Behaviour and cross-contamination of pathogenic bacteria in household kitchens – relevance to exposure assessment

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Contents

Abstract

Illness resulting from consumption of contaminated food is a continuous serious public health problem in the world. A proportion of this foodborne disease is attributable to improper preparation practices in the home, including cross-contamination.

Salmonella, *Campylobacter* and *Staphylococcus aureus* survive on stainless steel surfaces for hours or days, depending on the species, initial counts and the presence of food residues. Following a single contamination and air-drying at room temperatures, *Salmonella*, *Escherichia coli, Bacillus cereus* and *S. aureus* also remain on cleaning sponges for days, with or without the presence of food residues. The exposure to low water activity surfaces induces filamentation of *Salmonella* cells, some of which reach a size of 50 μm or more. Filamentous cells maintain their membrane integrity for days on these surfaces and are able to split in single cells under favourable conditions, resulting in the instantaneous appearance of a large number of viable cells.

Considering the common practice of surface cleaning, wiping countertops and other kitchen surfaces reduces microbial counts considerably, particularly when a clean cloth or an antibacterial-impregnated napkin is used. On the contrary, when the cloths are damp and contain high numbers of microorganisms, bacteria are readily spread from the cloths to the surfaces instead of removing bacteria from the surfaces.

Salmonella, *E. coli, S. aureus* and *B. cereus* survive the treatments with antibacterial dishwashing liquid in sponges. Application of this product under practical situations in households does not make a significant difference as compared to regular product. Furthermore, *Salmonella* and *S. aureus* demonstrate a better tolerance to household bleach to certain concentrations in cloths as compared to exposure in a suspension. Presence of food debris in the cloths, which occurs regularly in practical situations, decreases the effectiveness of antibacterial compounds or disinfectants. Therefore, other measures, such as heating, i.e. immersing in boiling water or washing by a dishwash or laundry cycle with detergents at $\geq 60^{o}$C is recommended to reduce bacterial contamination in cloths.

A quantitative analysis on cross-contamination of *Salmonella* and *Campylobacter* from contaminated chicken carcass to salad via kitchen surfaces has shown that it is realistic to expect that a proportion of the human exposure to, particularly, *Campylobacter* originates from cross-contamination in private kitchens during food handling. The probability that salads become contaminated with *Campylobacter* is higher than that with *Salmonella* since the prevalence and the levels of *Campylobacter* on chicken carcass are higher than that of *Salmonella*.

As foodborne disease occurrence continues over time, prevention and control measures must be managed on a continuous basis. Each individual contributes a critical role in preventing and controlling illness. Basic personal and kitchen hygiene can greatly help to defend against harmful microorganisms.

Chapter 1

General introduction

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Introduction

Infectious diseases are known as serious health risks since many centuries. The mortality rate of these diseases was a great concern even as recently as in the late eighteenth and the early nineteenth centuries. The increasing awareness of the importance of personal hygiene as well as the introduction of safe water supplies and sewage systems, milk pasteurisation, population wide vaccination schemes and the use of antibiotics resulted in successful control of acute infections in the course of the twentieth century (1, 67). However, epidemiological data indicate that infectious diseases remain globally a serious threat for public health (136). Previously unknown infections (emerging infectious diseases) and the reappearance of known diseases after a significant decline in incidence (re-emerging infectious diseases) cause enormous public health problems both locally and internationally.

With respect to foodborne disease it was particularly during the 1980s and the early 1990s that the international incidence increased considerably as a result of infections by (re-) emerging pathogens (100). Several factors contribute to the emergence and re-emergence of infectious diseases, but most can be linked with the increasing number of people living and moving around on the globe, including changes in human demographics and behaviour, changes in food production systems, rapid increases in international travel and commerce, microbial adaptation and change, and the breakdown of public health measures (55, 59). Understanding the route(s) of an infectious disease is critical in order to identify accessible targets for control strategies. For example, person-to-person transmission may be inhibited by proper hygiene and sanitary conditions and education. Vector-borne diseases may be prevented by control measures that either kill the vector or prevent its contact with humans. This Chapter presents an overview of potential aspects in the household kitchen environment implicated in the transmission of infectious diseases. Although there are different organisms present in the home, including bacteria, viruses, protozoa and fungi, as causal agents of diseases, this study only deals with the bacterial contaminations, since the kitchen plays an important role in transmission of bacterial diseases.

The role of household kitchens in transmission of foodborne infectious disease

Pathogenic organisms will continuously enter the home with foods (foodborne) or through water (waterborne), through food prepared in the home by an infected person (person-to-person spread), through the air, by insects or via pets (5). These are considered as the primary sources of potential harmful microorganism in the home.

In the domestic environment the kitchen is particularly important in spreading infectious disease. The first well-known bacterial transmission in the kitchen was documented in the early part of the twentieth century, when Mary Mallon, who worked as a cook in private New York households, was identified as a healthy chronic carrier of the typhoid fever bacterium. She had been spreading typhoid fever through the foods she prepared. Due to poor

personal sanitary habits, she caused more than thirty cases of typhoid fever with three deaths, while Mallon herself had never been sick with typhoid fever (48, 68, 97). This thorough epidemiological discovery work and the finding of typhoid bacteria in Mallon's stool proved the significant role of household environment in transmission of foodborne disease and had a great impact on the science of microbial hygiene (68).

Reviewing the mechanisms of transmission of foodborne infections in the United States between the years 1960 and 1982, Bryan (13) indicated that a colonised person handling the implicated foods (person-to-person spread) was the most frequently identified factor that contributed to staphylococcal food poisoning, shigellosis and typhoid fever. Cross-contamination dealt with 20% of reported salmonellosis and 22% of *Vibrio parahaemolyticus* gastroenteritis.

Over the past decade, up to 87% of reported foodborne disease outbreaks in Europe, the United States, Canada and Australia have been associated with food prepared or consumed in the home (100). Historically, *Salmonella* has caused the largest proportion of reported foodborne disease outbreaks associated with private homes. Some other bacterial infections associated with this environment are caused by *Campylobacter*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* (94, 136). The increase of incidence rates with *Salmonella* and *Campylobacter* as causal agents reflects the increasing of *Salmonella* and *Campylobacter* contamination rates of poultry products, which are found up to 60% and 80%, respectively (26, 34, 52). This fact illustrates the potential risk associated with cross-contamination during preparation of raw chicken in the domestic environment.

Accompanying the development of epidemiology and improved surveillance of foodborne disease, there is an increased interest in the collection of data that includes contributing factors to the outbreaks and place where food was contaminated, mishandled or consumed, next to the causative agents and incriminated foods. Hence, specific factors that contribute to the occurrence of foodborne disease have become apparent, and data detailing household food preparation practices from different countries are more and more documented.

Epidemiological data in different countries in Europe between 1993 and 1998 indicate that a considerable number of foodborne diseases are attributable to improper preparation practices in the domestic kitchen. A proportion of incidences is contributed to temperature misuse (44%) and to consumption of contaminated raw materials (20%). Inadequate handling including cross-contamination and insufficient hygiene, as well as environment factors such as contamination by persons who handle foods and contaminated equipment, are accounted for more than 27% of the reported outbreaks (136). Food safety studies at household level, however, indicated that most consumers (householders) failed to associate home food handling practices with foodborne infections (100). This fact is considered as a serious impediment to convince householders to change inappropriate food preparation behaviours (100) which is very important, since prevention of foodborne disease involves cooperation and responsibility of all stages in the food chain.

Bacterial contamination and cross-contamination in the kitchen

Until the late 1970s little attention has been paid on investigation of bacterial contamination and cross-contamination in the kitchen, as indicated by little published information in these areas in comparison to the detailed studies on bacterial contamination in the hospital environment (56, 117). This may be a result of assumptions that the home is normally occupied largely by healthy adults and that there is no special need for hygiene (7, 111). However, recent evidence has lead to a real acceptance of the home as an important environment in the chain of infection transmission and resulted in a resurgence of interest and public concern about bacterial contamination as well as hygiene and cleanliness in the home (7, 56, 111).

Early studies on bacterial contamination in the kitchen were conducted in the late 1960s investigating bacterial load of hand towels and the hygienic conditions of domestic dishcloths and tea towels (117). Such cloths were heavily contaminated with bacteria and suspected as one of the main vectors for spreading and dissemination of bacteria in the kitchen.

The current attention on bacterial contamination in the kitchen started in the late 1970s. Comprehensive studies of bacterial contamination in the home were carried out, which involved sampling various sites in the kitchen, bathroom and living room (29, 114). The sink area and dishcloths were found as the most frequent sites contaminated with *E. coli,* coagulase negative *Micrococcaceae* and *Bacillus* spp. (29), as well as Enterobacteriaceae (114). These results were supported by laboratory studies, which demonstrated that Gram-negative bacteria, such as *E. coli*, *Klebsiella* spp. and pseudomonads survived and were able to grow in cloths and in sink U-tubes (113). Furthermore, early studies on cross-contamination by de Wit et al. (24) indicated that after preparation of artificially contaminated chicken products, target organisms were spread over all the utensils and working surfaces used. Similar results were found after preparation of a dinner with artificially contaminated minced meat (10) and preparation of naturally contaminated chicken products in the kitchen (23). It was also demonstrated that when surfaces become contaminated, the bacteria were readily transmitted via hand or cloths to other surfaces (113).

An apparently renewed concern on home-hygiene started at about 1995 and is characterised by a recurring increase of interest on studies of home and personal hygiene, reflecting the trend of increasing incidence of illness resulting from foodborne infections. More than 80% of the household food safety studies from the past 25 years have been carried out since 1995 (100). The persistence of microorganisms, presence and density of pathogens and the potential spread of microbial contamination from contaminated food in the household kitchen have been extensively studied and re-examined. These studies indicated that domestic kitchen sites have been found repeatedly contaminated with a variety of bacterial contaminants, including *Listeria monocytogenes* (6, 117), *E. coli* and *Enterobacter cloacae* (117), *Salmonella* (27), *Campylobacter* (53) and *S. aureus* (38). Several kitchen sites,

particularly wet areas including sponges/dishcloths (6, 27, 38, 53, 104, 117), and sink drain areas (6, 53, 104, 117) continually appear to act as a reservoir that harbours and encourages the growth of potential pathogens.

The attention was not only pointed to the investigation of bacterial contamination in domestic environments, but also to the survival of bacteria in this setting. Detailed studies on bacterial survival on specific objects have been reported, including survival of *S.* Typhimurium on wooden and plastic chopping boards (32), attachment of *S. aureus* on domestic preparation surfaces (31) and survival of *Salmonella* and *Campylobacter* in a dry film on formica surfaces (41). Furthermore, cross-contamination also received additional attention. Zhao et al. (139) demonstrated that bacteria could be readily transferred to chopping boards after cutting and handling contaminated raw chicken. A large number of bacteria survived on the chopping boards for at least 4 hours and could cross-contaminate fresh vegetables if the boards were not cleaned sufficiently (139). Following preparation of chicken contaminated with *Salmonella* and *Campylobacter*, these bacteria could be isolated from the hands and food contact surfaces sampled (18). Moreover, a quantification study on bacterial cross-contamination in common food service tasks indicated that the transfer rates were highly variable depending on the nature of surfaces involved (17).

About the renewed attention that exists for the household kitchen environment, a special remark can be made, i.e. that most of the studies are qualitative assessments. Only a few areas have been examined quantitatively in any detail (17, 139). More and more quantitative data from systematic studies are needed for the purposes of risk assessment and risk management in the domestic environment. Continued quantitative research in microbial contamination and persistence in this environment is essential for improving the understanding of factors contributing to foodborne disease.

Measures for preventing cross-contamination in kitchen environments

Infectious diseases have raised the need for effective hygiene in the home since many years ago. In practice, cleaning is not the only important issue; knowing how to prevent contamination is just as crucial. Effective hygiene in the home is therefore the total sum of measures used to prevent contamination with pathogens aiming to avoid the occurrence of infectious disease. This measure includes hygiene during food preparation as well as personal hygiene.

Simple personal hygiene including soap utilization was the silent success of public health in the pre-disinfectant era (33). Increased life expectancy since the first half of the twentieth century can be attributed to improved personal hygiene status, resulting in decreased infectious disease incidence. Then came the era of disinfectants. Together, these have made a lasting effect on public health. However, while men can make choices of action that will protect their health, in response to the increasing media attention to life-threatening microbial agents, the public has recently apparently adopted a new definition of “clean”.

Rather than being washed free of dirt and other substances, sites must be free of microorganisms (69). This was prompted particularly by the promotion of hundreds of antibacterial products touted to eliminate microorganisms from homes and persons.

Several discussions have been initiated in order to determine what level of clean up will be required to be satisfactory from the point of view of public health in the home. Drying of cloths and surfaces, for example, as well as cleaning with detergent result in reduction of bacterial populations. But, it was shown that drying alone cannot be relied upon to prevent the transfer of infectious microorganisms from household surfaces to the householder and reduction by detergent cleaning is only a temporary event when the cloths are kept moist (112). If a high rate of reduction of microorganisms from sponges or cloths is a final target of measure, soaking in a solution of bleach should be incorporated, or alternatively, the cloths should be heated for one minute in a microwave or immersed in boiling water for 5 minutes (47). Heat is an effective form of disinfection, although it may not be applicable to large surface areas and may be unreliable in unskilled hands (5). Chemical disinfectants or hygienic cleaners can be used for decontamination of sites and surfaces in situations where the former methods are either impractical or deemed to be inadequate for the particular situation. However, the effects may be relatively short lived and recontamination of these sites may occur quite rapidly either as a result of transfer of microorganisms or by re-growth of residual survivors on surfaces that remain damp. This clearly indicates that to be effective, hygiene procedures should be applied for a specific purpose, rather than as a part of a routine cleaning process (5). The question of how safe is safe enough is a real concern in case there are vulnerable household members in the home, including young children, pregnant women, elderly persons and people who are extremely ill or undergoing therapies which compromise their immune systems and their host defences. People may now find themselves questioning how at-risk they are and what they can do to protect themselves. While effective hygiene is undoubtedly essential, the use of disinfectant may not necessarily be aggressive, especially if it is meant for household use with a lower human and environmental safety profile compared to a hospital (33, 69). Their use, however, should have a role as a part of an overall hygiene strategy within the home.

Recently, guidelines for home hygiene (45) have been introduced in order to respond to the need for improvements in hygiene awareness and hygiene practices in the home. The key features of these guidelines are based on the concept of risk assessment and risk prevention. Also, in The Netherlands a ‘Hygienic code of the private household’ based on hazard analysis critical control points (HACCP) has been drawn up (133). It was considered that such guidelines draw on all aspects of home hygiene related to infectious disease control and give comprehensive and consistent information on procedures to prevent infection and the transfer of pathogens in the home (5).

Reflecting to the increase of infections by pathogens that (re)emerged in recent times, the effectiveness of measures for preventing cross-contamination should be continuously evaluated. Hygienic codes that have been developed need also to be continuously updated and

justified, if necessary, to minimize the bacterial transmission in the domestic environments by any newly identified or previously known pathogens. Any effective measures to control or reduce the microorganisms in the home would reduce the public health concerns related to their exposure.

Behaviour of selected pathogens related with foodborne disease in domestic environment

Historically, *Salmonella* has caused the largest proportion of reported foodborne disease outbreaks associated with private homes, as described previously. The salmonellae are among the most ubiquitous microorganisms that cause bacterial diarrhoea. There is a widespread occurrence in animals, especially in poultry, cattle and swine. *Salmonella* lives in the intestinal tracts of animals and birds. Foods of animal origin become contaminated following faecal contamination of the environment and equipment and have been identified as vehicles for transmitting these pathogens to human beings and spreading them to kitchen environments. Cross-contamination is produced by contaminated raw foods during further processing and preparation. Although salmonellae do not form spores, they can survive for relatively long periods in foods and other substrates. *Salmonella* can grow at room temperature and albeit slow, at chill temperature. Salmonellae can also get established and multiply in the environment and in equipment of a variety of food-processing facilities (126).

Over the past few decades *Salmonella* Enteritidis was the most important cause of *Salmonella*-infection in Europe and the United States associated with the consumption of shelled eggs and poultry (94, 136). Stringent procedures for cleaning and inspecting eggs, implemented since the 1970s, have made salmonellosis caused by external faecal contamination of eggshell extremely rare. However, *S.* Enteritidis silently infects the ovaries of healthy appearing hens and contaminates the eggs before the shells are formed (14). Furthermore, like any other strain of *Salmonella*, which resides in chicken's and turkey's intestines, *S.* Enteritidis can find its way into the processed chicken carcass, where it can cause serious health risks to humans.

Campylobacter jejuni is now reported to be the leading cause of bacterial diarrhoea in humans in the countries where records are kept. Campylobacters (‘*Vibrio fetus’*) have been known as the cause of disease in animals since in the early 1900s, but they have been generally recognised only recently as a cause of the human disease. Campylobacters occur widely as part of the normal intestinal flora of many animals, especially chickens and turkeys, and enter the human food chain at slaughter of the animals. In addition, raw milk and poorly treated water supplies are also important sources of *Campylobacter* infections. *C. jejuni* will not multiply on chilled food or on shelf stable foods stored below 30^{o}C. This species survives better at chill temperature than at ambient temperature. It also survives for several months in frozen minced meat and poultry. Furthermore, it was thought that these bacteria were unable to persist on kitchen surfaces, but this may have been due to a limitation of the recovery technique used. If more sensitive methods are applied, campylobacters can be recovered from

surfaces 24 hours after contamination (41). Reflecting the fact that *C. jejuni* will not readily grow in food, it is believed that dissemination of the organism may occur through contamination of the environment and the hands of kitchen personnel with subsequent cross-contamination of prepared food (126).

Bacillus cereus is a bacterium that is common in the natural environment and in a variety of foods. The organism is so widespread, that it is almost impossible to keep it from contaminating certain foods. The bacterium has been isolated from dried beans, cereals, dried foods including spices and seasoning mixes, and potatoes. *B. cereus* is able to form spores that can survive long periods of dryness and mild heat treatments such as cooking. Hence, since *B. cereus* bacteria are common and widespread, preventing contamination of food with spores is virtually impossible. Consequently, effective prevention and control measures depend on inhibiting spore germination and preventing the growth of vegetative cells in cooked, ready-to-eat foods. Steaming under pressure, thorough roasting, frying and grilling are most likely to destroy cells and spores. Temperatures under 100^{o}C will allow for the survival of some spores. Not all strains of *B. cereus* are able to cause foodborne illness. Only those strains that are able to produce toxin(s) are able to cause illness. The toxins are actually destroyed by heating, but if the food, e.g. rice that is most commonly found contaminated by this bacterium, is just briefly reheated then the heat may not be sufficient to destroy all toxins. The bacteria cannot produce the toxin at refrigeration temperature therefore if the food is cooked ahead of time, it should be cooled as quickly as possible (125, 126).

Staphylococcus aureus is among the longest recognised of the pathogenic bacteria. This species constitutes a normal part of the microflora of the human and the animal body, as it is found on skin surfaces and hair, and in the nose, mouth and throat. Staphylococcal food poisoning occurs as the result of the ingestion of a heat-stable, preformed enterotoxin, produced by the organism during growth. *S. aureus* multiplies in food that is left out at room temperature. The products that are most often affected by *S. aureus* are high content protein and fat products with low numbers of competitive microorganisms including milk cream, smoked fish, poorly fermented meat products such as salami, and ready-made chicken and meat sandwiches. In general, *S. aureus* growth is repressed in the presence of competing microorganisms. The presence of a large number of *S. aureus* organisms in a food may indicate poor handling or sanitation due to human contact or cross-contamination. However, it is not sufficient evidence to incriminate a food as the cause of staphylococcal food poisoning. The isolated *S. aureus* must be shown to produce enterotoxins. Conversely, small staphylococcal populations at the time of investigation may be remnants of large populations that produced enterotoxins in sufficient quantity to cause food poisoning (125, 126).

In general, foodborne disease may occur when a susceptible individual consumes a food contaminated by (a) viable microbial pathogen(s), and or its toxin(s). However, not every exposure to a pathogen in food will result in infection or illness, and not all individuals in a given population are equally susceptible to all pathogens. The risk of foodborne disease is a combination of the likelihood of exposure to a pathogen in a food, the likelihood that

exposure will result in infection or intoxication and subsequent illness and the severity of the illness (64). Therefore, rational decisions about the kind of interventions, which would be most effective in reducing the impact of pathogens on human health, need a scientific-based approach facilitating estimation of the probability and severity of a health disturbance as a consequence of consumption of food (64).

Risk assessment approach in the household environment

The essence of microbial risk assessment is describing a system in which a microbial hazard reaches its host and causes harm. Risk assessment is a process that provides estimation of the probability and impact of adverse health effects attributable to potentially contaminated foods, or simply, risk assessment is a measure of risk and the identification of factors that influence it (64). This approach includes hazard identification, hazard characterization, exposure assessment, and risk characterization. Due to the structured approach, various options can be evaluated to assess the influence on the risk estimate. Mathematical modelling offers many possibilities in the quantitative estimation of the risks. Modelling should help to improve estimates and thereby allow quantification of food safety risks (30). Moreover, development of a model, although simplified and partially incomplete, can be a helpful tool to evaluate the relationship between risk and a factor that may be used to mitigate this risk (71). Once the model has been developed, the impact of various control strategies and trends can be simulated (65).

In developing suitable guidelines or control interventions for home hygiene, a structured approach is needed. A detailed risk assessment can be used to identify critical gaps in our knowledge base, to characterise the most important risk factor and to help identify strategies for risks reduction in this environment (65). In providing guidance to determine risk prevention a number of factors need to be taken into account. For example, reviews of microbial contamination of the homes may enable to identify sites and surfaces that most likely contribute to infection risks. However, as the risks associated with environmental contamination depend not only on whether the site is contaminated, but also on the probability of transfer either to food, to other surfaces or directly from hand to mouth, and whether the numbers exceed the level that can result in infectious disease, a total approach to home hygiene has additional benefit. It creates an understanding of the relative risks for different aspects of home hygiene (5).

The need for risk-informed decision making and planning is urgent in determining priorities in public health programs. In order to establish effective and acceptable decontamination for a public setting, including home setting, Raber et al. (99) indicated that public perception of risk to health, public acceptance of recommendations based on scientific criteria, time constraints and economic concerns must all be addressed in the context of a specific scenario. A risk-based approach means that clean up or decontamination guidelines should be based on a defined, ‘acceptable’ level of risk to health, while key issues are to

determine exactly what constitutes a safety hazard and whether decontamination is necessary or not for a particular scenario. This study indicates that clean up criteria are site dependent and population specific. Zero concentration of a biological agent and zero risk, in many cases is clearly not a necessity. It is likely that economic drivers will also influence population to accept higher risks (99). Furthermore, an important factor underlying each risk-based decision is the uncertainty and reliability of available data. Uncertainties in site specific features and prediction of natural attenuation or potential dilution effects all need to be considered to get to the decision about whether appropriate decontamination levels have been reached (99).

In order to provide a scientific basis for risk management strategies that minimise the level of undesirable bacteria on the hands and, therefore, reduce the risk of cross-contamination during food preparation, Montville et al. (82) described a risk assessment of hand washing efficacy. The risks associated with different hand washing techniques were quantified, including FDA (Food and Drug Administration) food code, i.e. soaping for 20 seconds and rinsing thoroughly, followed by drying with a paper towel. Proper hand washing has been recognised as one of the most effective measures to control the spread of pathogens, especially when considered along with the restriction of ill workers, and the controversial recommendation of no-bare-hand contact with ready-to-eat foods (82). Since foodborne pathogenic bacteria are transient in nature, with the exception of *S. aureus*, the models reflected hand washing efficacy with respect to the risk of bacterial contamination to a reasonable degree. The result indicated that when done properly, hand washing could reduce the risk of bacterial contamination on hands. The primary factors influencing final numbers of bacteria on the hand were sanitizer, soap and drying method (82).

The risk assessment at household level will be useful in providing guidance to make rational decisions about the kind of interventions to control transmission of foodborne infectious diseases in this environment. As relevant and accurate data are often lacking particularly in the exposure assessment, systematic studies are still needed to provide quantitative data for risk management efforts in domestic environment.

Aim and outline of this thesis

The aim of the research described in this thesis is to gain a quantitative understanding of survival, cross-contamination and stress response of foodborne pathogens in household kitchen environments and development of an exposure assessment model in this environment to be incorporated in microbiological risk assessment.

In **Chapter 2** the survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods are investigated. **Chapter 3** describes tolerance of *S.* Enteritidis and *S. aureus* to surface cleaning and household bleach. In this study the effect of surface cleaning by wiping using regular, micro fiber and antibacterial-treated cloths on these pathogens was investigated. The effect of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges is described in **Chapter 4**. In

Chapter 5 the morphological changes and cell viability of *S.* Enteritidis on reduced water activity surfaces was studied. The cross-protection to sodium hypochlorite was also investigated. **Chapter 6** describes a quantitative analysis on cross-contamination of *Salmonella* and *Campylobacter* via domestic kitchen surfaces. **Chapter 7** discusses the implication of the results that were found in this research on general home hygiene measures and its relevance to exposure assessment.

Chapter 2

Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods

Abstract

The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. In this study the survival of *Salmonella* Enteritidis, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces at different initial levels was determined at room temperature. The transfer rates of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods were also investigated. *S. aureus* was recovered from the surfaces for at least 4 days when the contamination level was high (10^5 CFU/cm^2) or moderate (10^3 CFU/cm^2). At low levels (10 CFU/cm^2) the surviving numbers decreased below the detection limit (4 CFU/100cm^2) within 2 days. *S.* Enteritidis was recovered from surfaces for at least 4 days at high contamination levels, but at moderate level the numbers decreased to the detection limit within 24 hours and at low level within 1 hour. *C. jejuni* was the most susceptible to slow-air-drying on surfaces; at high contamination levels the numbers decreased below the detection limit within 4 hours. The test microorganisms were readily transmitted from the wet sponges to the stainless steel surfaces and from these surfaces to the cucumber and chicken fillet slices, with the transfer rates varied from 20% to 100%. This study has highlighted the fact that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which a model of risks assessment in domestic setting could lead.

