• No attempt at washing ☐

• Dries hands adequately ☐

• Dries hands Inadequately ☐

• No attempt at drying ☐

Washes hands after touching raw chicken (RC)

Washes hands immediately after touching RC ☐

Contamination of the kitchen before washing ☐

• Touches tap before washing ☐

• Touches tap after washing ☐

Contamination of kitchen items within kitchen ☐

Adequacy of washing / drying hands

• Washes adequately ☐

• Washes Inadequately ☐

• No attempt at washing ☐

• Dries hands adequately ☐

• Dries hands inadequately ☐

Use of equipment and utensils for preparation of raw chicken and then salad vegetables for chicken salad

Salad veg. prepared before raw chicken Is handled ☐

Chopping Boards
• Use of same chopping board ☐
• Use of separate chopping board ☐

Adequacy of washing drying chopping boards between uses
• Scrub with hot water ☐
• Use of detergent ☐
• Rinsed with water ☐
• Wiped with cloth ☐
• Wiped with t-towel ☐
• No washing ☐
• Drying using paper towel ☐
• Use of clean t-towel ☐
• Use of unclean t-towel ☐
• Use of hand towel ☐
• No drying ☐

Knives
• Use of same knife ☐
• Use of separate knives ☐
Adequacy of washing / drying knives between uses
• Scrub with hot water ☐
• Use of detergent ☐
• Rinsed with water ☐
• Wiped with cloth ☐
• Wiped with t-towel ☐
• No washing ☐
• Drying using paper towel ☐
• Use of clean t-towel ☐
• Use of unclean t-towel ☐
• Use of hand towel ☐
• No drying ☐

Equipment / Utensils
• Use of same equipment / utensils ☐
• Use of separate equipment / utensils ☐

Adequacy of washing / drying equip./ uts between uses
• Scrub with hot water ☐
• Use of detergent ☐
• Rinsed with water ☐
• Wiped with cloth ☐
• Wiped with t-towel ☐
• No washing ☐
• Drying using paper towel ☐
• Use of clean t-towel ☐
• Use of unclean t-towel ☐
• Use of hand towel ☐
• No drying ☐

Preparation Actions

- Raw meat is washed under running water (contamination of preparation environment) ☐

- Direct contamination of preparation environment from **raw chicken** (✓ frequency). ....

  Preparation environment is followed by immed / efficient cleaning of contaminated area .....

- Direct contamination of preparation environment from **utensils contaminated with RC** (✓ frequency).

  ....

  Preparation environment is followed by immed / efficient cleaning of contaminated area ....

- Direct contamination of preparation environment from **raw chicken packaging**. ☐

  Preparation environment is followed by immed / efficient cleaning of contaminated area ☐

- Failure to wash contaminated equipment / utensils immediately after use ☐

- Chicken is cut into large (<2cm³) uneven pieces (inconsistent heat penetration). ☐

- Failure to wash tomato before use ☐

- Failure to wash lettuce before use ☐

- Failure to wash spring onion before use ☐

- Contamination of any salad vegetables directly from raw chicken packaging ☐

- Contamination of any salad vegetables directly from raw chicken ☐

- Salad ingredients touched with <u>hands</u> not adequately washed after handling RC ☐

- Ham touched with <u>hands</u> not adequately washed after handling RC ☐

- Touches contaminated <u>equip / part of kitchen</u> with chicken then touches salad ingreds ☐

- Touches contaminated <u>equip / part of kitchen</u> with chicken then touches ham ☐

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Heating

- Fails to preheat frying pan before heating   ❑

- Chicken pieces are not fried for at least 6 minutes   ❑

- Length of time chicken pieces are fried for (state)   ....

- Method for assessing heating completion   ....

Post Heating Handling (CU, U, H, CH) (*circle if within last minute of heating or pierced into the centre)

- Potential contamination of pasta after removal from heat   ....

- Potential contamination of chicken pieces / salad during assembly   ....

- Potential contamination of chicken salad for storage   ....

Cooling and Post Heating Storage (for consumption ~ 24 -36 hours time)

- Pasta is not cooled with cold water   ❑

- Chicken pieces are not transferred from frying pan to plate / bowl to cool   ❑

- Chicken pieces are covered during cooling   ❑

- Chicken pieces / pasta are placed into the salad immediately after heating   ❑

- Chicken salad is left at room temperature   ❑

Refrigerated storage

- Chicken salad is refrigerated within 30 minutes after removing chicken / pasta from heat   ❑

- State covering of chicken salad for storage   ....

- Chicken salad is not covered for storage   ❑

- State shelf Chicken salad is refrigerated on   ....