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Introduction

The importance of contaminated surfaces in relation to potential transmission of pathogens to food is apparent in food processing, catering and the domestic environment. Exposure of pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles. Some bacteria attach to surfaces as their predominant form of survival in nature and man-made ecosystems (72). Several studies indicated that various bacteria, including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp., survive on hands, sponges/cloths, utensils and currency for hours or days after initial contact with the microorganisms (51, 63, 113). In some other studies, the extent of bacterial survival and cross-contamination between hands and foods or various kitchen surfaces have been quantified (17, 81, 139). Dufrenne et al. (26) found that the most likely mode of infection of *Salmonella* or *Campylobacter* in The Netherlands was cross-contamination directly from raw poultry or indirectly via contaminated surfaces or niches in the household kitchen to ready-to-eat products. Early studies also indicated that cross contamination, from raw products via hands, cleaning cloths or sponges and utensils to foods not subjected to further cooking, contributed to the occurrence of outbreaks of food-borne salmonellosis in the United States (13). Quantifying the cross-contamination risk associated with various steps in the food preparation process can provide a scientific basis for risk management efforts in both home and food service kitchens (17).

The kitchen surfaces are a focal point in the kitchen for the preparation of food. Stainless steel has been the material of choice for work surfaces or kitchen sinks for many years because of its mechanical strength, corrosion resistance, longevity and ease of fabrication (39). Modern domestic sink and work top material such as polycarbonate, mineral resin and some enamelled steels would be as cleanable as stainless steel when new, but stainless steel, due to its resistance to abrasion or impact damage, is more likely to retain its hygienic properties throughout a domestic working life (39, 118). However, although food particles are usually cleaned from the surface when good hygienic practices are applied, bacteria attached to these surfaces are not visible to the eye and may therefore not be removed. In this study the survival of *Salmonella* Enteritidis, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces was determined. The transfer of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods was studied as to provide cross-contamination data for quantitative microbial risk assessments in domestic setting. The effect of food residues on the survival on surfaces was also investigated. Prior to the survival experiments, the efficiency of the sampling technique using contact plates was examined, in which *Bacillus cereus* spores were used in addition to the test microorganisms described before. The recovery of the pathogens from the surfaces using a single contact plate, as described by the standard procedure, was compared to the total recovery using five consecutive contact plates on the same surface area.

Materials and methods

Surfaces

Stainless steel (AISI type 304 standard, ODS, Barendrecht, the Netherlands) surfaces were prepared in two sizes: 20 x 20 cm^2 for survival experiments and 50 x 80 cm^2 for cross-contamination studies. Before use, the surfaces were disinfected with approximately 800 ppm hypochlorite solution (Glorix, Lever, The Netherlands) for 15 minutes; surfaces of 20 x 20 cm^2 were soaked and surfaces of 50 x 80 cm^2 were wiped. The surfaces were then washed with hot water with anionic-active detergent and rinsed with hot water. Prior to the artificial contamination the surfaces were sprayed with 70% (vol/vol) ethanol and subsequently dried.

Test suspensions and growth conditions

Salmonella Enteritidis (phage type 4, chicken product isolate) and *Staphylococcus aureus* 196E (human isolate, enterotoxin A producer) were obtained from the National Institute of Public Health and the Environment, The Netherlands (Rijksinstituut voor Volksgezondheid en Milieu, RIVM). *Campylobacter jejuni* (NCTC 81116) was from our culture collection and *Bacillus cereus* spores (Bacto cereus spore suspension) were obtained from Difco laboratories, Detroit, USA.

The stock cultures were maintained at -80 ^oC in cryo vials (Greiner Bio-one GmbH, Frickenhausen, Germany) containing a stationary-phase culture suspension in Brain Heart Infusion (BHI; Difco, Becton Dickinson, Maryland, USA) broth with 25% (vol/vol) glycerol (Fluka-chemica, Buchs, Switzerland) and glass beads (ø 2 mm, Emergo, Landsmeer, The Netherlands). Strains were cultured by transferring one glass bead to 10 ml of BHI broth followed by incubation for 20-22 h at 37^oC for *S.* Enteritidis and *S. aureus*, and for 40-48 h micro-aerobically (6% O_2) obtained by an Anoxomat-System (type WS9000, MART®, Lichtenvoorde, The Netherlands) at 42^oC for *C. jejuni*. *B. cereus* spores were used directly from the ampoules.

The test suspensions were prepared by making serial dilutions of the microorganisms in peptone saline solution (PSS: NaCl (Merck, Darmstadt, Germany) 8.5 g/l and Neutralised Bacteriological Peptone (Oxoid, Basingstoke, England) 1 g/l). For the final dilution, saline solution containing 0.1% Tween 80 (SS-0.1% Tween 80 (Merck, Hohenbrunn, Germany)) was used in order to obtain an equal spread of bacteria on the surface. Three different contamination levels were prepared: high contamination (approximately 10^7 colony forming units (CFU)/100cm^2), moderate contamination (approx. 10^5 CFU/100cm^2) and low contamination (approx. 10^3 CFU/100cm^2), obtained by spreading 1 ml of an appropriate solution on a surface of 20 x 20 cm^2 or 10 ml of an appropriate solution on a surface of 50 x 80 cm^2.

Selective agar media were used for the enumeration of pathogens: Mannitol Egg Yolk Polymixine Agar (Merck, Darmstadt, Germany) for *B. cereus*, incubated for 18-40 h at 30^oC; Mannitol Lysine Crystal Violet Brilliant Green Agar (Oxoid, Basingstoke, England) for *S.*

Enteritidis, incubated for 18-24 h at 37°C; Baird Parker Egg Yolk - Tellurite Agar (Oxoid, Basingstoke, England) for *S. aureus*, incubated for 24-48 h at 37°C; and Columbia-Blood-Preston Agar (Columbia agar base (Oxoid, Basingstoke, England) containing 5 % lysed, defibrinated sheep blood (bioTrading Benelux B.V., Mijdrecht, The Netherlands) and modified Preston Campylobacter supplement (Oxoid, Basingstoke, England)) for *C. jejuni*, incubated micro-aerobically for 40-48 h at 42°C.

Recovery of pathogens from surfaces

- Direct contact method using contact plates

The recovery of the viable counts from surfaces was carried out using contact plates (ø 55 mm, Greiner Bio-one GmbH, Glos, UK) with appropriate agar media. Applicator Count-Tact (bioMérieux, Marcy l'Etoille, France) was used during the sampling to obtain a consistent sampling condition of 10 seconds contact time with 500 g pressure per plate. To generate countable numbers at high contamination levels, the agar of the contact plates was suspended in 50 ml or 100 ml sterile PSS and subsequently homogenised in a Stomacher® (type 400 C*irculator*, Seward, Laboratory Blender, England) at 260 rpm for 60 seconds. Appropriate dilutions were spread on the selective media using a spiral inoculation apparatus (Eddy Jet, IUC, Barcelona, Spain).

- Single versus five contact plates on the same area

This experiment was carried out to examine whether any bacteria were still present on the surface after sampling using a single contact plate. The surfaces were contaminated with low numbers to obtain countable numbers on the contact plates. Therefore, 1 ml of the test suspension of an appropriate dilution was put on 20 x 20 cm^2 stainless steel surface to obtain a concentration of approximately 10^3 CFU/100cm^2. For *B. cereus* spores, the level of contamination was approximately 10^2 CFU/100cm^2. Polyester fiber-tipped applicator swabs (Falcon ™, Becton Dickinson, Sparks, USA) were used to spread the test suspensions over the surface.

The surface was sampled immediately after artificial contamination using a contact plate as described above and subsequently with four other contact plates. The recoveries of pathogens using a single plate were compared to the recoveries using five plates.

Survival of pathogens on stainless steel surfaces

The effects of different contamination levels on the survival of pathogens on dry surfaces for an extended period of time were investigated. For *S.* Enteritidis and *S. aureus,* three different contamination levels on 20 x 20 cm^2 surface area were examined: high, moderate and low levels. For the survival experiment of *C. jejuni* two levels were examined: high and moderate. The test suspensions were prepared as described before.

The contaminated surfaces were placed in a laminar hood without airflow at room temperature (22-25°C, 40-45 % RH). The viable counts were determined using a single

contact plate as described above immediately after contamination and at different time intervals for an extended period of time.

For the determination of the effect of food residues on the survival of pathogens, the surfaces were contaminated with 1 ml of pathogen suspension in commercial sterilised milk or a suspension of raw chicken breast fillet (obtained from local retail supermarket). The suspension of raw chicken breast fillet was negative for selected pathogens, enumerated using appropriate selective media. The pathogen suspensions were prepared by diluting the cultures in PSS as described previously, but for the final dilutions 9 ml of commercial sterilised milk or a suspension of raw chicken breast fillet in PSS (1: 9, blended at 260 rpm for 60 seconds) were used. These experiments were carried out using low contamination levels.

Cross-contamination via sponges and surfaces to foods

This experiment was carried out to determine the transfer rates of the pathogens from artificially contaminated sponges to stainless steel surfaces and subsequently from these surfaces to the foods. Kitchen sponges (Lola, 9cm x 7cm x 3cm; Nedac, Duiven, The Netherlands) were artificially contaminated with 10 ml of an appropriate dilution of bacterial suspension and used to wipe 50 x 80 cm^2 stainless steel surfaces. The contaminated surfaces were placed in a laminar hood without airflow at room temperature (22-25^oC, 40-45 % RH).

To simulate subsequent contamination of food, slices of cucumber or roasted chicken fillet ($\pm$ 0.5 cm thick, approximately 25 cm^2) were put on the surface for 10 seconds, with and without pressure of 500 g per slice. The numbers transferred to the food were determined by suspending the food in 50 ml sterile PSS and subsequently homogenising in a Stomacher® for 60 seconds at 260 rpm. Appropriate dilutions were spread on the selective media using a spiral inoculation apparatus. The levels of bacteria on the surfaces were investigated using a single contact plate as described above.

The transfer rates to cucumber or roasted chicken fillet were determined immediately after artificially contamination and 15 minutes after contamination. The transfer rates were calculated based on the numbers of microorganisms present on surfaces that were recovered by a single contact plate, using the next formula:

% transfer rate = N_f/N_s x 100%

where N_f = CFU recovered from food

N_s = CFU on surfaces recovered by contact plate

Scanning Electron Microscopy

To visualise the arrangement of the cells on the dry surfaces, stainless steel (1.5 x 1.5 cm^2) specimens were prepared. These surfaces were contaminated with pathogens as described for the survival experiments and with pathogens suspended in water. After the exposure to room temperature for particular periods of time they were sputter-coated with 10 nm platinum and viewed in a JSM-6300F Scanning Electron Microscope (JEOL, Massachusetts, USA).

Statistical analyses

Each experiment, except scanning electron microscopy, was repeated at least three times at different days and no less than two replicates were used in each experiment. Data analyses were performed on the SPSS for Windows 95/98/NT/2000, release 10.1. A p-value of < 0.05 was considered statistically significant.

Results and discussion

Recovery using contact plates

The recoveries of *S.* Enteritidis*, S. aureus, C. jejuni* and *B. cereus* spores from surfaces using contact plates are shown in Table 2.1. The recoveries were calculated based on the contamination numbers that were applied on surfaces. Approximately 10% to 20% of spores or cells were removed during the spreading of the test organisms on the surfaces by the swabs (data not shown).

The recovery was dependent on the test organism ($p=0.01$). A single contact plate recovered 18% of the *B. cereus* spores applied on the surface. Since spores may survive on dry conditions for a long period of time (54), slowly drying of surfaces will hardly affect the numbers of viable spores. The difficulty of detachment was probably due to the ability of the spores to attach to the surfaces. It has been found that the spores of *B. cereus* adhered to stainless steel surfaces better than vegetative cells (54). The recovery of *S.* Enteritidis by a single contact plate with this technique was 23%. Although *S.* Enteritidis, being Gram-negative, might be sensitive to drying on surfaces, similar recovery to *B. cereus* spores was obtained. The lowest recovery was found for *C. jejuni* (7%), indicating that this strain was likely to be susceptible to the experimental conditions: dry surfaces and direct exposure to the air (41). *S. aureus* was recovered in higher numbers (46%) than the other test organisms, probably due to its clump-like structure that may provide a higher chance of detaching more cells during sampling.

The recovery using a single contact plate represented 50 to 60% of the recovery using five consecutive plates. This result indicated that after the first sampling bacteria were still found on surfaces. By further sampling on the same spot the bacteria were recovered in decreasing numbers (Figure 2.1). Tebbutt (122) suggested that this probably influenced by whether the bacteria have formed clumps on the surfaces. Since there was no abrasion during the sampling, clumps were not broken and organisms were not released easily from the test surface (109). However, the scanning electron micrographs showed no clumps of bacteria on the surfaces contaminated with low levels (10^3 CFU/100cm^2), except for *S. aureus* that forms clump-like structures in vivo as well (results not shown). This indicated that the cells were spread over the surfaces. The contact method is easy to use, but it might have limiting factors as it is based on the detachment of microorganisms from surfaces. The cells or spores that adhered to the surfaces would not be detached easily. Some bacteria have been found to be able to adhere to the stainless steel surfaces after short contact times (75).

Table 2.1. Recovery of pathogens from stainless steel surfaces using a single contact plate and five consecutive plates immediately after contamination

Organism	n	Contamination (CFU/cm^2)	Recovery count numbers		Recovery percentages[a]		Comparison of recovery using single and 5 plates[b] (%)
			Single plate (CFU/cm^2)	Five plates (CFU/cm^2)	Single plate (%)	Five plates (%)	
B. cereus spores	50	4 ± 0	1 ± 0	1 ± 1	18 ± 6	33 ± 11	55 ± 8
S. aureus	10	12 ± 4	5 ± 2	10 ± 3	46 ± 14	88 ± 26	52 ± 3
S. Enteritidis	14	50 ± 14	11 ± 2	20 ± 5	23 ± 6	42 ± 12	57 ± 5
C. jejuni	10	119 ± 22	8 ± 4	17 ± 8	7 ± 3	14 ± 7	49 ± 4

n indicates the number of experiments; with two parallel samplings for each experiment

$\pm$ indicates the standard deviation

[a] Calculated as ((counts of single plate/contamination) x100%) or ((counts of 5 plates/contamination) x 100%)

[b] Calculated as ((counts of single plate/counts of 5 plates) x 100%)

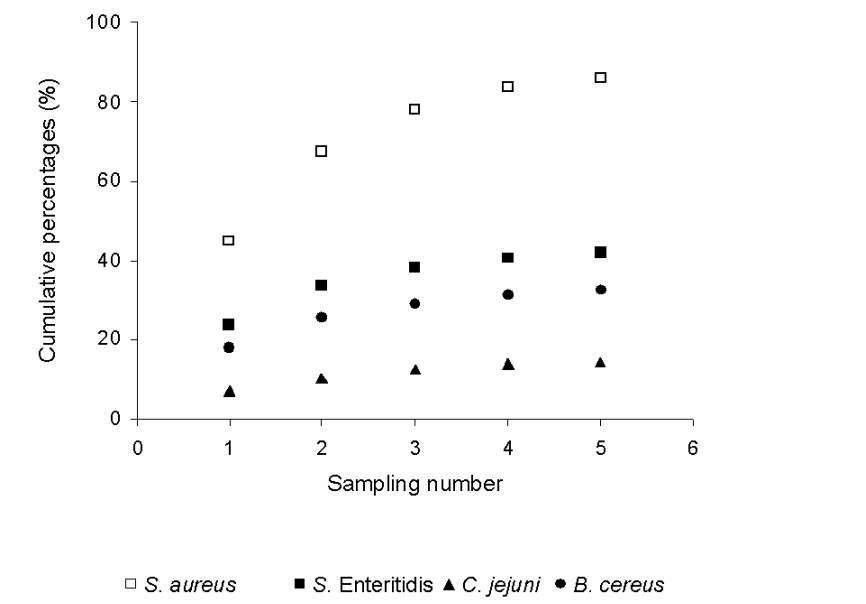

Figure 2.1. Cumulative percentages of microorganisms recovered from stainless steel surfaces on the same spot using five consecutive contact plates. The data obtained from 10 experiments for *S. aureus* (□), 14 experiments for *S.* Enteritidis (■), 50 experiments for *B. cereus* (●) and 10 experiments for *C. jejuni* (▲).

Niskanen and Pohja (90) indicated that the contact plate method was suitable for a flat, firm surface, considering both recovery and repeatability, whereas swabbing was better for flexible and uneven surfaces and for heavily contaminated surfaces. However, for heavily contaminated surfaces contact plates may be used for sampling, if the agar is homogenised and subsequently the bacterial content of the homogenate is examined (Baumgart and Kussmann, 1975 in Niskanen and Pohja (90)). During quantitative assessment of bacteriological contamination in domestic settings, the contact plate method was satisfactory for differentiation of hygiene level at environmental sites whilst facilitating the handling of large numbers of samples in a field survey (115). Both the contact method and the swab method are based on the detachment of surface-bound microorganisms. Validation and comparison studies on the recovery of microorganisms from the test surface by swabbing, contact agar plate, and Hygicult TPC dipslide, with a known microbial load spread onto the test surface, indicated that the method did not differ in practical terms either in yield or in precision (107). The sampling methods recovered 25-30% at the lowest, 18-20% at the middle and 16-21% at the highest levels of microorganisms from the test surfaces, with contamination numbers of 1.4 CFU/cm^2, 10.7 CFU/cm^2 and 43.6 CFU/cm^2 respectively.

Our study showed that the first contact plate sampled the fraction of cells that were easily removed. Since it has been known that a single contact plate represents 50 to 60 % of the total recovery, this technique was used in further experiments. This procedure was considered to be similar with the transfer process of microorganisms from surfaces to food products.

Survival on stainless steel surfaces

The survival of *S.* Enteritidis, *S. aureus* and *C. jejuni* on stainless steel surfaces is indicated in Figure 2.2. Three different contamination levels were used for the experiments with *S.* Enteritidis and *S. aureus,* whereas two initial levels were examined for *C. jejuni*.

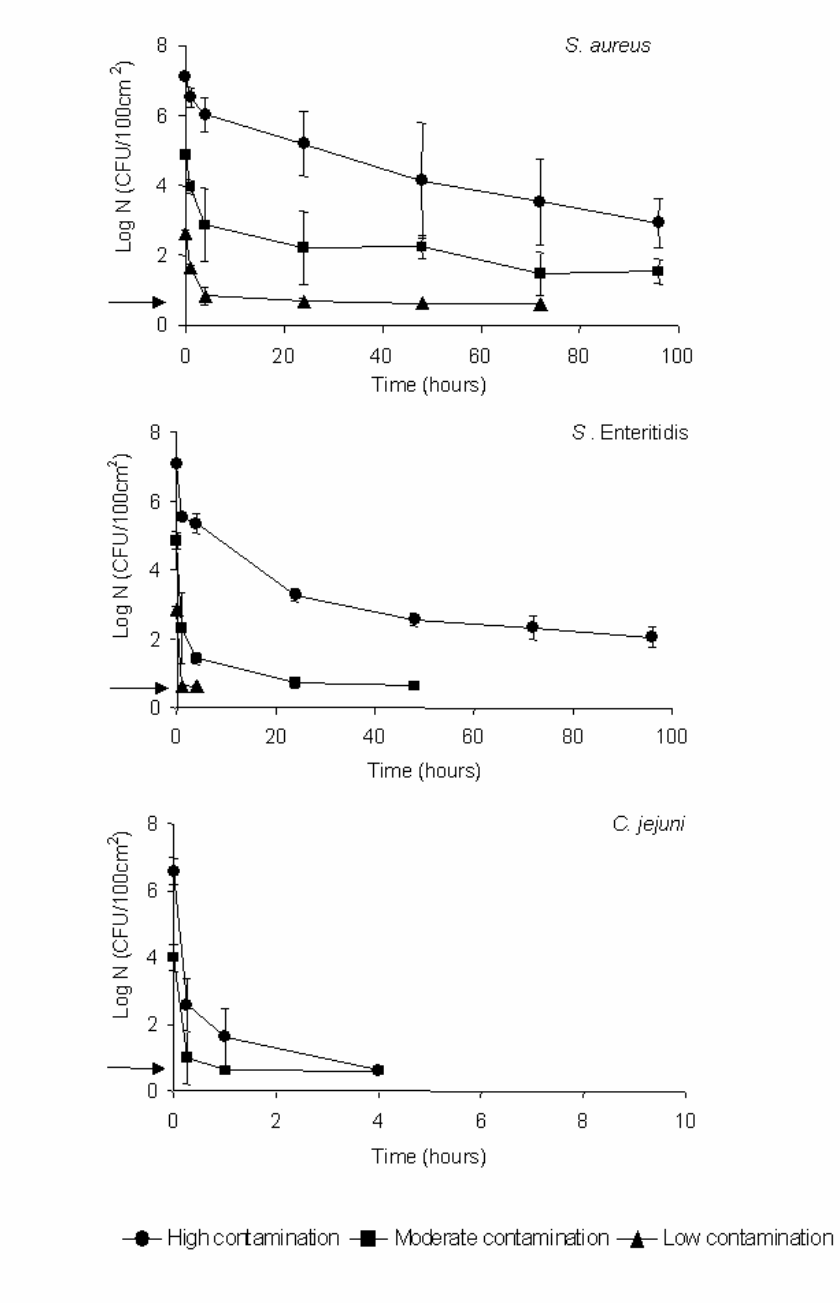

Figure 2.2. Survival of pathogens on stainless steel surfaces at room temperature (22-25^{o}C, 40-45% RH) at different contamination levels; (●) high, approximately 10^7 CFU/100cm^2; (■) moderate, approximately 10^5 CFU/100cm^2 and (▲) low approximately 10^3 CFU/100cm^2. Experiments with *C. jejuni* were carried out only at high and moderate contamination levels. The arrow bar (→) indicates the detection limit of sampling using a single contact plate (1 CFU/plate, correlated with 0.62 log CFU/100cm^2). The bars indicate the standard deviation from 3 experiments.

The results indicated that the survival of bacteria decreased rapidly, especially when the initial numbers on the surfaces were low. *S. aureus* could be detected on dry surfaces for at least 96 hours at high and moderate contamination levels, while at low levels the cells decreased below the detection limit (log cells numbers (N) = 0.62 CFU/100cm^2) within 48 hours after contamination. The surviving bacteria decreased rapidly within 4 hours, in which the cell numbers declined for about 1 or 2 log units, dependent on the initial levels of contamination. For *S.* Enteritidis the viable cells could still be detected after 96 hours when a high initial level was present, but at moderate initial numbers the amount of the surviving cells decreased within 24 hours below the detection limit. At low contamination levels, the count of *S.* Enteritidis decreased within 1 hour below the detection limit. The survival of these bacteria also decreased rapidly within 4 hours, with a 1.7 log reduction and 3.4 log reduction when high and moderate initial levels were present respectively. After 4 hours the cell numbers declined less rapidly. *C. jejuni* was the most susceptible to air-drying on surfaces at room temperature, since the results indicated that at high contamination levels, a 5-log unit reduction was obtained and the numbers decreased below the detection limit within 4 hours. For this reason the experiments at low levels were not carried out for *C. jejuni*.

The characteristics of an organism and its surrounding environment are among the important factors that may affect the survival of bacterial cells on surfaces. The presence of particular surface structures such as flagella, pili, and extracellular polysaccharides have been suggested to affect the adhesion and survival of bacteria (54). Scanning electron micrographs of surfaces that were contaminated with high levels of bacteria in water suspension show clumps of bacteria (results not shown). Clumps structure might form some protection to the innermost cells against drying (122). The scanning electron micrographs demonstrated also that some cells were found in crevices on the stainless steel surfaces.

The food residues on the surfaces improved the survival of pathogens (data not shown). When a low level of *S.* Enteritidis in saline solution was applied to surfaces, the numbers decreased 2.2 log units after 1 hour. In the presence of milk residues the number of *S.* Enteritidis decreased 1.0 log unit, whereas in the presence of chicken fillet suspension, the numbers decreased 1.9 log units. Since chicken fillet was suspended in peptone saline solution (1: 9), this suspension might contain less nutritional compounds than the milk, resulting in less protection on the survival on surfaces. Residues of chicken fillet suspension improved the survival of *C. jejuni* on the surfaces as well. The rate of decreasing survival on surfaces was reduced from 5.0 log unit reduction to 4.4 log unit reduction after 1 hour. The food residues, while slowly drying, probably formed a layer that might protect the cells on surfaces, resulting in a prolonged survival.

Our study has highlighted the fact that pathogens may remain viable on dry stainless steel surfaces and present a (re)contamination hazard for considerable periods of time. The presence of residual foods on these surfaces may have an important role as it may improve the survival.

Cross-contamination via sponges and stainless steel surfaces

The risk of cross-contamination during regular domestic cleaning is important since kitchen sponges were found to be potential vehicles of pathogens in domestic kitchens (38) and pathogens were able to survive in kitchen sponges for at least weeks (63). In this study the transfer rates of pathogens from artificially contaminated sponges to stainless steel surfaces were investigated (Table 2.2). The sponges that were contaminated with 10 ml of pathogen suspension, resulting in moisture contents of 50-65% (w/w), were used to wipe the surfaces and the transmission was determined directly after this artificial contamination. The transfer rates (21% to 43%) were not dependent on the test microorganisms (p= 0.07) or on the contamination levels (p=0.30). After the transmission process, some bacteria remained on the sponges (10^5 to 10^6 CFU/sponge, data not shown), which are also indicated by the transfer rates that were less than 50%. These residual bacteria could still be harboured and transferred during subsequent use of the sponge.

The transfer rates of pathogens from stainless steel surfaces to cucumber slices and to roasted chicken fillet slices are shown in Table 2.3 and Table 2.4 respectively. The transfer rates were based on the numbers of microorganisms that were recovered from surfaces using a single contact plate, which represents 50-60 % of total recovery using five consecutive plates. This resulted, for some cases, in transfer rates of more than 100% when bacteria were found more on food slices than on contact plates. When the transfer rates were determined based on the contamination numbers that were applied on the surfaces, the values were less than 100% (data not shown).

According to the statistical analysis, the moment of sampling (immediately or 15 minutes after contamination) did not affect the transfer rate of pathogens to cucumber (p=0.26 with pressure, p=0.46 without pressure), and to roasted chicken fillet when no pressure was applied (p=0.84). The transfer rate to roasted chicken fillet was dependent on the moment of sampling when pressure was applied (p=0.02). Furthermore, the type of microorganisms did not influence the transfer rates to roasted chicken fillet slices (with pressure p=0.77, without pressure p= 0.52) and to cucumber slices when pressure was applied (p=0.06). When a cucumber slice was placed on a contaminated surface without pressure, the transfer rate was dependent on the type of microorganism (p=0.00). However, from practical point of view, cross-contamination clearly took place from surfaces to food slices, with or without pressure.

Overall, the transfer rates varied from 50% to more than 100% for cross-contamination to cucumber slices, and from 25% to 100% for transmission to roasted chicken fillet slices. Roasted chicken fillet slices contained less moisture than cucumber and consisted of some fat. The moisture contents of cucumber surfaces might positively affect the ease of retrieval of bacteria from surfaces.

Table 2.2. Transfer of pathogens from sponges[a] to stainless steel surfaces immediately after contamination

Organism	n	Contamination (Log CFU/10ml)	Count numbers		Transfer rate
			Sponges (Log CFU/sponge)	Surfaces[b] (Log CFU/4000cm^2)	Sponges to surfaces (%)
S. aureus	3	High (8.8 ± 0.2)	9.0 ± 0.2	8.6 ± 0.2	38 ± 12
	6	Moderate (6.7 ± 0.1)	6.8 ± 0.1	6.4 ± 0.2	41 ± 17
S. Enteritidis	3	High (9.3 ± 0.1)	9.4 ± 0.2	8.8 ± 0.2	29 ± 23
	6	Moderate (7.3 ± 0.1)	7.3 ± 0.0	6.6 ± 0.2	21 ± 8
C. jejuni	3	High (9.4 ± 0.1)	9.4 ± 0.1	9.0 ± 0.0	43 ± 10
	6	Moderate (8.5 ± 0.1)	8.4 ± 0.1	7.8 ± 0.1	28 ± 13

n indicates the number of experiments; with two parallel sampling for each experiment

± indicates the standard deviation

[a] The sponges were artificially contaminated with 10 ml of appropriate pathogen suspension

[b] Sampled using a single contact plate

Table 2.3. Transfer of pathogens from stainless steel surfaces[a] to cucumber (n= 6) with or without pressure of 500g/slice

Organism	*Moment of sampling*	*Count numbers*			*Transfer rate*	
		Surface[b] (Log CFU/cm^2)	Cucumber		Surface to cucumber	
			Pressure (Log CFU/cm^2)	No pressure (Log CFU/cm^2)	Pressure (%)	No pressure (%)
S. aureus	Direct after contamination	2.8 ± 0.2	2.9 ± 0.1	2.8 ± 0.1	117 ± 48	95 ± 30
	15 min. after contamination	2.9 ± 0.1	2.8 ± 0.3	2.7 ± 0.2	100 ± 59	74 ± 41
S. Enteritidis	Direct after contamination	3.0 ± 0.2	3.0 ± 0.2	2.8 ± 0.2	105 ± 26	65 ± 21
	15 min. after contamination	3.1 ± 0.3	3.0 ± 0.3	2.8 ± 0.3	90 ± 27	50 ± 18
C. jejuni	Direct after contamination	4.2 ± 0.2	4.4 ± 0.1	4.4 ± 0.1	185 ± 75	177 ± 72
	15 min. after contamination	3.8 ± 0.5	3.7 ± 0.8	3.9 ± 0.5	134 ± 89	153 ± 99

n indicates the number of experiments; with two parallel sampling for each experiment

± indicates the standard deviation

[a] The surfaces were contaminated with artificially contaminated sponges with moderate contamination level (Log CFU/sponge); *S. aureus* (6.8 ± 0.1), *S.* Enteritidis (7.3 ± 0.0), *C. jejuni* (8.4 ± 0.1)

[b] Sampled using a single contact plate

Table 2.4. Transfer of pathogens from stainless steel surfaces[a] to roasted chicken fillet (n=3) with or without pressure of 500g/slice

Organism	Moment of sampling	Count numbers			Transfer rate	
		Surface[b] (Log CFU/cm^2)	Roasted chicken fillet		Surface to roasted chicken fillet	
			Pressure (Log CFU/cm^2)	No pressure (Log CFU/cm^2)	Pressure (%)	No pressure (%)
S. aureus	Direct after contamination	2.9 ± 0.2	2.8 ± 0.1	2.7 ± 0.1	76 ± 36	62 ± 28
	15 min. after contamination	2.9 ± 0.2	2.8 ± 0.0	2.7 ± 0.0	74 ± 17	56 ± 20
S. Enteritidis	Direct after contamination	3.1 ± 0.3	3.1 ± 0.2	2.8 ± 0.1	94 ± 42	49 ± 21
	15 min. after contamination	3.0 ± 0.0	2.8 ± 0.4	2.9 ± 0.0	55 ± 21	32 ± 9
C. jejuni	Direct after contamination	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.1	101 ± 42	66 ± 26
	15 min. after contamination	3.7 ± 0.4	3.4 ± 0.2	3.5 ± 0.4	24 ± 16	70 ± 83

n indicates the number of experiments; with two parallel sampling for each experiment

± indicates the standard deviation

[a] The surfaces were contaminated with artificially contaminated sponges with moderate contamination level (Log CFU/sponge); *S. aureus* (6.8 ± 0.1), *S.* Enteritidis (7.3 ± 0.0), *C. jejuni* (8.4 ± 0.1)

[b] Sampled using a single contact plate

The level of pressure used in our study, i.e. 500 g per slice (approx. 20 g/cm^2) was comparable to the pressure applied during sampling with the contact plates. Furthermore, the application of pressure in this experiment was to simulate the regular practice during food preparation in the kitchen. This pressure, however, was lower than that applied by Sattar et al. (108) who used a pressure of 0.2 kg/cm^2 with a contact time of 10 s to study the bacterial transfer from fabrics to hands and other fabrics. It was found that bacterial transfer from moist donor fabrics using recipients with moisture was always higher than that to and from dry ones (108).

Chen et al. (17) found that bacterial transfer rates varied by more than five orders of magnitude (0.0005% from hand to spigot, to 100% from chicken to hand) depending on the nature of the surfaces involved in the cross-contamination. A transfer rate of 100% was identified as the maximum value, although in some cases it was found that the recipients demonstrated higher numbers of microorganisms than the contributor of contamination. The least variability was observed for cutting board-to-lettuce cross-contamination, which was likely due to the relatively homogeneous surface of the cutting board. Our study resulted in less variability of the transfer rates due to the semi-controlled experimental conditions.

Cogan et al. (19) indicated that after meal preparation with *Salmonella*-contaminated chickens, *Salmonella* counts of more than 10^3 CFU/5cm^2 were found on 12.5% of the chopping boards. This level is comparable with the moderate contamination applied in our survival experiments. If it is assumed that the same numbers are present on stainless steel work surfaces, based on our results, the surviving numbers would be reduced to 10 CFU/5cm^2 after 1 hour. If foods are prepared on these surfaces, with a transfer rate of 30% and 50% to respectively roasted chicken fillet and cucumber slices (without pressure), the numbers of *Salmonella* would be 15 CFU and 25 CFU respectively, on 25cm^2 food slices. These numbers could represent a potentially infective dose, particularly when growth is taking place, since current estimates suggest that the infectious dose for *Salmonella* may be up to 10^6 but could be as low as 10 to 100 cells (19).

The risk of food-borne infection associated with cross-contamination depends on two factors: the level of contamination on the surfaces and the probability of its transfer to the foods being consumed (8). Our study has shown that *S.* Enteritidis, *S. aureus* and *C. jejuni* were still viable on dry stainless steel surfaces for hours (*C. jejuni*) or days after contamination (*S.* Enteritidis and *S. aureus*), dependent on their initial numbers. These pathogens were readily transferred from kitchen sponges to stainless steel surfaces and from these surfaces to the foods. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which risk assessment in domestic setting could lead to reduce, prevent or eliminate cross-contamination in the kitchen.

Chapter 3

Tolerance of *Salmonella* Enteritidis and *Staphylococcus aureus* to surface cleaning and household bleach

Abstract

The importance of effective cleaning and sanitizing of food preparation sites is apparent, since pathogens are readily spread to food contact surfaces after preparation of contaminated raw products. Tolerance of *Salmonella* Enteritidis and *Staphylococcus aureus* to surface cleaning by wiping using regular, micro-fiber and antibacterial-treated cloths was investigated. Wiping of surfaces using cleaning cloths resulted in a considerable reduction of microorganisms from surfaces, despite the fact that it was more difficult to remove *S. aureus* than *S*. Enteritidis. Depending on the cloth type, *S. aureus* were reduced from initial numbers of approximately 10^5 CFU/100cm^2 to count numbers ranging from less than 4 CFU/100cm^2 (below the detection limit) to 100 CFU/100cm^2 on surfaces. Directly after they were used to clean the contaminated surfaces, the cloths contained high numbers of bacteria (10^4 to 10^5 CFU/100cm^2), except for disposable antibacterial-treated cloths, where no bacteria could be detected. The tolerance of these pathogens to sodium hypochlorite was studied in the suspension test and in cloths. *S. aureus* showed a better tolerance against sodium hypochlorite than *S*. Enteritidis. Inactivation of microorganisms in cloths required a higher concentration of sodium hypochlorite than was needed in the suspension test. Repeated exposure to sodium hypochlorite, however, resulted in increase of susceptibility against this compound. This study provides essential information concerning bacterial transfer during wiping of surfaces and highlights the need of sufficient hygiene procedures considering the use of cleaning cloths in household environments to avoid cross-contamination.

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Introduction

Cross-contamination via contaminated equipment and poor personal hygiene during food preparation contributes to transmission of infectious disease (94). Pathogens are readily spread to food contact surfaces and cleaning tools after preparation of contaminated raw products (18, 23, 24). Proper cleaning and sanitizing of the kitchen sites is suggested to prevent the spread of microorganisms and cross-contamination to ready to eat food. This notion has led to promoting several commercial household sanitizers, disinfectants and cleaning cloths. A variety of active chemical agents are included in these products, many of which have been used for years in antiseptics, disinfectants or preservation products (80).

Under normal conditions, cleaning is adequate for household situations, but in some circumstances such as a diseased family member or the handling of potentially contaminated food, disinfection may be indicated (56). Sodium hypochlorite (household bleach) is widely recommended, particularly in the United States, for sanitizing of kitchen sites because of its biocidal activity at very low levels and its availability. In The Netherlands, approximately 10% of households used sodium hypochlorite to decontaminate kitchen work surfaces and dishcloths (132). The biocidal activity of sodium hypochlorite is dependent on some factors, such as chlorine concentration, pH, temperature, and presence of organic material. When disinfection needs to be applied, correct use is needed to achieve an optimal effect on the reduction of microorganisms. Laboratory studies have shown that adaptation to disinfectants can occur when microorganisms are exposed to sublethal concentrations of disinfectant (74). However, little is known about the effect of repeated challenge of sodium hypochlorite to pathogens in cloths.

In this study, the performance of antibacterial-treated cloths was investigated in comparison to regular and micro-fiber cloths with respect to reduction and/or elimination of *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) and *Staphylococcus aureus* from artificially contaminated surfaces. *S.* Enteritidis, a Gram-negative bacterium, is the most important cause of *Salmonella*-infections in Europe (136) and the United States (93) in the last five years. Being among the longest recognized of Gram-positive pathogenic bacteria, *S. aureus* is frequently found in domestic kitchen areas, in particular on dry surfaces. The effects of household sodium hypochlorite on these bacteria were determined in suspension test and in artificially contaminated cloths. Repeated exposure to sodium hypochlorite was studied in cloths. In addition, the efficacy of this product to reduce normal flora of household cloths was also determined.

Materials and methods

Bacterial strains and growth conditions

Two foodborne pathogens, *Salmonella* Enteritidis (phage type 4, chicken product isolate) and *Staphylococcus aureus* (196E toxin producer, human isolate), were obtained from the

National Institute of Public Health and the Environment, The Netherlands. The stock cultures were maintained at -80 ^{o}C in cryo vials (Greiner Bio-one GmbH, Frickenhausen, Germany) containing a stationary-phase culture in Brain Heart Infusion (BHI; Difco, Becton Dickinson, Maryland, USA) broth with 25% (vol/vol) glycerol (Fluka-chemica, Buchs, Switzerland) and glass beads (ø 2 mm, Emergo, Landsmeer, The Netherlands). Strains were cultured by transferring one glass bead to 10 ml of BHI broth, followed by incubation for 18-20 h (overnight) at 37^{o}C. Series of dilutions were prepared in peptone saline solution (PSS: NaCl 8.5 g/l and Neutralized Bacteriological Peptone (Oxoid, Basingstoke, England) 1 g/l). Tryptone Soy Agar (TSA, Oxoid) was used for enumeration of the test bacteria. In some cases, selective media were used, Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB, Oxoid) for *S.* Enteritidis and Baird Parker Egg Yolk - Tellurite Agar (BP, Oxoid) for *S. aureus*. All plates were incubated for 24 h at 37^{o}C.

Performance of regular and sanitizer-treated cloths

Five different cloths from local supermarkets, one disposable and four re-usable, listed in Table 3.1, were used. The antibacterial effect of the cloths was initially studied using the agar diffusion test. The cloths were cut in pieces with a diameter of approximately 1 cm, and placed on freshly prepared TSA surfaces that were inoculated with appropriate dilutions of overnight cultures. For the re-usable cloths, both new and used cloths were examined. The used cloths had been used once in a previous experiment, washed with hot water with anionic-active detergent, rinsed with water and allowed to dry at room temperature. The inhibition zones were measured after incubation for 24 h at 37^{o}C.

The performances of the cloths on cleaning and/or decontamination of surfaces were investigated on stainless steel (AISI type 304 standard, ODS, Barendrecht, the Netherlands) surfaces, placed in a laminar hood without airflow at room temperature (22-25^{o}C, 40-45 % RH). Surface areas of 50 x 80 cm^{2} were used. The surfaces were disinfected with 800 ppm of sodium hypochlorite solution (obtained from a local supermarket) for 15 minutes, washed with hot water with anionic-active detergent and rinsed with hot water. Prior to the artificial contamination the surfaces were sprayed with 70% (vol/vol) ethanol and subsequently dried. Ten-ml of an appropriate culture dilution was applied on the surface, to obtain initial counts of approximately 10^{5} to 10^{6} CFU/100cm^{2}, and spread using a kitchen sponge (Lola, Nedac, Duiven, the Netherlands). Approximately 15 minutes after contamination, each surface was wiped with the cloth in two directions, from left to right in a downward movement with a relatively uniform pressure, as applied during daily wiping. The surfaces were then sampled using contact plates (ø 55 mm, Greiner Bio-one GmbH, Glos, UK) containing TSA, immediately and 15 minutes after wiping. Applicator Count-Tact (bioMérieux, Marcy l'Etoille, France) was used during the sampling to obtain a consistent sampling condition of 10 seconds contact time with 500 g pressure per plate.

The cloth which was used for wiping the surface, was put in a stomacher bag and subsequently homogenized with 50 ml PSS in a Stomacher® (type 400 Circulator, Seward,

Laboratory Blender, England) at 260 rpm for 60 seconds. Appropriate dilutions were spread both on TSA and selective media using a spiral inoculation apparatus (Eddy Jet, IUC, Barcelona, Spain) to determine the bacterial counts of the cloths.

Suspension test

The cell suspension was prepared by centrifuging the overnight BHI cultures for 15 min at 3,660 x *g* and re-suspending in PSS to a concentration ranging between 1 x 10^8 and 1.5 x 10^8 CFU/ml. The effect of commercial household sodium hypochlorite (obtained from a local supermarket) was examined at final concentrations of 200, 300 and 400 ppm using the suspension tests following the European Standard (EN) 1276 (15). The available chlorine concentrations were confirmed by titration (3). Clean as well as dirty conditions are simulated by incorporating low (0.3 g/l) and high (3 g/l) amounts of protein load, respectively, added as bovine serum albumin (Sigma-aldrich, Steinheim, Germany) in the test procedure. The reductions of the log numbers were investigated at 10, 30 and 60 minutes after exposure with hypochlorite.

Effect of sodium hypochlorite on artificially contaminated cloths and repeated exposure experiment

The efficacy of sodium hypochlorite to decontaminate artificially contaminated cloths was investigated using 19 x 20 cm^2 viscose cloths (cloth B). Each cloth was placed in a stomacher bag, 10 ml of an appropriate dilution of an overnight culture were added to obtain initial counts of approximately 10^6 CFU/100cm^2, followed by adding of 10 ml of sodium hypochlorite solution at final concentrations of 500 ppm and 800 ppm. The reductions of the log numbers were investigated after 10, 30 and 60 minutes of exposure at room temperature, by homogenizing each treated cloth with 50 ml PSS in a Stomacher® at 260 rpm for 60 seconds. Appropriate dilutions were spread both on TSA and selective media using a spiral inoculation apparatus.

After sampling, the suspension was wrung out of the cloth by gloved hands in a normal household way and the cloth was then kept at room temperature for 24 hours in a stomacher bag to avoid contamination from the environment. The remaining water in the cloths was 74 $\pm$ 2% (w/w). The next day the cloths were sampled for the second time using the same procedure as described before.

The effect of repeated exposure to sodium hypochlorite was carried out in cloths that were contaminated with pathogens and treated with hypochlorite at final concentration of 650 ppm for 10, 30 and 60 minutes, prepared and sampled as described previously, and kept at room temperature in a stomacher bag.

Table 3.1. Types of cloth used in the study and their antibacterial effect by the diffusion test (n=3)

Code	Type of cloth	Antibacterial agents	Dimension	Inhibition zone (mm)			
				New cloths		Used cloths[b]	
				S. Enteritidis	*S. aureus*	*S.* Enteritidis	*S. aureus*
A	Reusable, micro fiber cloth[a]	-	20x20 cm2	0	0	0	0
B	Reusable, viscose cloth	-	38x40 cm2	0	0	0	0
C	Reusable, sponge cloth	-	18x20 cm2	0	1 ± 0.2	0	0
D	Reusable, sponge cloth	Unspecified	18x20 cm2	7 ± 1	22 ± 7	0	15 ± 6
E	Disposable, wetted napkins	Anionic surfactants	18x18 cm2	0	4 ± 1	n.a.	n.a.

[a] Micro fibers are fibers with a weight/length ratio < 1 decitex (1 decitex = 1 g/10,000m) and adsorb the dirt by their static electricity when used dry or by capillary force when damp (89).

[b] One time used and washed

± indicates the standard deviation

n.a., not applicable

The next day (after 24 hours) the cloths were challenged for the second time to hypochlorite at the same final concentration (650 ppm) for 10, 30 and 60 minutes, following by sampling by homogenizing each treated cloth with 50 ml PSS in a Stomacher® at 260 rpm for 60 seconds as described previously. The procedure was repeated with 500 ppm of hypochlorite solution.

Effect of sodium hypochlorite on naturally contaminated household cloths

Household cotton cloths were collected after approximately a week of use. Each cloth was cut in four pieces, and one piece of this cloth was immediately sampled using the procedure as described above. One-liter hypochlorite solution in tap water at a final concentration of 2400 ppm, as recommended by the manufacturer, was used to wash the other pieces of the cloth for approximately 30 to 60 seconds. Each piece cloth was then removed from the solution, kept in a stomacher bag at room temperature and sampled after 15 and 60 minutes, and after 24 hours. Plate Count Agar (PCA, Oxoid) was used for the enumeration of the total aerobic counts, and Violet Red Bile Glucose Agar (Oxoid) for the total *Enterobacteriaceae*, incubated for 24-48 h at 30^{o}C and for 24 h at 37^{o}C, respectively.

Scanning electron microscopy

In order to visualise the arrangement of the cells on cloths, scanning electron micrographs were prepared. Naturally and artificially contaminated sponge cloths were cut into pieces (0.5 x 0.5 cm^2) and fixed with 3.5% glutaraldehyde (Sigma, Aldrich) in 0.1 M Nacacodilate buffer (Sigma, Aldrich) pH 7.2. The cells were dehydrated once in 10, 30, 50, 70, 96 % ethanol and twice in 100 % ethanol, and critical point dried. Finally, cells were sputter coated with 10-nm platinum and viewed with model JSM-6300F scanning electron microscope (JEOL, USA).

Statistical analyses

Each experiment was carried out three or more times at different days and no less than two replicates were used in each experiment, except for the scanning electron microscopy. Data analyses were performed on SPSS for Windows 95/98/NT/2000, release 10.1. A p-value of < 0.05 was considered statistically significant.

Results

Performance of regular and sanitizer-treated cloths

The re-usable cloths A and B did not show inhibition of *S.* Enteritidis and *S. aureus* with the diffusion agar test (Table 3.1). Cloth C showed a small zone of inhibition on *S. aureus* plates when new (1 $\pm$ 0.2 mm), most likely due to the chemical residues from the production process. Only cloth D, which is available wetted with antibacterial solution, demonstrated a considerable zone of inhibition on *S.* Enteritidis plates (7 $\pm$ 1 mm), but the effect disappeared when the cloths were used once and washed. This cloth D showed the largest inhibition zone

with *S. aureus* (22 ± 7 mm) and the effect was still apparent at a lower level (15 ± 6 mm) when the cloths were used once. The disposable cloth that was available impregnated in chemical solution (cloth E), showed a slight antibacterial effect on *S. aureus* (4 ± 1 mm) but not on *S.* Enteritidis plates.

The performances of the cloths on bacterial removal and/or reduction from artificially contaminated stainless steel surfaces are shown in Table 3.2. In general, the results indicated that *S.* Enteritidis and *S. aureus* were still found on surfaces at a range of 0.6 to 2.0 log units (CFU/100cm^2), when the surfaces had just been cleaned, with the exception of the surfaces cleaned with cloth E. With the latter, the counts of bacteria were below the detection limit (log N < 0.6 CFU/100cm^2). For *S.* Enteritidis, the type and the condition (new or used) of the cloths did not influence the performance (p=0.16 and p=0.09, respectively). In addition, 15 minutes air-drying after wiping resulted in a significant reduction of the bacterial counts of *S.* Enteritidis on surfaces (p=0.00). In contrast, the cloth types did affect the removal or reduction of *S. aureus* (p=0.00), whereas 15 minutes air-drying did not significantly reduce the counts of this bacterium on surfaces (p=0.28). The condition of the cloths also did not influence the performance of the cloths on reduction of *S. aureus* (p=0.64).

Table 3.2a. Counts of *S.* Enteritidis on wiped surfaces[a] and in cloths[b]

Cloth	Condition	n	Counts on surfaces (Log CFU/100cm^2)		Counts in cloths
			Directly after wiping	15 min. after wiping	(LogCFU/100cm^2)
A	New	4	0.8 ± 0.3	0.8 ± 0.3	5.0 ± 0.1
	Used	4	0.6 ± 0.0	< 0.6 ± 0.0	5.4 ± 0.3
B	New	6	1.1 ± 0.4	0.8 ± 0.2	5.3 ± 0.2
	Used	6	1.1 ± 0.3	< 0.6 ± 0.0	5.3 ± 0.1
C	New	4	1.2 ± 0.4	< 0.6 ± 0.0	5.5 ± 0.6
	Used	4	0.9 ± 0.5	< 0.6 ± 0.0	5.1 ± 0.1
D	New	4	1.0 ± 0.4	< 0.6 ± 0.0	4.0 ± 0.3
	Used	4	0.8 ± 0.3	< 0.6 ± 0.0	4.6 ± 0.3
E	New	8	< 0.6 ± 0.0	< 0.6 ± 0.0	< 2.0 ± 0.0

[a] The log initial numbers on surfaces was 5.8 ± 0.6 CFU/100cm^2

[b] The initial numbers of target microorganism in cloths, both new and used, were below the detection limit (log N < 2.0 CFU/100cm^2)

n indicates the number of experiments, ± indicates the standard deviation

The log detection limit on surfaces was 0.6 CFU/100cm^2 and in cloths was 2.0 CFU/100cm^2

Data represent the enumeration on TSA.

Table 3.2b. Counts of *S. aureus* on wiped surfaces[a] and in cloths[b]

Cloth	Condition	n	Counts on surfaces (Log CFU/100cm^2)		Counts in cloths
			Directly after wiping	15 min after wiping	(LogCFU/100cm^2)
A	New	6	1.1 ± 0.7	1.0 ± 0.7	5.3 ± 0.2
	Used	4	1.1 ± 0.5	1.0 ± 0.5	5.1 ± 0.1
B	New	8	1.7 ± 0.9	1.5 ± 0.9	5.3 ± 0.1
	Used	6	2.0 ± 1.0	1.5 ± 0.7	5.2 ± 0.1
C	New	4	1.4 ± 0.2	1.2 ± 0.3	4.1 ± 0.1
	Used	4	1.4 ± 0.3	1.1 ± 0.4	4.1 ± 0.0
D	New	4	0.7 ± 0.2	0.8 ± 0.2	3.4 ± 1.2
	Used	4	1.0 ± 0.5	0.9 ± 0.5	4.1 ± 0.5
E	New	8	< 0.6 ± 0.0	< 0.6 ± 0.0	< 2.0 ± 0.0

[a] The log initial numbers on surfaces was 5.2 ± 0.2 CFU/100cm^2

[b] The initial numbers of target microorganism in cloths, both new and used, were below the detection limit (log N < 2.0 CFU/100cm^2)

n indicates the number of experiments, ± indicates the standard deviation

The log detection limit on surfaces was 0.6 CFU/100cm^2 and in cloths was 2.0 CFU/100cm^2

Data represent the enumeration on TSA.