- Refrigerated chicken salad is stored below raw ingredients in fridge   ❑

- Chicken salad is not transferred to a separate container for storage   ❑

ASSESSMENT OF KITCHEN ENVIRONMENT

Participant No..

Work Surfaces

1. Type of work surface (✓ as applicable)

- tiled ☐
- stainless steel ☐
- marble ☐
- smooth ☐
- textured ☐
- wood ☐
- laminate ☐

2. Number of preparation surfaces (✓ as applicable): 1.☐   2.☐   3.☐   more than 4 (specify)........

3. Approximate sizes of work surfaces / kitchen:

....

....

4. Work surfaces cluttered ☐
5. Breaks / crevices / chips on work surface ☐
6. No seal where preparation surface meets wall ☐

7. **Condition of work surface**

- Satisfactory condition; appears clean / free from visible debris ☐
- Moderate condition: crumbs and non stuck debris present ☐
- Unsatisfactory condition: dried goo on work surface, wet ☐

Ventilation / heating

- No ventilation system (no extractor fan) ☐
- No windows to be opened ☐
- Dampness present ☐
- Condensation on windows ☐
- Pets present in kitchen environment ☐
- Radiators / means of heating present in kitchen ☐
- Boiler / central heating system situated in the food preparation area ☐
- Washing machine / tumble dryer present in kitchen ☐
- Positioning of washing machine / tumble drier:

....

Kitchen Assessment

Floors

- Type of floor covering..
- Dirt / food debris present ☐
- Food debris present ☐

Rubbish bin

- No lid ☐
- Full / overflowing ☐
- Lid present, but not free from debris ☐

Storage Facilities

- Fridge door seal ineffective ☐
- Food debris / dirt visible in fridge ☐
- Freezer needs defrosting ☐
- Food debris / dirt visible in freezer ☐
- Temperatures of fridge ....

Equipment and Utensils

Chopping boards

Type(s) of chopping board(s) used in food preparation:	Number of chopping boards owned	Condition of chopping board(s)			
		Smooth, not scored, clean and dry	Moderate scoring and / or stained	Heavy scoring and staining	Very heavily scored, chipped, stained, dirty
Wood (hard or soft)	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Plastic	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Glass	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Marble	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Melamine	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Other (specify).....................	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐

Knives

- Clean, shining, dry ☐
- Marked slightly ☐
- Unclean, pieces of dried debris attached, chipped ☐

Kitchen Assessment 2

Cloths and Cleaning Materials

Type of cloth	Number of cloths present	In use for washing up	In use for cleaning surfaces
J cloth		❏	❏
Fine weave		❏	❏
Thick weave		❏	❏
Scourer		❏	❏
Non stick scourer		❏	❏
Sponge cloth		❏	❏
Vileda cloth		❏	❏
T'towels		❏	❏
Hand towel		❏	❏
T'towels		❏	❏

- **Condition / storage of cloths:**

	Washing up	Cleaning
⇒ No stains, not worn, not discoloured, no odour	❏	❏
⇒ Some wear, but not stained or discoloured	❏	❏
⇒ Some wear, some discoloration, screwed up	❏	❏
⇒ Worn, wet, soiled, smelly	❏	❏
⇒ Screwed up	❏	❏
⇒ Food Debris Visible	❏	❏
⇒ Remains wet	❏	❏

- **Cleaning materials available:**
  - ⇒ Antibacterial ❏
  - ⇒ Cream cleaner ❏
  - ⇒ 'Mr. Muscle' ❏
  - ⇒ Sanitiser ❏
  - ⇒ Other (state) ❏

- **Handwashing materials that are present in the kitchen**

	Present in home kitchens
⇒ Anti-bacterial soap pump	❏
⇒ Bar of soap	❏
⇒ Moisturising soap pump (liquid soap)	❏
⇒ Washing up liquid	❏
⇒ Hand cream	❏
⇒ Hand towel	❏
⇒ Nail brush	❏
⇒ Paper towels / kitchen roll	❏

- Smoking during food preparation ❏
- Protective clothing is visibly dirty ❏

Kitchen Assessment -3

Additional comments:

Kitchen Assessment

Appendix B. The scoring system used to calculate a risk score for participants behaviour food preparation sessions