Directly after using the cloths to clean the contaminated surfaces, the cloths contained high numbers of bacteria (10^4 to 10^5 CFU/100cm^2), except cloths E, in which the bacteria could not be detected (below the detection limit, log N < 2.0 CFU/100cm^2). The bacterial loads in cloths varied among the cloth types (p=0.00), both for *S.* Enteritidis and *S. aureus*, but were not significantly affected by the condition of the cloths (p=0.65 and p=0.28 for *S.* Enteritidis and *S. aureus*, respectively). These results indicate that during cleaning microorganisms were transferred from the surfaces to the cloths, and depending on the type of the cloths, with or without antibacterial component, the numbers of microorganisms were stable or reduced.

Effect of sodium hypochlorite in suspension test

The results of the suspension tests under addition of 3g/l bovine serum albumin as interfering substance, simulating dirty conditions, are shown in Figure 3.1. In general, *S. aureus* demonstrated a better tolerance to sodium hypochlorite than *S.* Enteritidis. At concentrations of 200 ppm, after 60 minutes of exposure *S. aureus* were reduced with 2-log units while *S.* Enteritidis decreased with approximately 4-log units. At 300 ppm, higher reductions were

found for *S.* Enteritidis as well as *S. aureus,* and at 400 ppm 5-log reductions were obtained for *S.* Enteritidis and *S. aureus* after 30 minutes and 60 minutes of exposure, respectively.

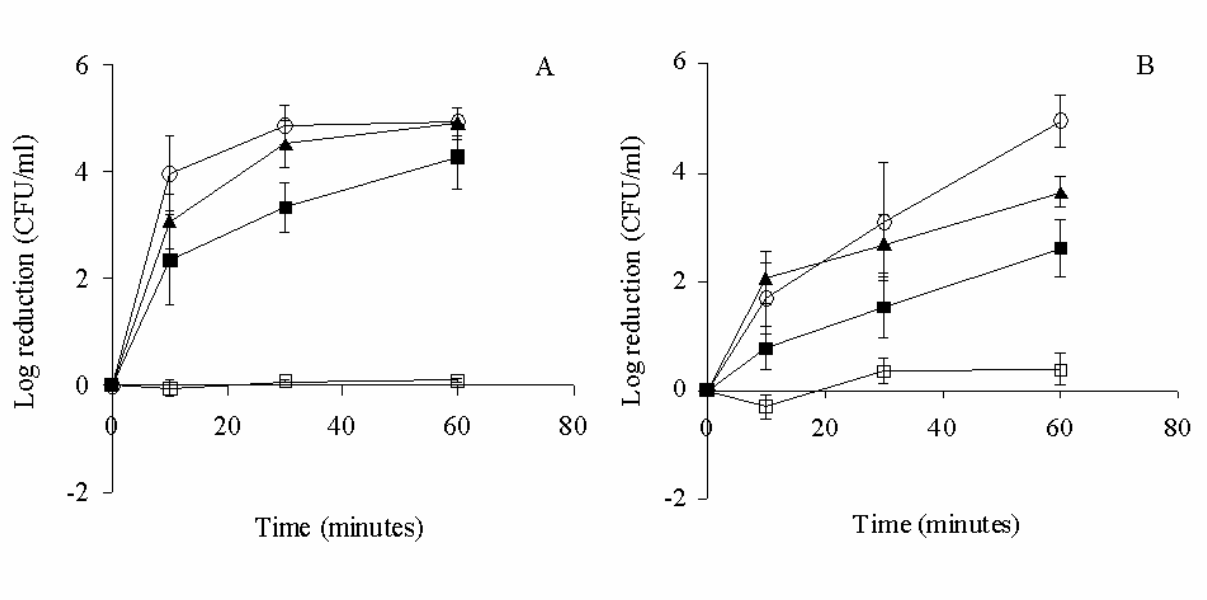


Figure 3.1. Effect of sodium hypochlorite solution on *S.* Enteritidis (A) and *S. aureus* (B) in suspension test (n=6). □ control, ■ 200 ppm, ▲ 300 ppm, ○ 400 ppm.

Effect of sodium hypochlorite on artificially contaminated cloths and repeated exposure experiment

The effect of commercial sodium hypochlorite solutions on reduction of *S.* Enteritidis and *S. aureus* in cloths are shown in Figure 3.2. Although the experiments were carried out in absence of food residues or interfering substances, decontamination of microorganisms in cloths required higher concentration of hypochlorite than in suspension tests. At 500 ppm hypochlorite, numbers of *S.* Enteritidis and *S. aureus* were slightly reduced in the exposure time of 60 minutes. When the cloths, after removal of the solution by wringing out, were left at room temperature for 24 hours, levels of microorganisms remained high (approximately 10^5 CFU/100cm^2).

At 800 ppm with 10 minutes exposure time, 2-log reductions were found for *S.* Enteritidis, while *S. aureus* was reduced with approximately 1-log unit. After 24 hours bacteria were still found in these cloths with approximately 10^3 CFU/100cm^2. At 30 and 60 minutes exposure time, approximately 3-log reductions were observed for *S.* Enteritidis and *S. aureus*, but the next day the counts in cloths decreased below the detection limit (log N < 2.0 CFU/100cm^2). These results indicate that inactivation of pathogens in cloths requires high concentrations of hypochlorite and a long exposure time.

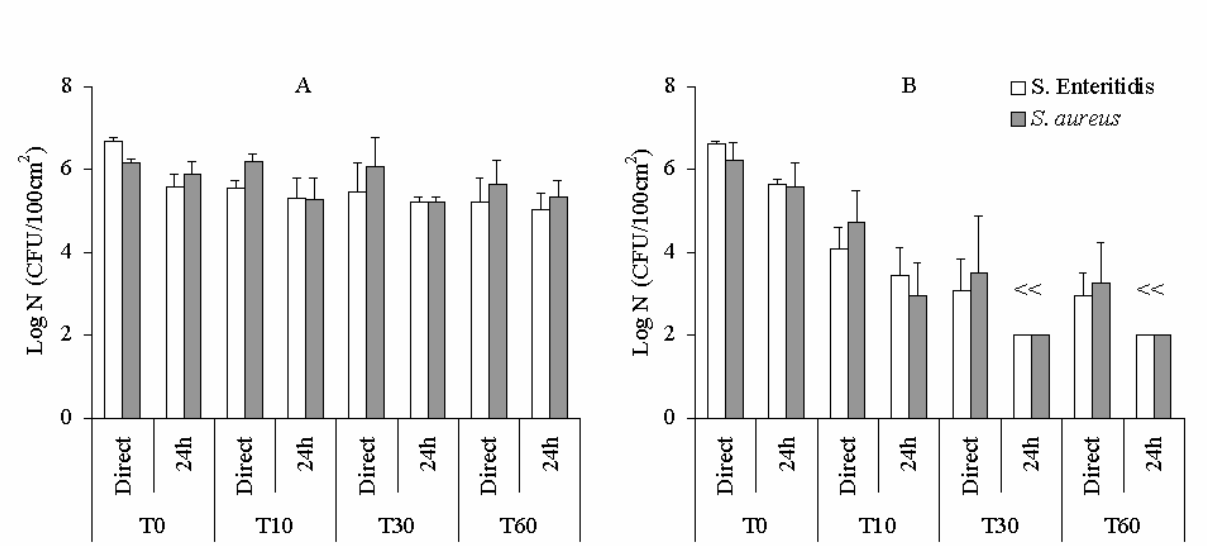


Figure 3.2. Effect of sodium hypochlorite at concentrations of (A) 500 ppm and (B) 800 ppm on *S.* Enteritidis and *S. aureus* in artificially contaminated cloths (n=3). Sampling was carried out at time zero, 10, 30 and 60 minutes, directly after exposure with hypochlorite and 24 hours after the cloths were removed from the hypochlorite solution and subsequently kept at room temperature. Data represent enumeration on TSA. << indicates that the count numbers are below the detection limit (Log N < 2.0 CFU/100cm^2)

The effect of repeated exposure to sodium hypochlorite was investigated in cloths. The first challenge to sodium hypochlorite at a final concentration of 650 ppm for 10, 30 and 60 minutes resulted in reductions of the count numbers of *S.* Enteritidis and *S. aureus* between 1- and 1.5-log units. When these cloths were subsequently removed from the solution, kept at room temperature after wringing out, and treated with the same concentration on the next day, the numbers of the microorganisms decreased below the detection limit. Experiments with a lower hypochlorite concentration (500 ppm) also resulted in increase of susceptibility against this product. At this concentration, the first challenge resulted in less than 1-log reduction of the count numbers of *S.* Enteritidis and *S. aureus* after exposure time of 10, 30 and 60 minutes. The second challenge on the next day resulted in 4-log reduction after 10 minutes exposure and in reduction until below the detection limit after 30 and 60 minutes exposure.

Effect of sodium hypochlorite on naturally contaminated household cloths

Eighteen cloths (18 x 18 cm^2), involved in daily use in households, contained total aerobic counts in a range between 10^8 to 10^9 CFU/cloth and total Enterobacteriaceae between 10^5 to 10^8 CFU/cloth. After the cloths were washed with hypochlorite solution at a concentration of 2400 ppm and subsequently left at room temperature for 15 minutes and 60 minutes, the total aerobic counts in cloths were reduced with approximately 4-log units (Figure 3.3). After 24 hours, the levels of the microorganism in the cloths were approximately 10^3 CFU/cloth, for

both total aerobic counts and total Enterobacteriaceae. High variability was observed among the count numbers of the cloths as indicated by large spreading of the standard deviation.

The scanning electron micrographs indicated that the cloths were constructed from a complex structure (Figure 3.4). This structure might offer a protective microenvironment for microorganisms where attachment could be facilitated. Furthermore, food residues were observed in household cloths covering the bacteria to some extent. This arrangement possibly facilitated additional protection to microorganisms against direct exposure with hypochlorite.

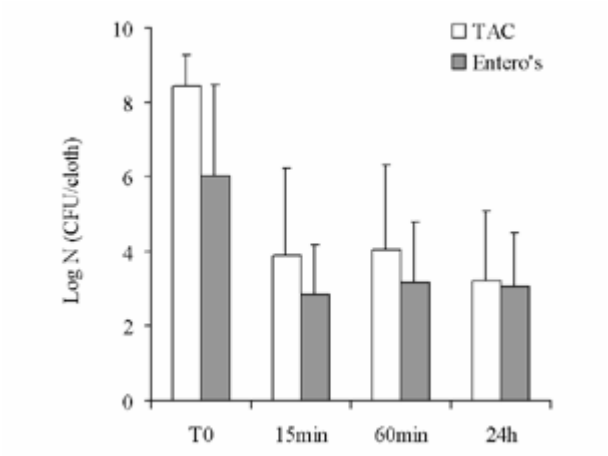
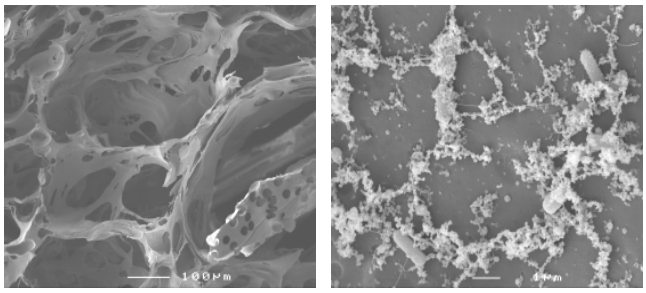


Figure 3.3. Effect of sodium hypochlorite solution (2400 ppm) on the total aerobic counts (TAC) and total Enterobacteriaceae (*Entero's*) of naturally contaminated household cloths (n=18).



A B

Figure 3.4. Scanning electron micrograph of naturally contaminated household sponge cloths, (A) x 2,000 and (B) x 10,000.

Discussion

Cleaning refers to the mechanical removal of dirt and soil from an object area. In addition, during cleaning microorganisms are removed from surfaces, as demonstrated in this study.

Different cloths which were used to wipe artificially contaminated surfaces, demonstrated similar considerable reduction of *S.* Enteritidis (about 4.5 to 5 log units), indicating that the performance of all cloths was comparable. Some variation may be associated with the amount of pressure applied to the cloths during wiping of the surfaces. The numbers of cells used in the experiments (approximately $2x10^5$ to $6x10^5$ CFU/100cm^2) were similar with *Salmonella* counts found on chopping boards after preparation of *Salmonella*-contaminated chicken carcasses (19).

The reduction of *S. aureus* by wiping, on the contrary, was influenced by the cloth type. The disposable cloth (cloth E) showed a better performance than the other cloths. The additional effect of this cloth on bacterial reduction was possibly due to the anionic surfactant left on surfaces, resulting in inactivation of the remaining microorganisms on this surface. A statistical test between the performances of the re-usable cloths, thus excluding cloth E, also demonstrated significant differences, indicating that some of these cloths performed better than others in reduction of *S. aureus* from surfaces. Although some variation may also be associated with the wiping practice, in general, it was more difficult to remove *S. aureus* from surfaces than *S.* Enteritidis using the cloths, most likely due to the characteristics of the microorganism. As found in our prior study, *S. aureus* survived better on stainless steel surfaces (62) than *S.* Enteritidis. Particularly at high initial levels, *S. aureus* was present in clumps that might provide some protection to the innermost cells against drying (122) and wiping. Possibly due to this clump structure, 15 minutes air-drying did not significantly reduce *S. aureus* on surfaces, next to intrinsically tolerance against dry conditions.

An important observation is that during cleaning microorganisms were transferred from surfaces to the cloths, which can potentially cause cross-contamination. Our previous study demonstrated that microorganisms were readily spread from kitchen sponges to surfaces with transfer rates of approximately 20 to 40% (62). Some kitchen cloths are specially treated with particular chemicals for disinfection purposes, such as cloths D (re-usable cloth) and E (disposable cloth). This treatment resulted in differences on bacterial counts remaining in the cloths, i.e. in cloth E the numbers were below the detection limit. Additionally, when the re-usable cloths were used once and washed, the antibacterial effect in these cloths was reduced or disappeared, as demonstrated with the agar diffusion test. However, single use did not result in lowering the disinfecting activity that could lead to statistical differences on the count numbers in cloths. Variations obtained between the results of the agar diffusion test and the exposure experiment in cloths were most likely due to the ease or difficulty of the diffusion of antibacterial component into the agar medium. As described above, cloths E showed no effect on *S.* Enteritidis and only a small inhibition zone on *S. aureus* by the agar diffusion test, but demonstrated significant bacterial reduction in the cloths. Furthermore, the antibacterial component in cloth D probably diffused better to the agar medium, resulting in considerable inhibition of *S.* Enteritidis and *S. aureus* on plates, although the disinfecting effect in cloths resulted in less than 2-log reduction in comparison to the regular cloths.

Some studies have described alternative procedures to reduce bacterial contaminants in cloths (47, 95), such as boiling in water for 5 minutes. In addition, recommendations for selection of suitable hygiene procedures for use in the domestic environment, including decontamination of cloths, have been published (45). When chemical disinfection is a choice for bacterial reduction in cloths, the correct use of these disinfectants, e.g. the correct concentration and the correct exposure time should be applied. The biocidal activity of disinfectants, such as sodium hypochlorite is dependent on some factors. The organic material in the environment interferes with the available chlorine, resulting in decreasing of the effectiveness of sodium hypochlorite as demonstrated in suspension tests under dirty conditions in comparison with clean conditions. In another study it was demonstrated that, in a suspension test, 9.2 times the concentration of hypochlorite solution was needed to achieve a 5-log reduction of *S. aureus* under dirty conditions, in comparison with clean conditions (4).

The intrinsic susceptibility (tolerance) of microorganisms to chemical agents varies, depending upon the species and the antimicrobial agent (37). Furthermore the test methodology used for the evaluation influences the tolerance of the microorganisms. Low concentrations of available chlorine (2 to 500 ppm) are active against vegetative bacteria in environments with low organic matter (4). However, at 500 ppm of hypochlorite, the levels of *S.* Enteritidis and *S. aureus* were not reduced noticeably when the test was carried out on cloths, even though no additional organic matter interfered. The structure of the cloths might offer a protective microenvironment where microbial attachment and survival is facilitated. Furthermore, in naturally contaminated household cloths, 2400 ppm of hypochlorite solution, as recommended by the manufacturer, did not result in a total reduction of the micro-flora of the cloths, although in some cases a reduction of more than 4 log-units was observed. Differences between cloths possibly due to the degree of bacterial contamination, present of organic materials including food residues, or attachment of bacteria on cloths. Organic matter could interfere with the hypochlorite or protected the microorganisms from direct contact with the disinfectants. As observed with scanning electron microscopy, bacteria were found in household cloths covered by organic matter from the environment.

Repeated exposure to hypochlorite, however, resulted in increased susceptibility of microorganisms against this product, most likely due to membrane damage. After the first exposure to hypochlorite with concentrations of 650 ppm in BHI broth for 30 minutes, membrane damage was observed in more than 70% of cell population, determined by epifluorescence microscopy after cell staining using LIVE/DEAD *Bac*Light bacterial viability kits (data not shown). The second challenge caused further damage, resulting in nearly 100% dead cells. The integrity of the cell membrane is important to maintain the folding of proteins and the integrity of the DNA (9). Both hypochlorous acid and hypochlorite ion are strong oxidizing agents that can attack cell walls and impair cellular membrane functionality, resulting in increased membrane permeability (77, 106).

This study has demonstrated the fact that effective cleaning of food preparation sites is important. Wiping of surfaces using regular cloths resulted in a considerable reduction of

pathogens such as *S.* Enteritidis. Subsequent air-drying resulted in further reduction to a level at which the risk for cross-contamination is very low. When bacteria such as *S. aureus* or other opportunistic bacteria have to be eliminated from surfaces, disposable chemical-impregnated cloths can be indicated. Our study also demonstrated that, during cleaning, microorganisms were transferred from surfaces to the cloths, and inactivation of microorganisms in cloths required higher concentration of sodium hypochlorite than in the suspension test, although the experiments were carried out in absence of interfering substances. When organic matters from the environment present in the cloths, which occurs regularly in practical situations, the effectiveness of such disinfectants will be less, requiring higher concentration of disinfectants. Instead, boiling of the cloths for 5 minutes or washing at $\geq 60^{o}$C could be indicated.

Chapter 4

Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges

Abstract

In response to increasing concern about home hygiene, the use of antibacterial products to reduce microorganisms in kitchen sponges and cleaning cloths is strongly promoted by some producers of detergent for domestic use. The effects of an antibacterial dishwashing liquid on *Escherichia coli, Salmonella* Enteritidis, *Staphylococcus aureus* and *Bacillus cereus* were investigated in a modified suspension test and in used sponges with and without food residues under laboratory conditions. A limited study was conducted in households to assess the efficacy of antibacterial dishwashing liquid as used by the consumer. In the suspension tests *S. aureus* and *B. cereus* were shown to be susceptible to low concentrations of antibacterial dishwashing liquid (0.5%), whereas *E. coli* and *S.* Enteritidis maintained their initial numbers for at least 24 h at 25^{o}C. At higher concentrations (2 to 4%), all test organisms decreased to below the detection limit after 24 h. Over a 24-h period, the antibacterial dishwashing liquid did not significantly reduce these organisms in used sponges in which food residues were present. The antibacterial product did not reduce the competitive microorganisms either. Similar results were found for sponges involved in daily household use. The results of this study demonstrate that the antibacterial dishwashing liquid was effective in reducing pathogens only in the suspension test but not in the used sponges. This finding indicates that to determine the efficacy of antibacterial products, their use in a household setting must be considered.

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Introduction

Previous studies have caused increasing concern about foodborne disease outbreaks in the home setting. These outbreaks have frequently occurred as a result of improper food preparation (2, 94, 111, 116), and cross-contamination in combination with inadequate storage or cooking has been implicated in many instances (2, 94). These previous studies have suggested that although raw material is probably the main source of contamination in the kitchen, the areas surrounding the kitchen could also act as sources of free-living populations of bacteria. Dishcloths and sponges have been recognized as potential agents in the spread of microorganisms, and it has been observed that bacteria persist in these vehicles (53, 104, 114, 117). Enteric pathogens such as *Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter cloacae* have been isolated, as have other types of pathogens (*Staphylococcus aureus*) and opportunistic pathogens (*Pseudomonas* spp.) (29, 114, 117).

Many antibacterial products are specifically manufactured for the reduction of bacteria in cleaning cloths and sponges. These include bleach solutions, detergent, and dishwashing liquid. Limited studies have addressed the effectiveness of these products in inactivating microorganisms on sponges. In previous studies it has been reported that the soaking of dishcloths or sponges for 5 min in bleach solution resulted in a >99.9% reduction of microorganisms (47, 112). However, the presence of food substances or other matter likely decreases this product's efficacy as a decontaminating agent (4). A product's effectiveness in reducing microorganisms also depends on the way the product is applied by the consumer (8), because the consumer does not always use the product as directed by the manufacturer.

In this study, the effects of an antibacterial dishwashing liquid on foodborne pathogens were investigated in a modified suspension test and in used sponges with and without food residues under laboratory conditions. The efficacy of the antibacterial version of this product in comparison with that of the regular version was studied under the conditions of practical use in a limited number of households.

Materials and methods

Sponges and dishwashing liquid

Synthetic yellow sponges with green pads (Lola, 9 by 7 by 3cm; Nedac, Duiven, The Netherlands) and dishwashing liquid (antibacterial product and regular product) were obtained from retail supermarkets. Used sponges were collected from earlier laboratory experiments on the survival of pathogens on new sponges (unpublished), washed with 0.05% dishwashing liquid in hot water (60 to 65^{o}C), air dried, and stored at room temperature for 1 to 2 weeks.

Test microorganisms

Four strains from our culture collection were used: *Escherichia coli* Ec-0025 (minced meat isolate, obtained from Inspectorate for Health Protection, The Netherlands), *Salmonella* Enteritidis Sa-0046 (PT4 - chicken product isolate), *Staphylococcus aureus* Sc-0010 (human isolate, enterotoxin A producer) and *Bacillus cereus* Ba-0001 (environmental isolate from a dairy processing plant). From overnight cultures (20 to 24 h) in Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit Mich, Becton Dickinson and Co., Paramus, N.J.), 0.75 ml suspension was added to cryo vials containing 0.25 ml glycerol and 2-mm-diameter glass beads and stored at -80 ^{o}C. They were activated by transferring one bead to 10 ml of BHI suspension followed by incubation for 20 to 24 h at 37^{o}C for *E. coli* Ec-0025, *S.* Enteritidis Sa-0046 and *S. aureus* Sc-0010, and at 30 ^{o}C for *B. cereus* Ba-0001.

Suspension test

Overnight cultures in BHI broth were diluted in saline solution (0.85% NaCl) and added to various concentrations of the antibacterial dishwashing liquid in saline solution (0, 0.5, 1, 2, and 4%) to final concentrations of about 10^4 CFU/ml. Enumeration of the test strains was performed directly after contamination and after 6 and 24 h contact time at 25^{o}C.

Effect of antibacterial dishwashing liquid in laboratory sponges

The used sponges were washed with antibacterial dishwashing liquid solution (0.05%) in warm water (40 to 45^{o}C) in a sink to simulate daily dishwashing and were treated with a squirt of dishwashing liquid as described in the manufacturer's instructions, resulting in 0.5 $\pm$ 0.2 g per sponge (3% $\pm$ 1.5% wt/wt).

Six sponges were each contaminated with 10 ml of test suspension and squeezed with gloved hands to distribute the suspension in the sponges. The test suspensions were prepared by diluting overnight cultures in BHI broth in saline solution and 10% commercial sterilized milk in saline solution to approximately 10^6 CFU/ml. In order to generate soiled conditions, the cultures were also diluted in 1% lettuce suspension in saline solution and 0.1% suspension of raw chicken breast fillet in saline solution.

Sponges were stored at room temperature (20 to 25^{o}C, 42 % $\pm$ 2 % relative humidity) and sampled on various days (days 0, 1, 2, 4, 7, and 10) by suspending the whole sponge in 100 ml of sterile peptone saline solution (0.1% peptone) with a Stomacher laboratory blender (Seward, UK) for 60 s.

Enumeration of test microorganisms

Appropriate dilutions were spread with a spiral plater (Eddy Jet, IUC) on selective media: Chromocult coliform agar (Merck, Darmstadt, Germany) for *E. coli*, incubated for 24 h at 37^{o}C; Mannitol lysine crystal violet brilliant green agar (Oxoid) for *S.* Enteritidis, incubated for 18 to 24 h at 37^{o}C; Baird Parker egg yolk - tellurite agar (Oxoid, Basingstoke, UK) for *S. aureus*, incubated for 24 to 48 h at 37^{o}C; and Mannitol egg yolk polymixine agar (Merck) for

B. cereus, incubated for 18 to 40 h at 30°C. Plate count agar (Oxoid) was used for total aerobic counts and was incubated for 24 to 48 h at 30°C. For identification of bacteria, Analytab Products kits (20E, 20NE, API Staph and 50 CHB; BioMéreux, France) were used.

Practical-use study

In eight households, the antibacterial dishwashing liquid, packed in a sterile plastic jar, was applied to sponges according to the manufacturer's instructions and used daily for a 2-week period. Its effect on the reduction of microorganisms was compared with that of the regular product, examined at the same households for another 2-week period. Microbiological investigations of the sponges for total aerobic counts, coliforms and pseudomonads were performed at days 3, 7, and 14 by sampling as described above. The coliforms were enumerated on Chromocult coliform agar and incubated for 24 h at 37°C, and the pseudomonads were enumerated on Pseudomonas agar (Oxoid) and incubated at 30°C for 48h.

Statistical analyses

Three replications were performed for suspension tests, and two were performed for laboratory sponges. Student's two-tailed paired t test was used to analyse the significance of the difference in the levels of the test organisms in used sponges at contamination and after 24 h. A one-way factorial analysis of variance was used for the other experiments. A *P* value of <0.05 was considered statistically significant.

Results

Suspension test

All test organisms demonstrated rapid growth in BHI broth (control) and a slow increase in numbers in saline solution (blank), as shown in Figure 4.1. Immediately after exposure to 0.5% of antibacterial dishwashing liquid in saline solution, the numbers of *B. cereus* were below the detection limit (log cell numbers (*N)* $\leq$ 1.3), and a >90% reduction of *S. aureus* was obtained. *E. coli* and *S.* Enteritidis maintained their initial numbers for at least 24 h in the presence of 0.5% of the product, whereas with a 1% concentration only a small reduction was observed. With higher concentrations (2 to 4%), all test organisms were below the detection limit after 24 h; for *S. aureus* and *B. cereus* the numbers decreased immediately after the test inoculum was added.

Effect of antibacterial dishwashing liquid in laboratory sponges

In uncontaminated laboratory used sponges the pathogens specified in this study were not found, and the total aerobic counts were about 10^5 to 10^6CFU per sponge (data not shown).

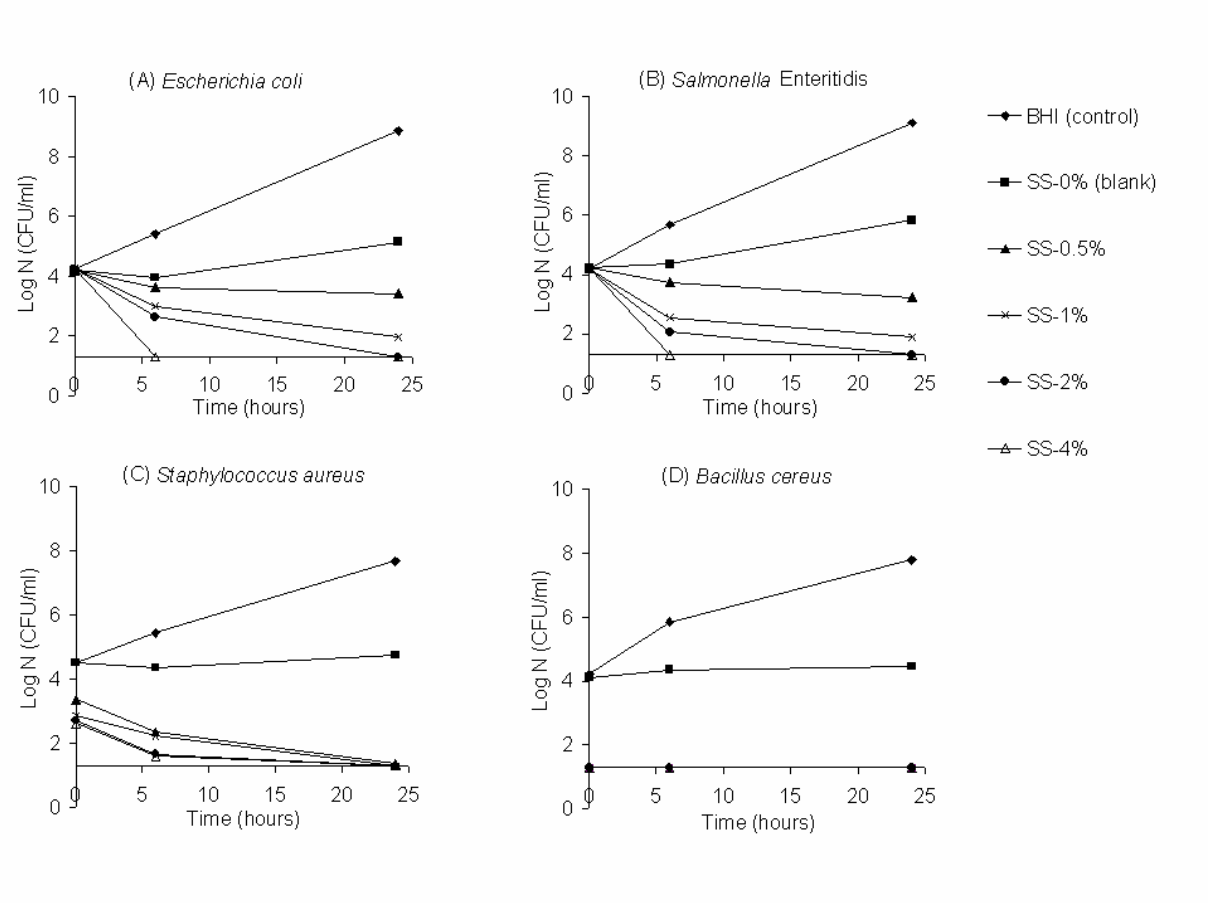

Figure 4.1. Effect of antibacterial dishwashing liquid on pathogens in the modified suspension test. Percentages indicate the concentrations of dishwashing liquid; time zero represents the number of the test organisms immediately after contamination.

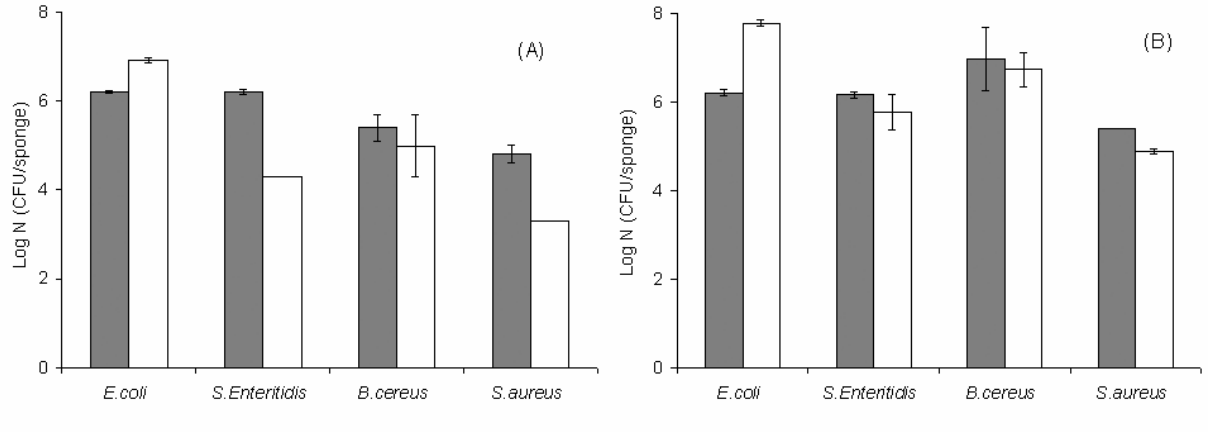

Figure 4.2. Effect of antibacterial dishwashing liquid on pathogens in artificially contaminated used sponges (A) contaminated with test organisms in saline solution suspension and (B) contaminated with test organisms in 10% milk-saline solution suspension. Symbols: ■, directly after contamination; □, after 24 h.

With the amount of dishwashing liquid added to the sponges (3% ± 1.5% wt/wt), *E. coli* increased in numbers during 24 h both with and without food residues, whereas the other test organisms decreased by <2 log units (Figure 4.2). The paired Student's *t* test demonstrated no statistically significance between the levels of all test organisms in sponges at contamination and after 24 h of storage at room temperature, with exception of *S.* Enteritidis and *S. aureus* without food residues.

Figure 4.3 shows the effect of the antibacterial dishwashing liquid on the test organisms and the competitive microflora in used laboratory sponges for a period of 10 days, during which the water content of the sponges declined from 80% to 7% (wt/wt). Without food residues (Figures 4.3A, 4.3C, 4.3E, and 4.3G), the test organisms decreased in numbers, with the exception of *B. cereus,* which survived and maintained its initial number. The total aerobic counts demonstrated substantial increases for 48 h (1 to 3 log units). With additional milk substances (4%, vol/wt), the total aerobic counts and the number of *E. coli* increased with a log factor of 2 to 3 for 48 h. Thereafter, their numbers decreased to the initial level (approximately 10^6 CFU per sponge). Only *S. aureus* demonstrated a significant decrease, of 3 log units. The levels of *S.* Enteritidis and *B. cereus* (approximately 10^6 CFU per sponge) did not change considerably during the test period (10 days).

In the presence of lettuce and chicken breast fillet suspension (Figures 4.3B, 4.3D, 4.3F, and 4.3H), *S. aureus*, *B. cereus* and the total aerobic counts showed results similar to those found in the presence of milk substances. *E. coli* and *S.* Enteritidis survived better in these sponges than in sponges without food residues but not as well as they did in the presence of milk substances.

Analytab Products identification of colonies isolated from plate count agar plates showed that the predominant microorganisms were *Pseudomonas putida* (55%) and *Pseudomonas fluorescens* (23%). In Chromocult plates *Enterobacter cloacae* was the most frequently isolated bacterium (60%). These microorganisms were found in all sponges.

Practical-use study

The sponges involved in daily use in households were in contact with the dishwashing liquid at least once a day. The total aerobic counts during the test period of 14 days (approximately 10^6 CFU per sponge) did not change dramatically (Figure 4.4) when either the antibacterial product or the regular product was used. Daily application of the antibacterial dishwashing liquid to the sponges had only a slight effect on the numbers of coliforms and pseudomonads. There was no clear indication that one of these groups became the predominant microflora in the sponges during the 2-week examination.

In only one of eight households did analysis of variance demonstrate significant differences ($P < 0.05$) between the levels of microorganisms (less than log factor 2) in the sponges with the antibacterial product and with the regular product. This finding suggests that the effect of daily application of this antibacterial product by the consumer was not really different from the effect of that of the regular product.

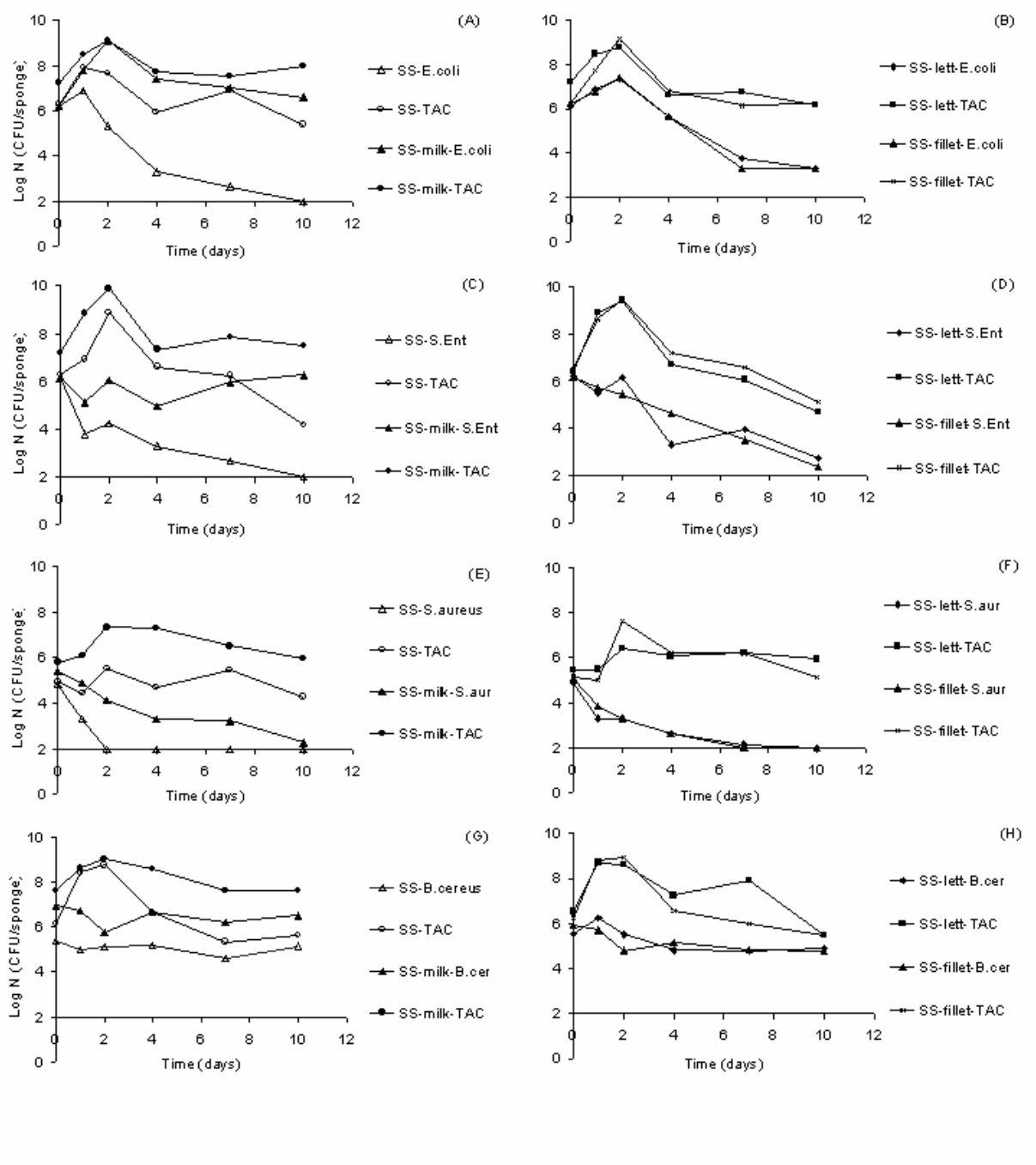

Figure 4.3. Effect of antibacterial dishwashing liquid on the survival of pathogens and their competitive microflora (total aerobic counts) in used sponges artificially contaminated with: (A) *E. coli* in saline solution and in 10% milk-saline solution suspension, (B) *E. coli* in lettuce and chicken fillet suspension, (C) *S.* Enteritidis in saline solution and in 10% milk-saline solution suspension, (D) *S.* Enteritidis in lettuce and chicken fillet suspension, (E) *S. aureus* saline solution and in 10% milk-saline solution suspension, (F) *S. aureus* in lettuce and chicken fillet suspension, (G) *B. cereus* in saline solution and in 10% milk-saline solution suspension, and (H) *B. cereus* in lettuce and chicken fillet suspension.

Discussion

Antibacterial dishwashing liquid, which for some brands is distinguished from a regular product by the addition of one or more antibacterial compounds, is supposed to reduce or inactivate bacteria. The efficacy of such products is usually tested under laboratory conditions using suspension tests in which a generally accepted requirement for disinfectants is a log reduction of $\geq$5 (a reduction of $\geq$99.999%) during 5 min of contact (104). Some of these products are able to meet the test requirements, but there is little evidence with regard to whether and to what extent the product can reduce cross-contamination under conditions of actual household use.

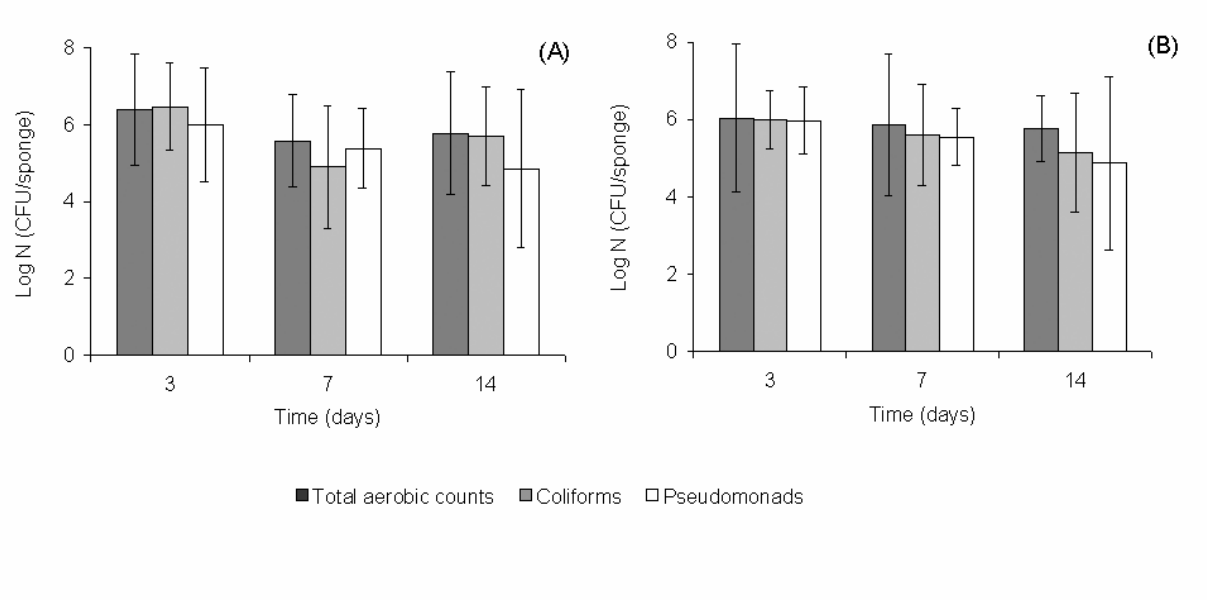

Figure 4.4. Effect of (A) antibacterial dishwashing liquid and (B) regular dishwashing liquid on microflora in household sponges.

Because the outcome of suspension tests might be a poor predictor of efficacy under practical conditions, especially with regard to bacteria attached to surfaces and in the presence of food debris, a variety of test procedures have been designed to mimic these conditions (131). There are many variants of the test whereby distribution of the disinfectant solution over a small defined test surface (tile, microscopic slide, PVC, stainless steel disk) is followed by contamination with a standardized inoculum and determination of the survivors after a given exposure time using a rinsing technique (101).

In this study, some modifications were made to the standard suspension tests to simulate practical conditions. Therefore, the level of artificial contamination was relatively low (approximately 10^4 CFU/ml) and the tested concentrations of the product were in a range of dilutions that are generally applied in a household situation: 0.1% $\pm$ 0.05 % in water for

dishwashing and 3% ± 1.5% for application to sponges. The effect of antibacterial dishwashing liquid in used sponges was studied for a relatively long period (10 days under laboratory conditions and 14 days under practical conditions) to include the possible changes in composition of the microflora in the sponges.

In the suspension test, *S. aureus* and *B. cereus* were shown to be more susceptible to the antibacterial product than were *E. coli* and *S.* Enteritidis at low concentrations (0.5%). This result might be due to the tolerance of these bacteria to the antibacterial compounds in the product. Gram-positive bacteria are generally sensitive to membrane-active disinfectant (119). Anionic surfactants that are included in the composition of the product in a relatively high concentration (15 to 30%) might contribute to the inactivation of the gram-positive bacteria, because these compounds, as typical targets, affect cytoplasmic membrane integrity and membrane-bound enzymes and cause cell leakage (25, 119).

Increasing the concentration of the antibacterial product (2 to 4%), which in the suspension tests reduced all test organisms to below the detection limit after 24 h, did not result in a considerable effect for the sponges used under laboratory conditions. The numbers of *E. coli, S.* Enteritidis*, B. cereus* and total aerobic counts were hardly influenced by the antibacterial activity of the product, particularly if food residues were present. The cavernous structure of the sponge might offer a protective microenvironment in which microbial attachment and survival is facilitated (38). In this structure, food residues left after dishwashing might form a biofilm that inactivates the antibacterial compounds (4, 25, 101). Comparative studies examining the effect of a low and high protein loads on the antibacterial activity of quaternary ammonium compounds and hypochlorite demonstrated that high protein load affects the application concentration as well as the contact time needed to inactivate the microorganisms (4). Our results are similar with those of Scott and Bloomfield (113), who found that soiling in cleaning cloths encouraged regrowth of residual survivors (113). Overnight storage at an ambient temperature after disinfection of cleaning cloths by hypochlorite or a phenolic solution demonstrated regrowth of residual survivors, particularly if the cloths were heavily contaminated with competitive microorganisms (112).

Although drying has been shown to reduce the number of organisms on dishcloths (113), the decreasing moisture contents in the sponges during the test periods did not affect the total aerobic counts. The numbers of these competitive microorganisms increased when the sponges were still moist, probably as a result of characteristic differences between the structure of the sponges and dishcloths. The microstructure of the sponge offers a degree of protection compared with the more exposed surface of the dishcloth and hence facilitates maintenance of its bacterial load (38).

In eight households, both the antibacterial product and the regular product were applied for 2 weeks, and it was shown that neither product significantly reduced total aerobic counts, coliforms or pseudomonads. In agreement with the results in this study, sponges used in households were shown by Hilton and Austin (38) to harbour large numbers of bacteria during early use (within 3 days), and this maximal load did not increase or decrease

significantly over extended periods. This finding indicates that although an antibacterial product is believed to be more effective in reducing or inactivating microorganisms, in practical situations its effect is not always better than that of a regular product.

Our results and those of other studies (38, 112, 113) suggest that bacterial inactivation in sponges depends on a number of factors and is largely unpredictable. Because the number of bacteria increases rapidly under favourable conditions in a used sponge and there is a constant risk of contamination transfer from this surface, disposable sponges or cloths should be considered for use whenever possible. When reusable sponges have to be used, they should be thoroughly cleaned and dried after use or immersed in boiling water for 5 min, an effective means of decontamination (47, 112, 113). Recommendations for suitable hygiene procedures in the domestic environment have been published (45).

In this study, the antibacterial dishwashing liquid was shown to be effective in reduction of pathogens only in the suspension test. In the used sponges to which application of the product is recommended, the numbers of *E.coli, S.* Enteritidis*, B.cereus* and total aerobic counts were hardly influenced. The presence of food residues strongly reduces the product's efficacy. This finding indicates that to determine the efficacy of an antibacterial product and other similar products, practical conditions must be considered in the test.

Chapter 5

Morphological changes, cell viability and development of increased tolerance to hypochlorite of *Salmonella enterica* serovar Enteritidis on surfaces with reduced water activity

Abstract

The survival of microorganisms on dry(ing) surfaces, resulting in exposure to low water activity (a_w), may affect the morphology and physiological activity of the cells. In this study the morphological changes and cell viability of *Salmonella enterica* serovar Enteritidis challenged to low a_w surfaces were analysed. Viability was determined using the Live/Dead *Bac*Light bacterial viability kit with epifluorescence microscopy and flow cytometry. The results indicated that exposure to reduced a_w surfaces induced filamentation of the cells. The amount of filamentous cells at a_w 0.94 was up to 90% of the total number of cells. Cellular damage was evidenced by the inability of a proportion of the stressed cells to form colonies on selective medium. Surviving filamentous cells maintained their membrane integrity after exposure to low a_w surfaces for a long period of time and were able to split in single cells under favourable conditions, resulting in the instantaneous appearance of a large number of viable cells. Furthermore, both short and elongated cells pre-challenged to low a_w surfaces demonstrated better tolerance against sodium hypochlorite than control cells. These tolerant cells are able to survive disinfection and therefore could be a source of contamination of foods coming in contact with surfaces. This points to the need of increased attention in the disinfection procedures of surfaces in processing plants or household environments.

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Submitted for publication

Introduction

Salmonella enterica serovar Enteritidis (*S. enterica* serovar Enteritidis) is the most important cause of *Salmonella*-infections associated with the consumption of shelled eggs and poultry in Europe (136) and the United States (94). Cross contamination directly from raw poultry to ready-to-eat products or indirectly via contaminated surfaces or niches in the household kitchen, is the most likely mode of infection (26), next to the consumption of contaminated raw product. During transmission, the microorganism often experiences environmental stresses, such as nutrient starvation, osmotic shock and/or temperature variation.

The risks associated with cross-contamination of *S. enterica* serovar Enteritidis from surfaces have been recognized, since this microorganism has the ability to survive on dry surfaces for hours or days, dependent on the initial counts and the presence of food residues (42, 62). On surfaces, the cells are exposed directly to the air that may lead to water removal from the cells and adjustment of cytoplasmic solvent composition. Osmotic stress is one consequence of the initial stage of the air-drying of cells on surfaces (21, 91, 98). Little is known about the response of *S. enterica* serovar Enteritidis to dry(ing) surfaces or surfaces with low water activity (a_w) (e.g. $a_w < 0.96$) and the consequences of cellular adaptation on these surfaces to subsequent stress exposure, such as disinfection with sodium hypochlorite. Hypochlorite is generally used as chemical sanitizer because of its effective activity against a wide variety of microorganisms.

Mattick et al. (78) have shown that filamentous cells were formed by three wild type strains of *S. enterica* serovar Enteritidis at low a_w in broth. Lowering of a_w values was achieved by addition of sucrose, glycerol and sodium chloride (NaCl). With the latter compound in the medium the elongated cells appeared to be longer and more numerous (78). Furthermore, in another study, it has been shown that air-dried *Salmonella* cells become more heat tolerant (57). In this study the survival, morphological changes and cell viability of eight wild type strains of *S. enterica* serovar Enteritidis of different phage types after exposure to surfaces with reduced a_w at different temperatures were investigated. Agar surfaces with reduced a_w, obtained with NaCl, were used as a model to study the response of bacteria to dry surfaces. The cross-protection against hypochlorite solutions as a result of pre-challenge to reduced a_w surfaces was studied in suspension tests.

Materials and methods

Bacterial strains and growth conditions

Six human isolates of *S. enterica* serovar Enteritidis (1438 (phage type, PT, 13), 1439 (PT 4), 1444 (PT 25), 1391 (PT 21), 1389 (PT 1) and 1514 (PT 28)), a chicken isolate (1138, PT 28) and a chicken meat isolate (1448, PT 4) were obtained from the National Institute of Public Health and the Environment, The Netherlands. The stock cultures were maintained at -80^{o}C in cryo vials (Greiner Bio-one GmbH, Frickenhausen, Germany) containing a stationary-phase

culture in Brain Heart Infusion (BHI; Difco, Becton Dickinson, Maryland, USA) broth with 25% (vol/vol) glycerol (Fluka-chemica, Buchs, Switzerland) and glass beads (ø 2 mm, Emergo, Landsmeer, The Netherlands). Strains were cultured by transferring one glass bead to 10 ml of BHI broth, followed by overnight incubation (20-22 h) at 37°C.