INADEQUATE PRACTICE	TOTAL DEMERIT MARKS AWARDABLE
Handling and Preparation	
1.1 Washing and drying of hands after handling raw chicken packaging	
• No washing and drying of hands *OR* washing and drying of hands immediately after handling raw chicken packaging after contamination of equipment, utensils or preparation environment after handling raw chicken packaging *OR* unhygienic washing and drying of hands immediately after handling raw chicken packaging	100
• Hygienic washing / and drying of hands immediately after handling raw chicken packaging	0
1.2 Washing and drying of hands after handling raw chicken	
• No washing and drying of hands *OR* washing and drying of hands immediately after handling raw chicken after contamination of equipment, utensils or preparation environment after handling raw chicken *OR* unhygienic washing and drying of hands immediately after handling raw chicken	100
• Hygienic washing / and drying of hands immediately after handling raw chicken	0
1.3 Washing / Drying of CHOPPING BOARDS after raw chicken and before lettuce, tomato, spring onion, ham	
• No washing and drying or unhygienic washing and drying of the same chopping board for raw chicken and then lettuce, tomato, spring onion or ham	1000
• Use of separate chopping boards for raw chicken and then lettuce, tomato, spring onion or ham *or* hygienic washing and drying of chopping board for raw chicken and then lettuce, tomato, spring onion or ham	0
1.4 Washing / Drying of KNIVES after raw chicken and before lettuce, tomato, spring onion, ham	
• No washing and drying or unhygienic washing and drying of the same knives for raw chicken and then lettuce, tomato, spring onion or ham	1000
• Use of separate knives for raw chicken and then lettuce, tomato, spring onion or ham *or* hygienic washing and drying of knives for raw chicken and then lettuce, tomato, spring onion or ham	0
1.5 Washing / Drying of EQUIP / UTENSILS after raw chicken and before lettuce, tomato, spring onion, ham	
• No washing and drying or unhygienic washing and drying of the same equip / utensils for raw chicken and then lettuce, tomato, spring onion or ham	1000
• Use of separate equip / utensils for raw chicken and then lettuce *or* hygienic washing and drying of equip / utensils for raw chicken and then lettuce	0

INADEQUATE PRACTICE	TOTAL DEMERIT MARKS AWARDABLE
1.6 Handling of raw chicken and contamination of preparation environment	
• Washes raw chicken	100
• Contamination of preparation environment with **raw chicken** (other than from sections 1.1 – 1.5)	100
⇒ Contamination of preparation environment followed by efficient cleaning of contaminated area	0
• Contamination of preparation environment with **utensils** contaminated with raw chicken (other than from sections 1.1 – 1.5)	100
⇒ Contamination of preparation environment followed by efficient cleaning of contaminated area	0
• Contamination of preparation environment with **raw chicken packaging** (other than from sections 1.1 – 1.5)	100
⇒ Contamination of preparation environment followed by efficient cleaning of contaminated area	0
• Failure to wash / dry utensils / equipment contaminated with raw chicken immediately after use	100
• Chicken pieces are cut into large uneven pieces	10
• Failure to wash tomato before use	10
• Failure to wash lettuce before use	10
• Failure to wash spring onion before use	10
• Contamination of any salad vegetables from raw chicken packaging	1000
• Contamination of any salad vegetables from raw chicken	1000
• Salad ingredients touched with hands not adequately washed after handling raw chicken	1000
• Ham touched with hands not adequately washed after handling raw chicken	1000
• Touches equipment / part of kitchen contaminated with raw chicken and then touches salad ingredients	1000
• Touches equipment / part of kitchen contaminated with raw chicken and then touches ham	1000

INADEQUATE PRACTICE	TOTAL DEMERIT MARKS AWARDABLE
1.7 Heating	
• Frying pan is not preheated	10
• Failure to heat chicken efficiently: Fried chicken is not heated for 6 minutes	1000
1.8 Post Heating Handling	per malpractice (no limit)
• Potential contamination of pasta with utensils or hands after removal from heat	100
• Potential contamination of pasta with contaminated utensils or contaminated hands after removal from heat	1000
• Potential contamination of **chicken pieces** with utensils or hands during heating (by piercing the centre of chicken piece)	100
• Potential contamination of chicken pieces with contaminated utensils / hands during the final 1 minute of heating (by piercing the centre of chicken piece)	1000
• Potential contamination of **chicken salad / chicken pieces** with utensils or hands during assembly	100
• Potential contamination of chicken salad / chicken pieces with contaminated utensils or contaminated hands during assembly	1000
• Potential contamination of chicken salad with utensils or hands for storage	100
• Potential contamination of chicken salad with contaminated utensils or contaminated hands for storage	1000
1.9 Cooling and Post Heating Storage	
• Pasta pieces are not cooled using cold water	10
• Fried chicken pieces remain in frying pan for cooling	10
• Fried chicken pieces are covered during cooling	10
• Chicken pieces / pasta are transferred to the salad immediately from the heat	10
• Chicken Salad is left at room temperature	100
• Chicken salad is refrigerated within 30 minutes of heating chicken pieces or pasta	10
• Chicken salad is not covered	10
• Chicken salad is stored on shelf 2 or 3	10
• Chicken salad is not transferred to separate container for storage	10

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