Survival in environments with reduced a_w

Survival at low a_w surfaces was studied on Tryptone Soy Agar (TSA, Oxoid, Basingstoke, England) surfaces containing 4%, 6% and 8% NaCl (Merck, Darmstadt, Germany) in petri dishes, resulting in a_w of 0.97, 0.95 and 0.94, respectively. The a_w was measured using a water activity meter (Novasina, Zurich, Switzerland) based on the relative vapour pressure. Appropriate dilutions of culture suspension in peptone saline solution (PSS: NaCl 8.5 g/l and Neutralized Bacteriological Peptone (Oxoid, Basingstoke, England) 1 g/l) were applied on these surfaces using a spiral inoculation apparatus (Eddy Jet, IUC, Barcelona, Spain) and the plates were sealed with parafilm (M®, Pechiney, Plastic Packaging, Inc., USA) to avoid evaporation of water during incubation at 25°C and 37°C. The colony counts were determined when the visible colonies were observed: after 2, 4 or 6 days, depending on the a_w of the media and the incubation temperature. The recovery percentages were calculated as quotients of the colony counts found on surfaces with reduced a_w and those on TSA without additional humectants.

On glass surfaces (2 x 7 cm^2), 0.2 ml of overnight culture of *S. enterica* serovar Enteritidis diluted in fresh BHI broth with a concentration of approximately 10^5 CFU/ml, were applied and spread with a sterile loop, and incubated at 25°C and 37°C in petri dishes. Microscopic examination of the glass surfaces was performed 24 h, 48 h and 72h after incubation.

Morphological changes

The morphological changes of the cells, i.e. the cell elongation, were observed by viewing the cells from agar surfaces, prepared on an object glass, using the x 100 phase contrast objective of a Zeiss-standard 20 light microscope (Carl Zeiss, Germany). Images were passed through a Hyper HAD, CCD-Iris/RGB colour video camera (Sony, Japan) to a Protocol computer system (Synoptics, Ltd., UK), to generate digital photomicrographs. The percentages of the elongated cells, calculated from the total cells, were determined using the direct microscopic count procedure on a Bürker-Türk objective (Schreck-Hofheim, Germany). Since under optimal growth condition the cells of *S. enterica* serovar Enteritidis were found between 2 to 3 μm, cells longer than two times of the maximal length, i.e. 6 μm, were considered as elongated cells.

Cell viability

The cells grown on the surfaces at a_w 0.95 and a_w 0.94, incubated for 6 days at 25°C, were transferred using a sterile loop into an eppendorf tube (Greiner Bio-one GmbH,

Frickenhausen, Germany) containing 1 ml of PSS. The cell suspensions were centrifuged (BHG, Hermle, Gosheim, Germany) at 4,000 x *g* at 4°C for 5 minutes, and the pellets were re-suspended in PSS to a concentration ranging between 1.0 to 1.5 $x10^8$ CFU/ml. The culturability of these cells was determined on TSA and Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB, Oxoid, Basingstoke, England), a selective medium for isolation of *Salmonella*.

The membrane integrity of the cells from surfaces with reduced a_w was determined using LIVE/DEAD *Bac*Light Bacterial Viability Kits (Molecular Probes, Inc., Eugene, Oregon, USA) according to the protocols provided with the kit. This kit utilizes mixtures of 3.34 mM SYTO® 9 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, propidium iodide (PI) 20 mM. With a 1:1 mixture of the SYTO® 9 and PI stains, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red. The cell suspensions were microscopically analysed with an Axioskop epifluorescence microscope equipped with a 50-W mercury arc lamp, a fluorescein isothiocyanate filter set (excitation wavelength, 450 to 490 nm; emission wavelength, >515 nm), a x 100 1.3 numerical aperture Plan-Neofluar objective lens, and a camera (Carl Zeiss, Oberkochen, Germany). Photomicrographs were made with simultaneous light and epifluorescence microscope, a low light intensity, a magnification of x 1,000, and an exposure time of 15 to 45 seconds, on Kodak 400 ASA colour films. In these photomicrographs both the SYTO® 9 and PI- labelled cells could be counted.

Flow cytometry

The cells from surfaces with reduced a_w, labelled with *Bac*Light bacterial viability kits, were analysed with a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry System, San Jose, Calif., USA) equipped with a 15 mW blue light at 488 nm, air-cooled argon ion laser. The side scatter signal was used as a trigger signal. The green fluorescence from SYTO® 9-stained cells was detected through a 515 to 545 nm band-pass filter (FL1 channel), and the red fluorescence of the PI signal was collected in the FL3 channel (>670 nm long-pass filter). FACSFlow solution (Becton Dickinson, USA) was used as the sheath fluid. The cells were measured at a low flow rate corresponding to 150 to 500 cells per second, and 10,000 events were collected for further analysis. A combination of FSC (forward scatter) and SSC (side scatter) signals was used to discriminate bacteria from the background and to characterize the morphology of the cells. All signals were collected using logarithmic amplifications. Data were analysed by using the Windows Multiple Document Interface computer program (WinMDI; Joseph Totter, Salk Institute for Biological Studies, La Jolla, Calif.; available at http://facs.Scripps.edu/software.html)

Cross-protection to sodium hypochlorite challenges

The cell suspensions were prepared from the population on TSA surfaces and surfaces with reduced a_w, incubated at 25°C for 6 days, to a concentration ranging between 1.0 and 1.5 x 10^8

CFU/ml, as described for examination of the cell culturability. Sodium hypochlorite (Acros Organics, New Jersey, USA) solutions were prepared at chlorine concentrations of 300 and 400 ppm. The available chlorine concentrations were confirmed by titration (3). The preparation of the solutions and the suspension tests were carried out following the European Norm (EN) 1276 (15). Bovine serum albumin (Sigma-Aldrich, Steinheim, Germany) was used as interfering substance to simulate dirty conditions. The reductions of the log numbers were determined at 10, 30 and 60 minutes of exposure to hypochlorite.

Statistical analyses

Each experiment (n) was carried out at least twice at different days and no less than two replications were used in each experiment. Except flow cytometry data, data analyses were performed on SPSS for Windows 95/98/NT/2000, release 10.1. A p-value of <0.05 was considered to be statistically significant.

Results

Survival in reduced a_w environments

Survival and growth of eight strains of *S. enterica* serovar Enteritidis were studied on TSA surfaces with reduced a_w. All strains formed visible colonies 2 days after incubation at 37°C at a_w 0.97 and 0.95, while at 25°C the colonies appeared after 4 and 6 days, respectively. Strikingly, on surfaces at a_w 0.94 a thin film of bacterial growth was found instead of separate colonies. The recovery percentages, calculated as quotients of the colonies on surfaces with reduced a_w and those on TSA without additional humectants were in a range of 70 to 95% at a_w 0.97 and 30 to 70% at a_w 0.95 (data not shown). The overall recovery on surfaces was not dependent on the temperature ($p=0.45$), but was affected by the a_w of the surfaces ($p=0.01$).

Our study also indicated that when an aliquot of bacterial suspension in fresh BHI broth was applied on glass surfaces, an increase in cell numbers was microscopically observed after slow air-drying for 24 hours at 37°C and for 48 hours at 25°C, although the surfaces were visually dry after approximately 3 and 24 hours, respectively (data not shown).

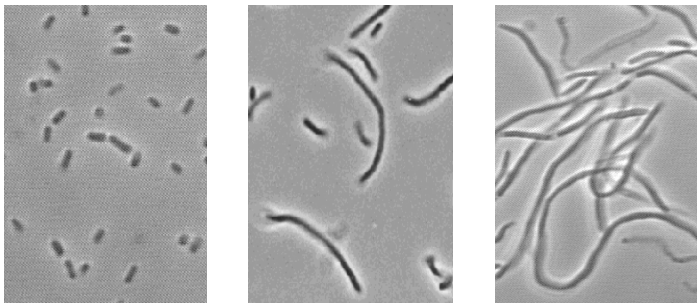
Morphological changes

Microscopy revealed that all strains of *S. enterica* serovar Enteritidis formed elongated cells (longer than 6μm) on surfaces at a_w 0.95 and a_w 0.94. On surfaces at a_w 0.97 no elongated cells were found. Direct microscopic counting indicated that on surfaces at a_w 0.95, the percentage of the elongated cells was between 24 to 40% and at a_w 0.94 between 82 to 90% of the total cell numbers (Table 5.1). Particularly at 25°C on surfaces at a_w 0.94, elongated cells with a size of 50 μm or more were found (Figure 5.1).

Table 5.1. Percentages of elongated cells in broth and on surfaces with reduced a_w after 6 days at 25°C, determined by direct microscopic counting.

Strains	Percentages of elongated cells (Average ± SD %)[a]	
	On surfaces a_w 0.95	On surfaces a_w 0.94
1138	29 ± 6	88 ± 4
1389	24 ± 5	94 ± 2
1391	40 ± 5	90 ± 3
1444	38 ± 6	94 ± 2
1448	24 ± 5	93 ± 2
1438	34 ± 4	82 ± 6
1439	29 ± 4	87 ± 3
1514	35 ± 3	90 ± 6

[a] Averages from two experiments, with 5 observations for each experiment



A B C

Figure 5.1. Effect of low a_w on morphology of *S. enterica* serovar Enteritidis strain 1448. (A) Control cells, (B) Cells on surfaces at a_w 0.95, and (C) Cells on surfaces at a_w 0.94, after 6 days at 25°C

The elongated cells were also found on glass surfaces in low percentages (approximately 3% of the cell population, data not shown). These results indicated that filamentation of the cells may occur on *Salmonella*-contaminated wet surfaces after a slow-drying process.

Data obtained by flow cytometry indicated that cells challenged to a_w 0.95 showed slightly higher signals on FSC and SSC (Figure 5.2B) in comparison to the control cells grown on TSA surfaces (Figure 5.2A). Noticeable increase of FSC and SSC signals was observed in cell populations grown on surfaces at a_w 0.94, which resulted in a complete shift of a population on both detector signals (Figure 5.2C). These findings indicated that particularly on surfaces at a_w 0.94 cells with large dimensions were observed, which was confirmed by the microscopy analysis.

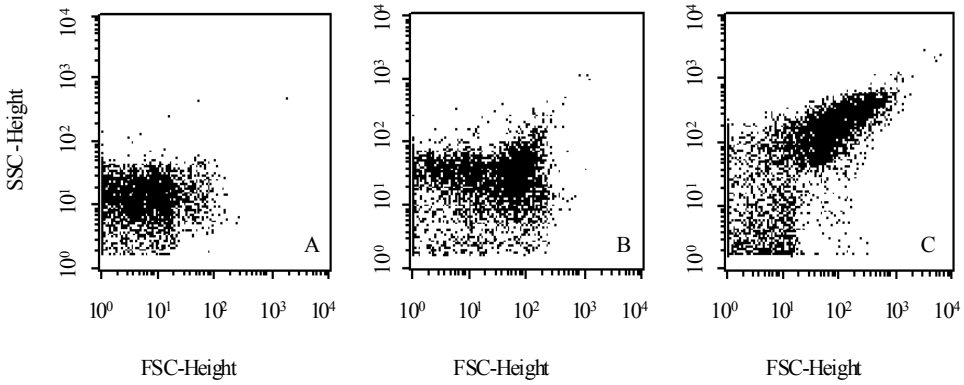


Figure 5.2. Forward scatter- (FSC) and Side scatter (SSC) signals, obtained by flow cytometry, of *S. enterica* serovar Enteritidis strain 1448. (A) Grown on TSA surfaces, (B) Challenged to surfaces at a_w 0.95 and (C) at a_w 0.94, after 6 days at 25°C.

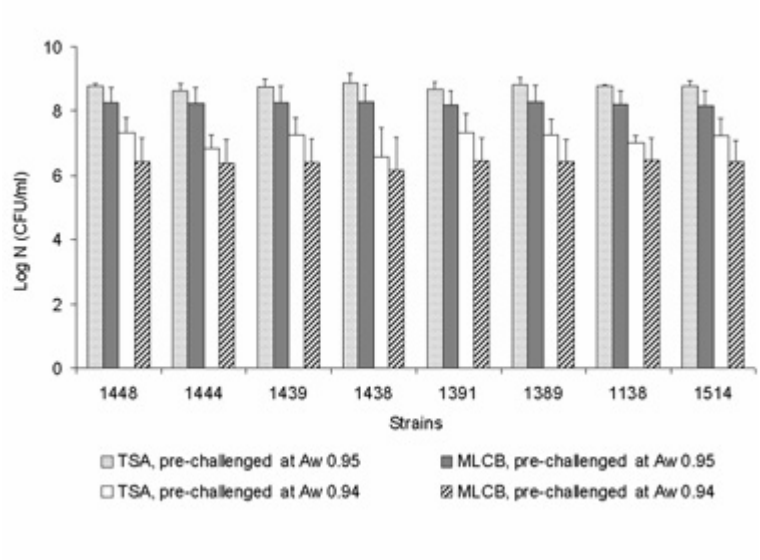


Figure 5.3. Culturability on TSA and MLCB agar of *S. enterica* serovar Enteritidis strains pre-challenged for 6 days at 25°C to surfaces at a_w 0.95 and a_w 0.94, n=2.

Cell viability

The culturability on TSA and MLCB of cells pre-challenged to surfaces at a_w 0.95 and 0.94 at 25°C for 6 days are shown in Figure 5.3. Control cells demonstrated equal colony counts on MLCB and TSA (data not shown), indicating that viability of the control cells was not affected by the selective agents present in MLCB. A number of cells that were grown on surfaces with reduced a_w demonstrated inability to form colonies on MLCB, resulting in differences up to 1-log unit in comparison to the log numbers on TSA.

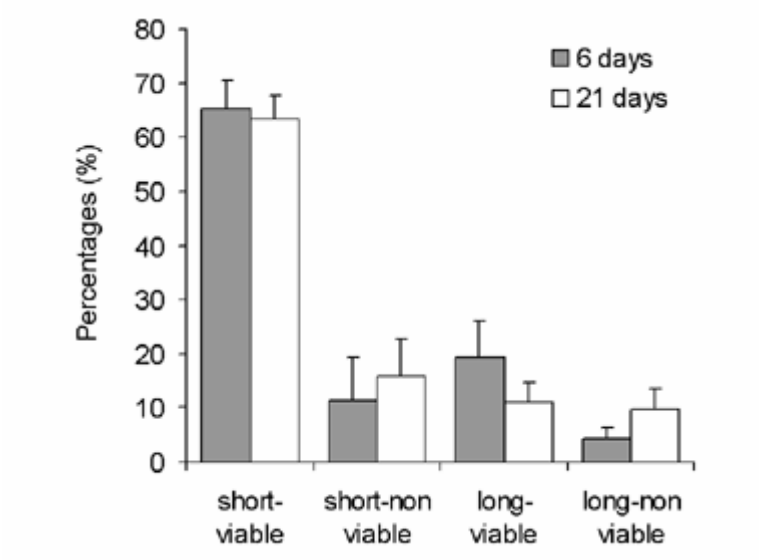


Figure 5.4. Viability of *S. enterica* serovar Enteritidis strain 1448 challenged to surfaces at a_w 0.95 at 25°C, determined by direct microscopy counting after staining with Live/Dead *Bac*Light viability kit, n=2. Cells longer than 6 µm were considered as elongated cells.

Epifluorescence microscopy of the stressed cells revealed the existence of four sub-populations composed of viable short and elongated cells as well as non-viable short and elongated cells (Figure 5.4). The viability was based on assessment of intact or damaged membranes of individual cells. Dead cells with damaged membranes accumulated PI and were fluorescent red. Notably, all elongated cells showed homogeneous fluorescence without indentation. On surfaces at a_w 0.95 at 25°C about 80% and 70% of the total number of the cells were still viable after 6 and 21 days, respectively. Among the elongated cells, the percentage of the viable cells was approximately 75% after 6 days and decreased to 50% after 21 days of exposure. At a_w 0.94, about 50% of the cell population lost their viability after 6 days (data not shown), indicating that exposure at a_w 0.94 resulted in more rapid loss of viability than at higher a_w. Furthermore, when the elongated cells were recovered in BHI broth and incubated at 37°C, the majority of the filaments split up and the division was complete within approximately 3 hours as was revealed by microscopy analysis (data not shown).

Figure 5.5 shows dot plots of events collected by flow cytometry of the control cells (A) and cells challenged to surfaces at a_w 0.94 (B), subsequently stained with the *Bac*Light kit. FL1 is a measure for viable, green fluorescence of SYTO® 9-stained cells. The control cells demonstrated low signals on SSC with high signals on FL1, indicating that almost all cells were viable. Cells challenged to surfaces at a_w 0.94 revealed a shift in the population to higher SSC signals, indicating the formation of elongated cells as shown before in figure 5.2C. This population consisted of two sub populations either with high and low signals on FL1, indicating viable and dead cells, respectively (Figure 5.5B). These results confirmed the findings obtained by the fluorescence microscopy analysis, which indicated that after exposure to surfaces at 0.94 for 6 days at 25°C the majority of the cells were elongated and approximately 50% of the cells were viable.

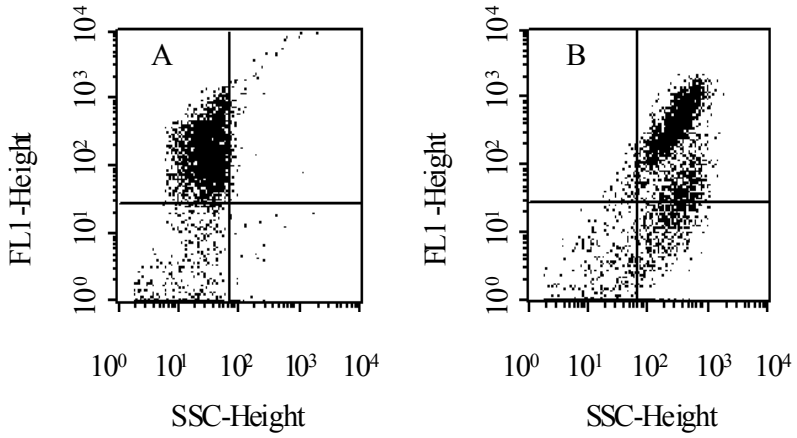


Figure 5.5. Green fluorescence- (FL1) versus Side scatter signals (SSC), obtained by flow cytometry, of control cells of *S. enterica* serovar Enteritidis (A) and cell challenged to surfaces at a_w 0.94 (B) at 25°C for 6 days, subsequently stained with Live/Dead *Bac*Light viability kit.

Cross-protection to sodium hypochlorite

The tolerance to hypochlorite of cells grown on TSA surfaces (control) and pre-challenged to surfaces with reduced a_w at 25°C is shown in Figure 5.6. The test was carried out with addition of bovine serum albumin (3g/l) as interfering organic substance, simulating practical conditions. At a chlorine concentration of 300 ppm, a 3-log reduction was observed for control cells after 60 minutes of exposure. Cells that were pre-challenged to a_w 0.95 were the most tolerant to the treatment, followed by the cells challenged to a_w 0.94 and 0.97. Here log reductions of 1.2, 2.5 and 2.6 log unit, respectively, were found.

At 400 ppm, a higher killing efficiency was found with the same trend. After 60 minutes of exposure, more than a 5-log reduction was observed for the control cells, while cells pre-challenged to a_w 0.95 were reduced with approximately 3-log units. The cells that

were pre-challenged to a_w 0.97 and 0.94 decreased with approximately 4.5-log units. Overall, the cells challenged to reduced a_w surfaces demonstrated better tolerance to hypochlorite than the control cells.

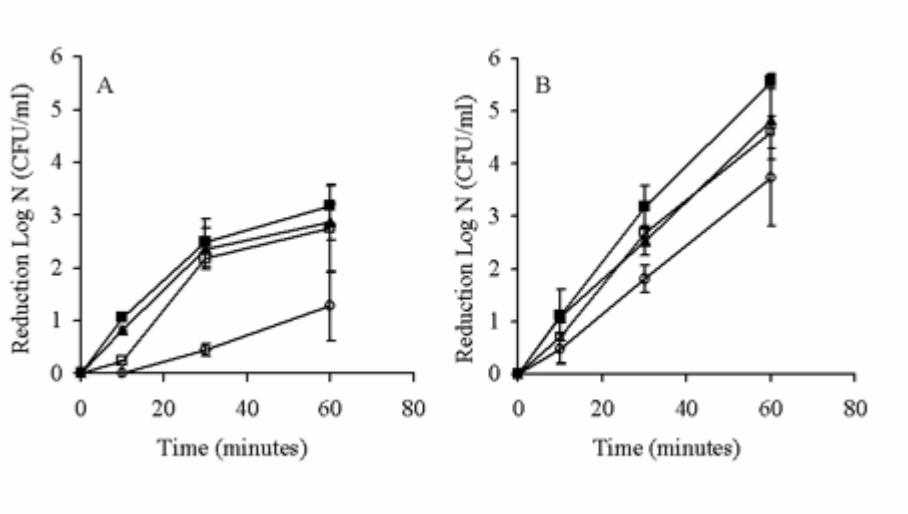


Figure 5.6. Tolerance to sodium hypochlorite of *S. enterica* serovar Enteritidis strain 1448 at (A) 300 ppm and (B) 400 ppm, n=3. (■) Control cells, (▲) cells pre-challenged to surfaces at a_w 0.97, (○) cells pre-challenged to surfaces at a_w 0.95 and (□) cells pre-challenged to surfaces at a_w 0.94.

Discussion

In this study, the response of eight wild type strains of *S. enterica* serovar Enteritidis to reduced a_w environments was analysed. Challenge to surfaces at a_w 0.95 and 0.94 resulted in cell elongation of *S. enterica* serovar Enteritidis. The percentage of filamentous cells at a_w 0.94 was up to 90% of the total number of cells. On *Salmonella*-contaminated glass surfaces cell filamentation also occurred in low percentages after a slow-drying process. It is conceivable that cell elongation resulted from inactivation or inhibition of cell division proteins, which in turn block the septation during the cell division (76, 103). It has been reported that FtsZ is by far the most highly conserved of the known cell division proteins, and is also present in most species of bacteria (76, 103). Next to FtsZ, when any one of the cell division proteins, including FtsA, FtsI, FtsK, FtsL, FtsN, FtsQ, FtsW and ZipA is non-functional or absent, cells grow without dividing, which leads to the formation of filaments (16).

The filamentation of the cells resulted in higher signals on FSC and SSC in flow cytometry analysis. FSC light is laser light diffracted around the cells and is related to cell surface area. SSC light is reflected and refracted laser light, and is related to the internal complexity or granularity of a cell (22). Using flow cytometry a large population of cells, i.e.

10,000 events, was measured simultaneously, offering substantial information on the morphological heterogeneity of this particular bacterial population.

In a preliminary experiment in this study it was observed that the filamentation of the cells resulted in an increase of the optical density in broth without apparent increase in colony forming units (data not shown), indicating that the filamentous cells form single colonies on plates. However, when these elongated cells were recovered under favourable conditions, the filaments could split up and form numerous single cells after approximately 3 hours. This could give a significant impact on the public health risk when it would occur in practical situations.

Since not all cells were elongated, as particularly observed on surfaces at a_w 0.95, the response of *S. enterica* serovar Enteritidis strains to a_w reduction was most likely at the level of the individual cells. Booth (9) suggested that response to stress largely takes place at the single cell level, and can lead to heterogeneity in a bacterial population. This heterogeneity is a recognized property of bacterial populations and a consequence of the mechanisms adopted by bacteria to ensure adaptability to a diversity of niches. Any protein that is required for survival is capable of contributing to the heterogeneity (9).

Essential to survival under stress conditions are the integrity of the cell membrane, maintenance of the folding of proteins and the integrity of the DNA (9). Discrimination between intact and permeable cells by fluorescent stains has been used in many studies on viability of bacteria (22). Propidium iodide is commonly used as a cell death marker because intact membranes exclude this probe. The fluorescence conferred by this probe is therefore generally associated with cells that have lost their membrane integrity. Examining the cell viability using fluorescent techniques highlighted the heterogeneity of bacterial populations in response to challenge to low a_w surfaces since viable short- and elongated cells, and non-viable short- and elongated cells were observed. Moreover, fluorescent techniques in combination with flow cytometry supply detailed information on the heterogeneity in morphology and viability of bacterial populations at the single cell level.

Studies have demonstrated the fact that *Salmonella* cells adapted to certain stress conditions show cross protection against other stresses (79, 105). In this study we investigated the effect of sodium hypochlorite on cells pre-challenged cells to low a_w surfaces. When mixed with water, sodium hypochlorite dissociates and forms hypochlorous acid (HOCl), an active form of chlorine. HOCl is an effective disinfectant partly because most microorganisms do not posses specific enzymatic mechanisms for detoxification of HOCl such as they do for other oxidants like reactive oxygen species (70). This study indicated that the cells pre-challenged to the low a_w showed better tolerance against sodium hypochlorite than the control cells. The cells that were exposed to a_w 0.95 demonstrated better coping with hypochlorite than the cells that were exposed to a_w 0.94. As indicated before, at a_w 0.95 after 6 days more than 80% of the cells retained their membrane integrity, while at a_w 0.94 approximately 50% of the population demonstrated membrane damage.

The survival of *S. enterica* serovar Enteritidis on surfaces with reduced a_w, until at least a_w 0.94 increases the risk of cross-contamination since these tolerant cells are likely to come into contact with foodstuff placed and/or processed on these surfaces. The possible presence of elongated cells on surfaces should also be considered as potential infection risk, since these filaments are still viable for days and can split up under favourable conditions in foodstuff rapidly, resulting in a large number of viable cells. Furthermore, the existence of a population tolerant to hypochlorite after challenge to low a_w surfaces poses an important implication for public health. These populations are possibly better able to survive disinfection in processing plants or household surfaces and thus require increased attention in the disinfection procedures of surfaces in these environments.

Acknowledgement

The authors thank Kaouther Ben-Amor for her valuable comments on flow cytometry assessments.

Chapter 6

A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* via domestic kitchen surfaces

Abstract

Epidemiological data indicate that cross-contamination during food preparations in the home contributes noticeably to the occurrence of foodborne diseases. Inclusion of a cross-contamination model in exposure assessments will therefore be helpful in development and evaluation of interventions to control the spreading of pathogenic bacteria to prevent the occurrence of foodborne diseases. A quantitative analysis was carried out to estimate the probability of contamination and the levels of *Salmonella* and *Campylobacter* on salad as a result of cross-contamination from contaminated chicken carcass via kitchen surfaces. Data on prevalence and numbers of these bacteria on retail chicken carcasses and the use of unwashed surfaces to prepare foods were collected from literature. The rates of bacterial transfer were collected from laboratory experiments and literature. Monte Carlo simulations with incorporation of input parameter distributions were used to estimate the contamination of the product. The results have shown that the probability of *Campylobacter* contamination on salads is higher than that of *Salmonella* since both the prevalence and the levels of *Campylobacter* on chicken carcass are higher than those of *Salmonella*. It is realistic to expect that a fraction of the human exposure to, particularly, *Campylobacter* originates from cross-contamination in private kitchens during food handling. The probability of illness caused by *Campylobacter* is generally 3 orders of magnitude higher than that caused by *Salmonella*. It is important to use separate surfaces or to properly wash the surfaces between preparation of raw and cooked foods or ready to eat foods to cut the cross-contamination route.

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Submitted for publication

Introduction

Salmonella and *Campylobacter* are zoonotic pathogens, with many animal species serving as reservoirs. These microorganisms are the most common cause of human enteritis in western European countries (136) and the United States (94) during the last five years. Surveillance data often associate *Salmonella* and *Campylobacter* related illness with the consumption of raw or undercooked contaminated product and cross-contamination during food preparation (136).

In order to develop strategies to reduce the risk of infection with *Salmonella* or *Campylobacter,* a number of risk assessment studies have been carried out in poultry meat production chains (12, 35, 36, 86). Risk assessment includes hazard identification, hazard characterization, exposure assessment, and risk characterization. Exposure assessment is the estimation of how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of the microorganisms are likely to be ingested (64). In the later phase in a risk assessment these variables are combined with the other estimates, including the consumption patterns and the dose/response relationship to estimate the probability of illness that might occur per serving in a given population (64).

Exposure assessment at a consumer level is of particular interest, because it is less controlled than the other phases in food processing (85), and at this point the contamination levels on foods are directly related to the public health. As preparation of safe foods is important, incorporation of cross-contamination models in exposure assessments during food handling at this level will greatly help to design control interventions for the risks. Modelling can facilitate to improve estimates and allow quantification of food safety risks (30). Once the model has been developed, the impact of various control strategies and trends can be simulated (65).

The objective of this study was to estimate the probability and the levels of contamination of *Salmonella* and *Campylobacter* on foods due to cross-contamination. The probability of illness by consuming the contaminated foods was also predicted. Cross-contamination is defined as the transmission of pathogens from naturally contaminated sources to the finished product. As an example, bacterial transfer from contaminated chicken carcass via unwashed cutting board to salad vegetables was studied in this paper. Assuming that the prevalence of *Salmonella* and *Campylobacter* on raw product and their transfer rates do not have a single, constant value, they were described by probability density functions. Using these functions, both variability (random effect of chance) and uncertainty (lack of precise knowledge) of the variables can be incorporated (64). Monte Carlo simulations were used to estimate the distribution of prevalence of contamination and the levels of microorganisms on contaminated salad. The model obtained will be useful to develop and evaluate strategies to control the risks caused by cross-contamination during food preparation.

Materials and methods

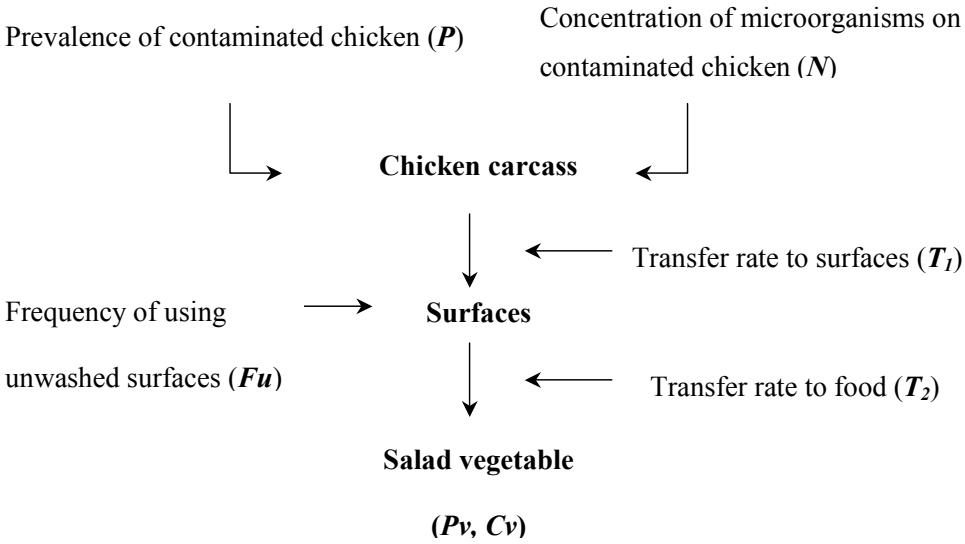

Figure 6.1. Model of cross-contamination from contaminated retail chicken carcass to salad vegetable via unwashed surfaces

Cross-contamination model

A model of bacterial cross-contamination from retail chicken carcass to salad vegetable, e.g. cucumber slices, is shown in Figure 6.1. The probability that salad vegetables are contaminated (*Pv*), and the estimation of the level of contamination (*Cv*) on salad in CFU/cm^2, are determined as:

$Pv = P \times Fu$ *(1)*

$Cv = N \times T_1/100 \times T_2/100$ *(2)*

with *P:* prevalence of contaminated chicken carcass at retails (-)

Fu: frequency of using unwashed surfaces (-)

N: levels of microorganisms on contaminated chicken carcass (CFU/cm^2 carcass)

T_1: transfer rates from chicken carcass to surfaces
(CFU/cm^2 surface)/(CFU/cm^2 carcass) x 100%

T_2: transfer rates from surfaces to salad vegetable
(CFU/cm^2 salad vegetable)/(CFU/cm^2 surface) x 100%

Data collection

Data on prevalence and levels of *Salmonella* and *Campylobacter* on raw chicken carcass from retails, frequency of using unwashed surfaces, and consumption of vegetables were collected from the literature.

The transfer rates of *Salmonella* and *Campylobacter* from chicken carcass to surfaces were collected by laboratory experiments. For this, 5 ml of bacterial cell suspension of

approximately 10^6 CFU/ml was applied onto approximately 150 g portion of raw chicken breast meat with skin and held at room temperature for 15 minutes to facilitate attachment. The levels of microorganisms on these artificially contaminated chicken breast meats were determined by sampling of a 5 x 5 cm ^{2} area using cotton swabs, which thereafter were suspended in 10 ml of peptone saline solution and subsequently enumerated on Tryptone Soy agar and selective media as described in Kusumaningrum et al. (62). The chicken portion was then put on a stainless steel surface (20 x 20 cm^2) with unsampled part on the contact surface. After 5 minutes contact the chicken portion were removed, followed by sampling of the surface area where the chicken portion was placed. The preparation of cell cultures and dilutions as well as sampling and enumeration of the microorganisms on surfaces were carried out following procedures described in previous work (62).

The transfer rate data of *Salmonella* and *Campylobacter* from stainless steel surfaces to cucumber slices were collected from previous work (62) and additional experiments that were carried out with the same procedures. In addition, data on transfer of *Enterobacter aerogenes* from chicken breast meat via cutting board to lettuce reported by Chen et al. (17) were also included and compared with the results of this study.

Data analysis

Data from literature were compiled and tabulated. As bacterial contamination levels on chicken carcass are expressed in CFU/carcass, the numbers were converted to CFU/cm^2 carcass using the following formula:

Total CFU/cm^2 carcass = CFU on carcass/ total carcass area (cm^2) *(3)*

with CFU carcass = CFU/ml x ml used to rinse the carcass

Total carcass area (cm^2) = 0.87 w + 635 (127)

w: weight of carcass (g), assumed to be 1,415 g (128)

From the laboratory experiments, the total bacterial counts of each sample were determined and the appropriate transfer rate was calculated as:

Transfer rate (%) = (CFU/cm^2 on destination)/(CFU/cm^2 on source) x 100% *(4)*

Dot plots were generated using Excel software (Microsoft, Redmond, WA). Both bacterial counts and percentages of transfer rates were log transformed to obtain normally distributed errors. Statistical analysis was performed using univariate analysis of variance in SPSS software with significance level of 5% (SPSS Inc., Chicago, USA).

The serving size of salad vegetable was generated from daily vegetable consumption in The Netherlands (40). As the consumption size was expressed in a weight unit (g), while the levels of contamination on cucumber were expressed in CFU/cm^2, the weight of serving size was transformed to cm^2 using formula:

cm^2 serving = $(w_{consumption} / w_{slice}) \times \pi d^2/4$ *(5)*

with $w_{consumption}$: weight of vegetable consumption (g)

w_{slice}: weight of a cucumber slice with approximately thickness of 0.3 cm (g)
d: diameter of cucumber (cm);
w_{slice} and *d* cucumber were measured during experiments

Probability distribution functions and Monte Carlo simulation

Literature and experimental data were transformed into appropriate probability distribution functions. Data sets for T_1 and T_2 were fitted to theoretical distributions using Bestfit (@Risk software version 4, Palisade Corporation, Newfield, N.Y., USA). The accuracy of fit of a distribution was ranked using the Anderson-Darling (A-D) test that highlights equal emphasis on fitting a distribution at the tails as well as the main body (134).

Monte Carlo simulation with Latin-Hypercube sampling was carried out to simulate the distribution of contamination probability and levels of *Salmonella* and *Campylobacter* in salad vegetable as a result of cross-contamination (@Risk software; 10,000 iterations). The result is a probability distribution of contamination levels of salad vegetables.

Probability of illness (P_{ill}) by consumption of contaminated salad was estimated with the exponential model (123):

$$P_{ill} = 1 - e^{-(rCe)} \qquad (6)$$

with *r*: specific constant for the pathogen
Ce: level of exposure (CFU), obtained by multiplication of contamination levels on salad (*Cv,* CFU/cm^2) with serving size (cm^2)

Results

Prevalence and levels of Salmonella and Campylobacter on retail chicken carcass

Presence of *Salmonella* and *Campylobacter* were qualitatively found in 4% to 53% and 26 to 83% of retail chicken carcasses, respectively (Tables 6.1 and 6.2). These data were collected from studies that were carried out between 1999 and 2002 to reflect recent situations as most as possible. Among these data sets, however, quantitative numbers of *Salmonella* on chicken carcass have only been indicated in one study in the UK, in which 0.8% of chicken carcass was positive by direct counting, although 25% positive by enrichment (52). Countable levels of *Campylobacter* have been indicated in 41% and 18% of chicken carcass in the UK (52) and The Netherlands (26), respectively. For further assessment, the levels of *Salmonella* and *Campylobacter* on chicken carcass were generated from Jørgensen et al. (52) since the levels in that study are expressed as log CFU/carcass, not as MPN/carcass as indicated by Dufrenne et al. (26).

Table 6.1. Prevalence of *Salmonella* on retail chicken carcass

Source	No. of samples	No. of positives	Prevalence (-)	$F(x)^d$	Reference
Zhao et al., 2001	212	9 [c]	0.04	0.21	(138)
van der Zee et al., 2002	241	34 [c]	0.14	0.45	(130)
Dufrenne et al., 2001	89	19 [a]	0.21	0.54	(26)
Jørgensen et al., 2002	241	60 [c]	0.25	0.77	(52)
Uyttendaele et al., 1999	133	45 [b]	0.34	0.91	(129)
Harrison et al., 2001	95	50 [c]	0.53	1.00	(34)
Total	1011	217	0.21		

[a] Defined positive if MPN/carcass > 10

[b] Defined positive if > 1 CFU/100cm^2 or 25 g

[c] Positive by enrichment

[d] *F(x)* is the cumulative probability, with $n_i/(n+1)$, where n_i is the number of the observed data point and n is the sum of the number of data points (number of samples).

Table 6.2. Prevalence of *Campylobacter* on retail chicken carcass

Source	No. of samples	No. of positives	Prevalence (-)	$F(x)^d$	Reference
Uyttendaele et al., 1999	133	34 [b]	0.26	0.11	(129)
van der Zee et al., 2002	241	81 [c]	0.34	0.32	(130)
Dufrenne et al., 2001	89	56 [a]	0.63	0.39	(26)
Zhao et al., 2001	184	130 [c]	0.71	0.55	(138)
Harison et al., 2001	95	73 [c]	0.77	0.63	(34)
Jørgensen et al., 2002	241	199 [c]	0.83	0.83	(52)
Kramer et al., 2000	198	165 [c]	0.83	1.00	(60)
Total	1181	738	0.62		

[a] Defined positive if MPN/carcass > 10

[b] Defined positive if > 1 CFU/100cm^2 or 25 g

[c] Positive by enrichment

[d] *F(x)* is the cumulative probability, with $n_i/(n+1)$, where n_i is the number of the observed data point and n is the sum of the number of data points (number of samples).

As different types of samples were used by Jørgensen et al., including neck-skin, carcass-rinse without skin, carcass-rinse with whole skin, and carcass-rinse with neck-skin, in the present study, only data from carcass-rinse with whole skin were used. For Campylobacter, the data set includes 91 positives from 101 samples, in which 41 samples of the positives are countable by direct plating. As the detection limit of the direct counting was 800 CFU/carcass, the levels of the positive samples below this detection limit, were assumed to be between 0 to 2.89 log CFU/carcass. These data were transformed to log CFU per cm^2 with formula *(3)* using an average *w* value of 1,415 g (128), and are shown in Table 6.3.

Table 6.3. Levels of *Salmonella* and *Campylobacter* on retail *Salmonella* and *Campylobacter* positive chicken carcass [a]

Bacteria	Levels (Log CFU/carcass)	Calculated levels [d] (Log CFU/cm2)	n	*F(x)* [e]
Salmonella[b]	0 - 2.89 [f]	(-3.27) - (-0.38) [f]	58	0.95
	2.9 - 3.99	(-0.37) - 0.72	1	0.97
	4.0-4.99	0.73 - 1.72	1	0.98
		Total	60	
Campylobacter[c]	0 - 2.89 [f]	(-3.27) - (-0.38) [f]	50	0.54
	2.9 - 3.99	(-0.37) - 0.72	9	0.64
	4.0-4.99	0.73 - 1.72	13	0.78
	5.0-5.99	1.73 - 2.72	14	0.93
	6.0-6.99	2.73 - 3.72	4	0.98
	9.0-9.99	5.73 - 6.72	1	0.99
		Total	91	

[a] Generated from Jørgensen et al., 2002 (52)

[b] Using 60 positives of 241 samples

[c] Using carcass-rinse with whole skin data, with 91 positives of 101 samples

[d] Calculated with formula = CFU on carcass/(0.87*w* + 635), (127); with *w* = 1,415 g (128)

[e] *F(x)* is the cumulative probability, with $n_i/(n+1)$, where n_i is the number of the observed data point and n is the sum of the number of data points (number of samples)

[f] Positive by enrichment but below the detection limit by direct counting (<800 CFU/carcass)

Table 6.4. Prevalence of using unwashed cutting board between preparation of raw and cooked or ready to eat food

Source	Number	Unwashed use		$F(x)$ [a]	Remark	Reference
	of samples	Number	Prevalence (-)			
Voedingcentrum, The Netherlands, 1999	145	4	0.03	0.04	use as is/wiped with damp cloths	(132)
Worsfold and Griffith, UK, 1997	108	9	0.08	0.06	use dirty surfaces	(137)
Food Safety Authority Ireland, 1998	1,000	180	0.18	0.31	rarely/never washed	(49)
Klontz et al., US, 1995	1,620	421	0.26	0.70	use as is/wiped with damp cloths	(58)
Jay et al., Australia, 1999	1,203	457	0.38	1.00	use as is/wiped with damp cloths	(50)
Total	4,076	1,071	0.26			

[a] $F(x)$ is the cumulative probability, with $n_i /(n+1)$, where n_i is the number of the observed data point and n is the sum of the number of data points (number of samples).

Frequency of using unwashed surfaces

Prevalence of using unwashed surfaces between preparation of raw and cooked foods or ready to eat foods is shown in Table 6.4. On average, 26% of the consumers did not wash the surfaces between preparation of raw and cooked foods or ready to eat foods. However, the same studies also indicated that only approximately 60% of the consumers always washed the surfaces between the preparations of raw and ready to eat foods (49, 50, 58, 132). This indicates that the prevalence of risky practices that could lead to cross-contamination are likely even higher than the data given in Table 6.4.

Cross-contamination from chicken to foods via surfaces

The transfer rates of *Salmonella* and *Campylobacter* from artificially contaminated chicken carcass to stainless steel surfaces and from these surfaces to cucumber slices are presented in Figures 6.2 and 6.3, respectively. In addition to these data, the transfer rates of *E. aerogenes* from artificially contaminated chicken carcass to plastic cutting board and subsequently to lettuce from Chen et al. (17) are included. Statistical analysis indicated that the transfer rates of *E. aerogenes* from chicken carcass to plastic cutting board were significantly different ($p<0.05$) from *Salmonella* and *Campylobacter* from chicken carcass to stainless steel surfaces. With the assumption that *E. aerogenes* is an indicator bacterium with attachment characteristics similar to that of *Salmonella* (17, 139), it is likely that the transfer rates of bacteria from chicken carcass to plastic cutting board were higher than that to stainless steel surfaces.

It is not possible to compare the bacterial transfer rates from stainless steel surfaces and from plastic cutting board to salad vegetables as different microorganisms, source of surfaces and destinations were implied.

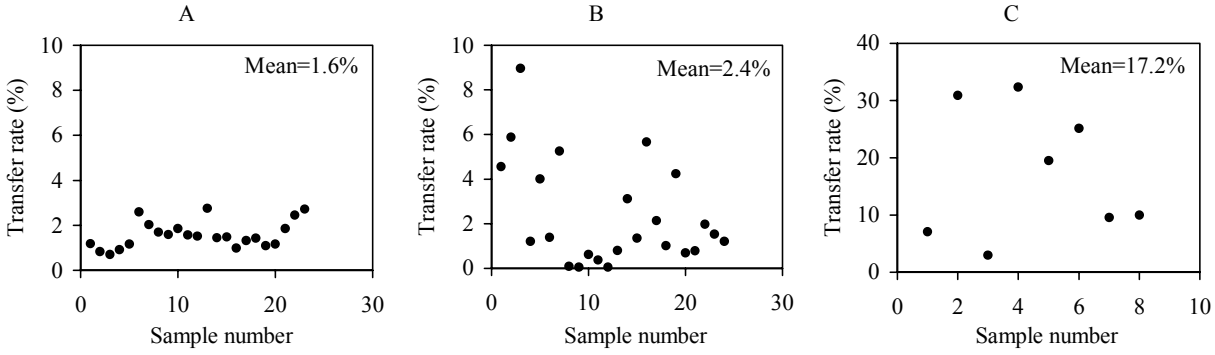

Figure 6.2. Transfer rates of *Salmonella* (A) and *Campylobacter* (B) from chicken carcass to stainless steel surfaces, and *E. aerogenes* (C) from chicken carcass to plastic cutting boards (17)

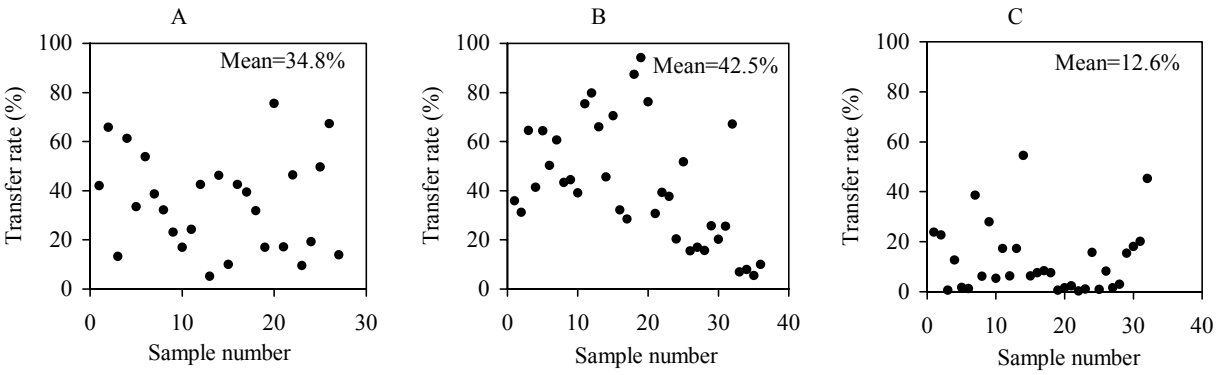

Figure 6.3. Transfer rates of *Salmonella* (A) and *Campylobacter* (B) from stainless steel surfaces to cucumber slices (62), and *E. aerogenes* (C) from plastic cutting boards to lettuce (17)

Input distributions

Distributions of prevalence of *Salmonella* and *Campylobacter* on retail chicken carcass were generated from the data sets in Tables 6.1 and 6.2 and are given in Table 6.5. The prevalence was described by a cumulative distribution assuming a minimum and maximum prevalence of 1 and 60%, respectively, for *Salmonella,* and of 1 and 90%, respectively, for *Campylobacter*. Cumulative distributions were also used to describe the levels of bacteria on positive contaminated chicken carcass, assuming a minimum and maximum probability of –2.0 and 2.0 log CFU/cm^2, respectively, for *Salmonella,* and of –2.0 and 7.0 log CFU/cm^2, respectively, for *Campylobacter*. Since the data were given in log level ranges, the mean log level of each range was used in the estimation of the contamination levels. The cumulative distribution was also chosen to describe data of using unwashed surfaces. Therefore, a minimum and a maximum prevalence of 1 and 40%, respectively, were assumed.

Normal distributions were selected to describe the log-transformed transfer rates from one surface to another surface for all test microorganisms because of their adequate goodness of fits and statistical convenience. Normal distributions were usually ranked first or second in goodness of fit using the A-D test. Next to normal distributions, logistic distributions were the best fit describing particular transfer rates. The normal distributions included in Table 6.5 indicate the mean values and the standard deviations of the log transfer rates.

Table 6.5. Input distributions for respective parameters

Bacteria	Variable	Distribution	Rank[a]
Salmonella	*P*	RiskCumul(0.01,0.60,{0.04,0.14,0.21,0.25,0.34,0.53},{0.21,0.45,0.54,0.77,0.91,1.00})	
	N (Log)	RiskCumul(-2,2,{-1.82,0.175,1.225},{ 0.95,0.97,0.98}) [b]	
	T_1 (Log %)	RiskNormal(0.171, 0.162)	1
	T_2 (Log %)	RiskNormal(1.458, 0.298)	2
Campylobacter	*P*	RiskCumul(0.01,0.90,{0.26,0.34,0.63,0.71,0.77,0.83,0.83},{0.11,0.32,0.39,0.55,0.63,0.83,1.00})	
	N (Log)	RiskCumul(-2,7,{-1.82,0.175,1.225,2.225,3.225,6.225},{ 0.54,0.64,0.78,0.93,0.98,0.99}) [b]	
	T_1 (Log %)	RiskNormal(0.098, 0.606)	2
	T_2 (Log %)	RiskNormal(1.535, 0.320)	2
E. aerogenes	T_1 (Log %)	RiskNormal(1.146, 0.452)	1
	T_2 (Log %)	RiskNormal(0.769, 0.626)	1
	Fu	RiskCumul(0.01,0.40,{0.03,0.08,0.18,0.26,0.38},{0.04,0.06,0.31,0.70,1.00})	

[a] Normal distribution rank by A-D test

[b] Using the mean of the range of log values

Simulations and scenarios

Simulated distributions of the probability that salad vegetable will be contaminated with *Salmonella* and *Campylobacter* and the levels of these pathogens on salad vegetable, due to cross-contamination via surfaces from chicken carcass, were obtained (Figures 6.4 and 6.5). The y-axis gives the probability density. The mean value of the probability of occurrence of contamination with *Salmonella* was 4% with a 90% confidence interval of 0.3 to 10 %. Contamination with *Campylobacter* was estimated to occur in a higher percentage than *Salmonella* with a mean value of 13% and a 90% confidence interval of 1 to 27%.

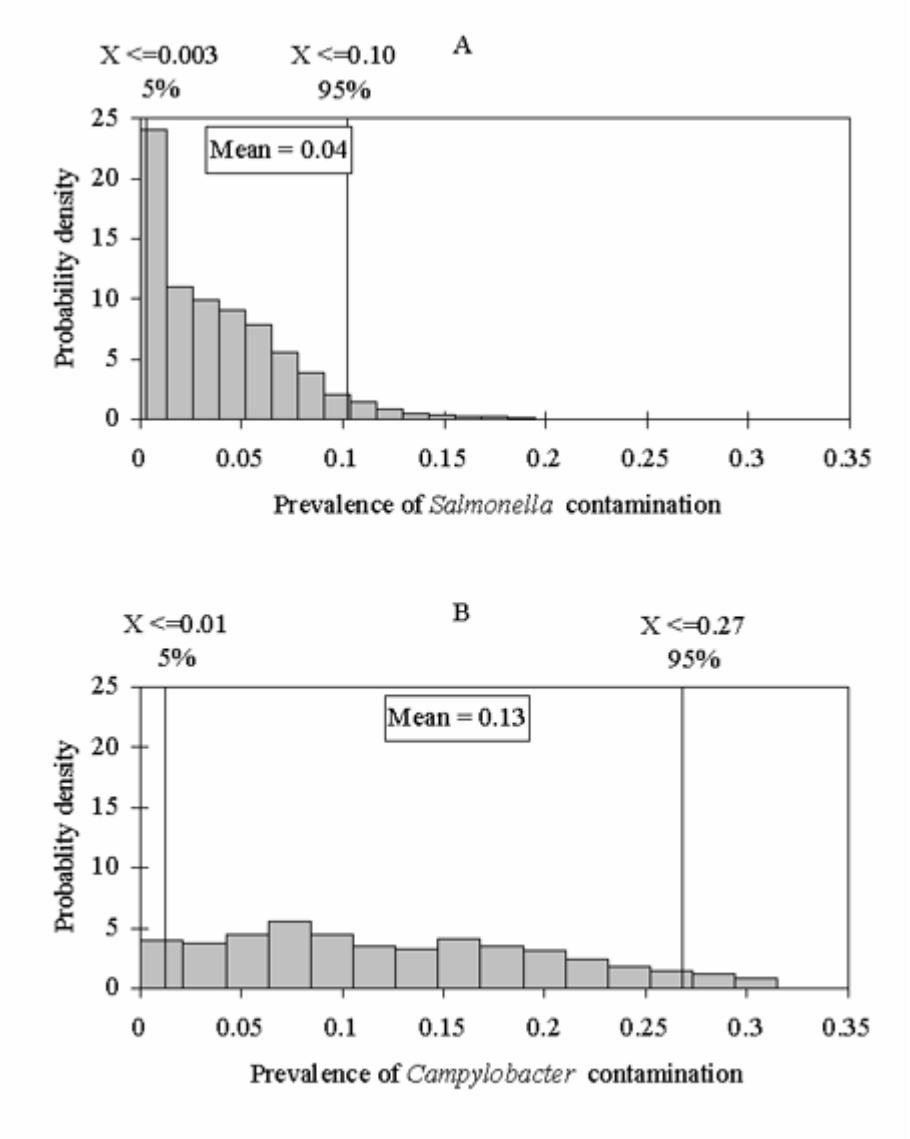

Figure 6.4. Probability density of prevalence that cucumber is contaminated with (A) *Salmonella* and (B) *Campylobacter* due to cross-contamination via unwashed surfaces

The levels of *Salmonella* on salad vegetable due to cross-contamination via unwashed surfaces expressed as log CFU/cm^2 was estimated with a mean value of –4.2 (Figure 6.5). For contamination with *Campylobacter,* the cucumber slices may have a mean value of –2.9 log

CFU/cm^2 with a 90% confidence interval of –5.2 to 0.5 log CFU/cm^2. This means that 5% of the cucumber slices may be contaminated with *Campylobacter* with a level of less than 1 CFU/cm^2, but also 5% of the cucumber slices may be contaminated with 3.2 CFU/cm^2 or more. Prevalence of *E. aerogenes* contamination could not be estimated due to lack of data of the frequency of contamination of these bacteria on chicken carcass.

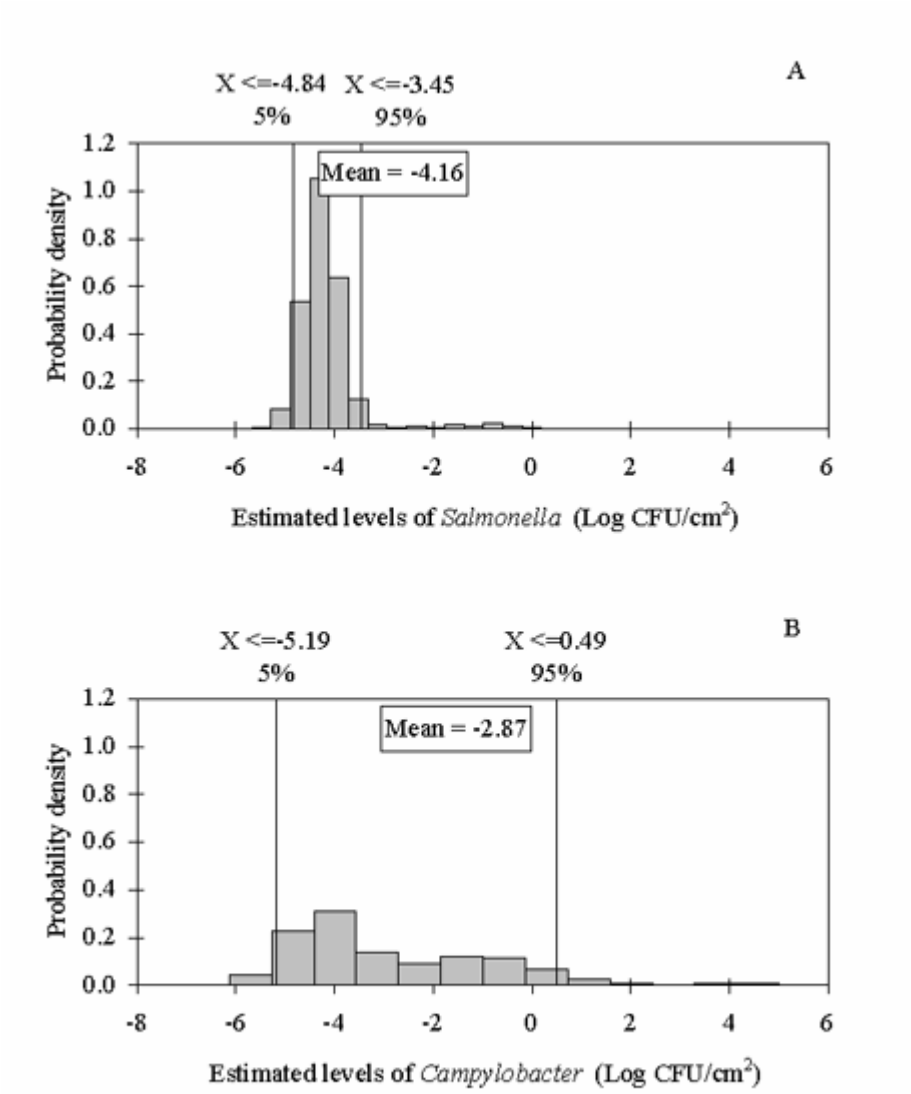

Figure 6.5. Probability distributions of estimated contamination levels of (A) *Salmonella* and (B) *Campylobacter* on cucumber slices due to cross-contamination via unwashed surfaces

Table 6.6 shows the results of a deterministic approach and Monte Carlo simulation to estimate the probability and the levels of pathogens on salad due to cross-contamination and the probability of illness caused by consuming contaminated salads. For this, the log transformed data of the contamination levels on chicken carcasses and the transfer rates were used.

Table 6.6. Prevalence of contaminated salad (*Pv*), levels of contamination on salad (*Cv*), and probability of illness (*Pill*) due to cross-contamination, estimated by deterministic calculation and simulation using the log-transformed data

Variable/Process		Unit/Formula	*Salmonella*			*Campylobacter*		
			Mean [a]	Worst Case	Mean-Monte Carlo simulation	Mean [a]	Worst case	Mean-Monte Carlo simulation
Input								
P	Prevalence contaminated chicken	-	0.21	0.53	0.18	0.62	0.83	0.59
Fu	Frequency using unwashed surface	-	0.26	0.38	0.22	0.26	0.38	0.22
N	Contamination levels on chicken	Log CFU/cm^2	-1.74 [b]	1.72	-1.79	-0.26	6.72	-0.51
T1	Transfer rates chicken to surfaces	Log %	0.17	0.44	0.17	0.10	0.95	0.10
T2	Transfer rates surfaces to salad	Log %	1.46	1.88	1.46	1.53	1.97	1.53
Output								
Pv	Prevalence contaminated salad	*P * Fu* (-)	0.06	0.20	0.04	0.16	0.32	0.13
Cv	Estimated levels on contaminated salad (log CFU/cm^2)	*LOG(10^N*10^T1/100 *10^T2/100)*	-4.11	0.04	-4.16	-2.63	5.65	-2.87
Ce	Exposure (log CFU)	*LOG(10^Cv*serving)* [c]	-1.62 [e]	2.52	-1.68 [e]	-0.14 [e]	8.13	-0.39 [e]
Pe	Probability of illness eating contaminated salad	*1-e^-(r*10^Ce)* [d] (-)	3.71x10^{-8}	5.18x10^{-4}	3.26x10^{-8}	2.55x10^{-6}	1.00	1.44x10^{-6}
Pill	Probability of illness per serving	*Pe * Pv* (-)	2.09x10^{-9}	1.04x10^{-4}	1.24x10^{-9}	4.19x10^{-7}	3.17x10^{-1}	1.84x10^{-7}

[a] Calculated from the data sets

[b] Calculated as (Σ(avg log level$_i$ x n$_i$))/n from Table 6.3

[c] Serving = 306 cm^2, generated from Hulshof et al. (40) and transformed to cm^2 using formula *(5),* with weight of a cucumber slice of 6.4 $\pm$ 0.8 g and diameter of 4.0 $\pm$ 0.2 cm, measured by experiments

[d] *r* (*Salmonella*) = 1.55x10^{-6}; *r* (*Campylobacter*) = 3.52x10^{-6} (123)

[e] theoretical value

Variations were found between the results estimated with Monte Carlo simulation in comparison with that by deterministic calculation. During simulation randomly selected single values were used to calculate a mathematical solution, and this sequence was repeated with different set of values for the inputs selected at each iteration, until a predetermined number of iterations (10,000 iterations) have been reached. In a normal situation, the mean value of the prevalence of salad contamination with *Salmonella* was 4% and with *Campylobacter* was 13%, both with levels less than 1 CFU/cm^2, estimated by Monte Carlo simulation. The deterministic estimates of the prevalence of *Salmonella* and *Campylobacter* contamination were 6% and 16%, respectively, both also with levels less than 1 CFU/cm^2. In a worst-case scenario 20% of preparation events results in contamination of cucumber with *Salmonella* with levels of 1.0 CFU/cm^2. Prevalence of contamination with *Campylobacter* in a worst-case scenario was estimated to be 32% with level of $4.5x10^5$ CFU/cm^2.

The serving size was derived from the Dutch national consumption survey that was carried out by Hulshof et al. (40). A mean daily vegetable consumption of 157 $\pm$ 9 g/day by men and women with overall mean age of 43 year was used. This consumption was transformed to cm^2 with formula *(5)*. With an average weight of a cucumber slice of 6.4 $\pm$ 0.8 g and diameter of 4.0 $\pm$ 0.2 cm, obtained by experiments, and with assumption that only one side of cucumber was in contact with surfaces, the calculation resulted in a serving size of 306 cm^2. The mean exposures with *Salmonella* and *Campylobacter* per serving, both by deterministic calculation and Monte Carlo simulation, were estimated to be <1 CFU as indicated by negative log values. These results suggest that, for example, when *Pv* is 0.06 and *Ce* is –1.62 log CFU (0.02 CFU) per serving, the prevalence of contamination is 0.06 x 0.02 = 0.0012, or 1 of 800 servings of salads that prepared will be contaminated with 1 CFU.

Assuming that *r* for *Salmonella* is $1.55x10^{-6}$ and for *Campylobacter* is $3.52x10^{-6}$ (123), the probability of getting ill by consumption of contaminated salad was obtained. If this probability was multiplied with the prevalence of salad contaminated with *Salmonella* and *Campylobacter,* the overall probability of illness were in magnitude of 10^{-9} and 10^{-7} per serving, respectively. This indicates that the infections with *Salmonella* and *Campylobacter* can occur with a proportion of 1 from 1,000 million people and 1 from 10 million people per serving, respectively. For the worst-case situation the proportions will be 1 to 10,000 and 1 to 3 people, respectively.

Table 6.7 shows a deterministic approach to estimate the probability and the levels of pathogens on salad and the probability of illness caused by consuming contaminated salads, using the mean values of contamination levels and transfer rates from the actual data. Numbers are higher since the higher values weigh relatively stronger if compared with that on log scale. With this approach, the overall probability of illness caused by *Salmonella* and *Campylobacter* can occur with proportion of 1 from 10 million people and 1 from 20 people per serving, respectively. The proportion of being ill caused by *Campylobacter* is high.

Table 6.7. Prevalence of contaminated salad (*Pv*), levels of contamination on salad (*Cv*), and probability of illness (*Pill*) due to cross-contamination, estimated by deterministic approach using the actual data

Variable/Process		Unit/Formula	*Salmonella*		*Campylobacter*		
			Mean	Worst case	Mean 1	Mean 2	Worst case
Input							
P	Prevalence contaminated chicken	-	0.21	0.53	0.62	0.62	0.83
Fu	Frequency using unwashed surfaces	-	0.26	0.38	0.26	0.26	0.38
N	Contamination levels on chicken	CFU/cm^2	7.30×10^{-1} [a]	5.24×10^{1}	3.19×10^{4} [a]	1.78×10^{2} [a,b]	2.89×10^{6}
T1	Transfer rates chicken to surfaces	%	1.58	2.76	2.38	2.38	8.97
T2	Transfer rates surfaces to salad	%	34.77	75.58	42.47	42.47	94.20
Output							
Pv	Prevalence contaminated salad	*P * Fu* (-)	0.06	0.20	0.16	0.16	0.32
Cv	Estimated levels on contaminated salad	*N*T1/100*T2/100* (CFU/cm^2)	4.02×10^{-3}	1.09	3.22×10^{2}	1.79	2.44×10^{5}
Ce	Exposure	*Cv*serving* [c] (CFU)	1.23	3.34×10^{2}	9.86×10^{4}	5.49×10^{2}	7.47×10^{7}
Pe	Probability of illness eating contaminated salad	*1-e^-(rCe)* [d] (-)	1.91×10^{-6}	5.18×10^{-4}	2.93×10^{-1}	1.93×10^{-3}	1.00
Pill	Probability of illness	*Pe * Pv* (-)	1.07×10^{-7}	1.04×10^{-4}	4.81×10^{-2}	3.17×10^{-4}	3.17×10^{-1}

[a] Calculated as $(\Sigma(\text{avg level}_i \times n_i))/n$ from Table 6.3

[b] Without 1 sample with contamination level of 10^9 to $10^{9.9}$ CFU/carcass

[c] Serving = 306 cm^2, generated from Hulshof et al. (40) and transformed to cm^2 using formula *(5),* with weight of a cucumber slice of 6.4 $\pm$ 0.8 g and diameter of 4.0 $\pm$ 0.2 cm, measured by experiments

[d] r (*Salmonella*) = 1.55×10^{-6}; r (*Campylobacter*) = 3.52×10^{-6} (123)

Table 6.8. Scenarios to examine the effect of strategies to minimize the probability of illness as a result of consumption of contaminated salad due to cross-contamination with *Campylobacter* using the actual data

			Mean case				Worst case			
	Process/Variable	Unit/Formula	Value	Scenario1	Scenario2	Scenario 3	Value	Scenario1	Scenario2	Scenario3
Input										
P	Prevalence contaminated chicken	-	**0.62**	**0.06**	0.62	0.62	**0.83**	**0.08**	0.83	0.83
Fu	Frequency using unwashed surfaces	-	**0.26**	0.26	0.26	**0.03**	**0.38**	0.38	0.38	**0.04**
N	Contamination levels on chicken	CFU/cm2	**$1.78x10^2$**	$1.78x10^2$	**$1.78x10^1$**	$1.78x10^2$	**$2.89x10^6$**	$2.89x10^6$	**$1.15x10^3$**	$2.89x10^6$
T1	Transfer rates chicken to surfaces	%	2.38	2.38	2.38	2.38	8.97	8.97	8.97	8.97
T2	Transfer rates surfaces to salad	%	42.47	42.47	42.47	42.47	94.20	94.20	94.20	94.20
Output										
Pv	Prevalence contaminated salad	*P * Fu* (-)	0.16	0.02	0.16	0.02	0.32	0.03	0.32	0.03
Cv	Estimated levels on contaminated salad	*N*T1/100*T2/100* (CFU/cm2)	1.79	1.79	$1.79x10^{-1}$	1.79	$2.44x10^5$	$2.44x10^5$	$9.76x10^1$	$2.44x10^5$
Ce	Exposure	*Cv*serving*[a] (CFU)	$5.49x10^2$	$5.49x10^2$	$5.49x10^1$	$5.49x10^2$	$7.47x10^7$	$7.47x10^7$	$2.99x10^4$	$7.47x10^7$
Pe	Probability of illness eating contaminated salad	*1-e^-(rCe)* [b] (-)	$1.93x10^{-3}$	$1.93x10^{-3}$	$1.93x10^{-4}$	$1.93x10^{-3}$	1.00	1.00	0.10	1.00
Pill	Probability of illness	*Pe * Pv* (-)	**$3.17x10^{-4}$**	**$3.17x10^{-5}$**	**$3.17x10^{-5}$**	**$3.17x10^{-5}$**	**0.32**	**0.03**	**0.03**	**0.03**

[a] Serving = 306 cm^2, generated from Hulshof et al. (40) and transformed to cm^2 using formula *(5),* with weight of a cucumber slice of 6.4 $\pm$ 0.8 g and diameter of 4.0 $\pm$ 0.2 cm, measured by experiments

[b] *r* (*Campylobacter*) = $3.52x10^{-6}$ (123)

Scenario 1: Reduction of prevalence of carcass positive; Scenario 2: Reduction of contamination levels on carcass; Scenario 3: Reduction of the use of unwashed surfaces

This is due particularly to a high contamination level on raw chicken carcass, which was up to $>10^9$ CFU/carcass. However, this extra high level was only found in one sample of 91 *Campylobacter* positive carcasses. The other samples were in a range from below 800 (detection limit) to 10^7 CFU/carcass. Neglecting the sample with levels $>10^9$ CFU/carcass, the contamination levels on chicken carcass was estimated to be 1.78×10^2 CFU/cm^2 and resulted in probability of illness of 3.17×10^{-4}, or in a proportion of 1 from 3,000 people per serving.

In Table 6.8 some scenarios are presented to examine the effect of strategies to minimize the probability of illness as a result of consumption of contaminated salad due to cross-contamination with *Campylobacter*. Three distinct ways of reducing the probability of exposure and illness were analysed: by reducing the prevalence of *Campylobacter* positive retail chicken carcass, by reducing the concentration of *Campylobacter* on the contaminated chickens, and by improving the relative level of using washed and/or clean surfaces during food handling. In general mean case, to obtain a reduction in human cases by, for example, a factor 10, the prevalence of contaminated chicken carcass or the level of *Campylobacter* contamination should be reduced by a factor 10. A similar reduction in the number of human cases could be obtained by improving the level of food hygiene in private kitchens by a factor 10. In the worst-case scenario, a 10-factor reduction in the number of human cases could be obtained by reducing the level of *Campylobacter* on the contaminated chickens by a factor 2,500 (3.4 log units), or by the reduction of contaminated chicken carcass as well as decreasing the use of unwashed surfaces by a factor of 10. The fact that in the worst-case scenario a large reduction on the level of *Campylobacter* on chickens is necessary to obtain a 10-factor reduction in the number of human cases due to the very high exposure (*Ce*) value, which is not in the linear part of the dose response curve. As the consumers do not know the degree of contamination on the chicken carcasses that enter the homes, it is obviously important to use separate surfaces or to wash the surfaces between preparation of raw and cooked foods or ready to eat foods.

Discussion

Development of a model, although simplified, can be a helpful tool to evaluate the relationship between cross-contamination and probability of occurrence of related foodborne illness. The results of modelling, however, should be interpreted with care considering the origin of data and assumptions made.

During the development of this exposure assessment model, a number of data gaps were identified. The main data gap concerned the lack of quantitative numbers of contamination levels of *Salmonella* and *Campylobacter* on chicken carcass. Published data generally indicated the prevalence of these bacteria, i.e. the percentage of samples positive, and not its numbers. In some studies quantification of the contamination levels was also carried out. However, it was evident that the *Salmonella* numbers on retail chicken carcass were very low, as samples did not show sufficient numbers to allow their detection by direct

counting (26, 130). Although a lognormal distribution has been used to describe the distribution of salmonellae in chicken products (12), in the present study cumulative distributions were used, since the use of a theoretical or parametric model would be hard to justify with the limited data set and with the limited knowledge on the prevalence and levels of *Salmonella* and *Campylobacter* (71, 134).

Furthermore, the distribution for prevalence and levels were based on the analysis of samples at retails, neglecting transportation to the home and possible storage at home situations. This can possibly lead to an underestimation of the levels of particularly *Salmonella*, as these bacteria can grow at room temperature. Incorporating the effect of transportation and possible storage in the kitchen before food preparation on the contamination levels on chicken carcass, when data are available or by prediction, will give a more accurate description of reality. However, since *Campylobacter* do not multiply at room temperature (44), the levels of *Campylobacter* will not considerably be affected by ignoring these effects.

In this study, stainless steel surfaces were used to prepare the salads. This could lead to an overestimation of the transfer rates to foods, as a preliminary study indicated that the transfer rates from stainless steel surfaces to a rodac plate as a model of salad vegetable were higher than that from plastic cutting boards, with an average percentage of 25% and 6.3%, respectively (data not shown). However, the transfer rates of bacteria from chicken carcass to stainless steel surfaces were likely lower than that to plastic boards. When *Salmonella* and *E. aerogenes* data are compared (Table 6.5) it can be seen that the T_1 value for plastic board is 6 times higher than that for stainless steel surfaces. These results indicate that in a non-continuous contact with bacteria (a single contamination), microorganisms are transferred to stainless steel surface to a less extent than to plastic cutting board, but once a stainless steel surface is contaminated the bacteria are easier transferred to foods. Transfer rates calculated in this study are fail-safe since it is assumed that bacterial transfer takes place immediately until 15 minutes after contamination. Longer time intervals should result in reductions of the surviving numbers (62).

Whether foodborne disease occurs depends on the number of pathogens ingested and the virulence of the pathogen. In some cases the number of microorganisms that is transferred from contaminated product or items to other surfaces will be too low to cause illness. Only in cases where the infectious dose is very low, e.g. *Campylobacter*, or when persons at risk are involved, numbers will be sufficiently high to cause foodborne disease. It follows that in other cases of microbial transfer, subsequent growth of the pathogens will often be necessary to cause illness. The results indicate that the probability of illness caused by *Campylobacter* by the consumption of contaminated cucumber salads due to cross-contamination is higher than that with *Salmonella* since both the prevalence and the contamination levels of *Campylobacter* on chicken carcass are higher. The use of washed and/or separate surfaces will cut one of the cross-contamination routes of bacterial contamination to ready to eat foods and avoid the occurrence of food infection.

This study illustrates how cross-contamination during food preparation in domestic kitchens can be modelled by linking currently available data and experimental data, and has shown that it is realistic to expect that a fraction of the human exposure to, particularly, *Campylobacter* originates from cross-contamination in private kitchens during food handling. The model needs to be validated for strains and products other than those that are used in this study. It should be kept in mind that the quantitative exposure assessment procedure is not a static document as data, assumptions and the models used may be changed as new information is available. This model can be used to assess the importance of cross-contamination and can be used to evaluate interventions. Furthermore, the models can be used in a more extensive microbiological risk assessment to assess the influence of cross-contamination on foodborne disease.

Chapter 7

General Discussion

Behaviour of pathogenic bacteria in household kitchens

Survival on surfaces and cleaning cloths

Domestic kitchen environments are potentially places for spreading pathogenic bacteria, including *S.*Enteritidis, *C. jejuni* and *S. aureus*. Even after a single contamination event, these pathogens survive on stainless steel surfaces for hours or days, depending on the species, initial counts and the presence of food residues (Chapter 2). In the case of repeated or continuous contamination, e.g. in kitchen sink drains, development of biofilms in particular by *S.* Enteritidis and *S. aureus* can be expected, and will result in a longer survival. Following a single contamination and air-drying at room temperatures, pathogens also remain on cleaning sponges for days, with or without the presence of food residues (Chapter 4). With food residues present in the sponges the survival is prolonged.

The common practice of surface cleaning, wiping countertops and other kitchen surfaces reduces microbial counts considerably, particularly when a clean cloth is used. Subsequent air-drying or application of disposable antibacterial-impregnated napkins results in additional reduction of bacterial on surfaces (Chapter 3). However, these napkins are often only intended for specific purposes instead of for daily cleaning activities in the kitchen because of the higher costs as compared to the regular cloths. The use of re-usable wiping cloths remains popular (132); some people are using them in combination with a detergent. It seems that detergents rather than disinfectants are still extensively applied in combination with physical removal (wiping) for surface cleaning in everyday household cleaning activities, particularly in the Netherlands.

The wiping practice, however, tends to transfer microorganisms from the surface to the cleaning cloth when a clean cloth is used (Chapter 3). On the contrary, when the cloths are damp and contain high numbers of microorganisms, bacteria are readily spread from the cloths to the surfaces during wiping (Chapter 2). Keeping the cloth clean is thus obviously an important measure in order to prevent the spreading of microorganisms.

About satisfactory reduction of bacteria in cleaning cloths or sponges there have been extensive discussions in literature particularly in relation with application of antibacterial compounds or disinfectants. The present study indicates that pathogens, including *S.* Enteritidis, *S. aureus* and *B. cereus* survived the treatments with antibacterial dishwashing liquid with or without the presence of food residues (Chapter 4). Application of this product under practical situations in households did not make a significant difference as compared to regular product.

Furthermore, pathogens demonstrated a tolerance to household bleach to certain concentrations in cloths as compared to exposure in a suspension (Chapter 3). Presence of food debris in the cloths, which occurs regularly in practical situations, decreases the effectiveness of antibacterial compounds or disinfectants, leading to the requirement of higher concentrations of these compounds. Food residues interfere with the hypochlorite or antibacterial compounds, and possibly protect the microorganisms from direct contact with

the sanitizers. The neutralising effect of food residues and other organic matter on most sanitizers makes thorough cleaning highly desirable before disinfection to achieve a satisfactory result. Since it is impossible to know the degree of soiling, it is difficult to apply a correct concentration of sanitizer. Therefore, other measures, such as heating, i.e. immersing in boiling water or washing by a dishwash or laundry cycle with detergent at $\geq 60^{o}C$ is recommended to reduce bacterial contamination in cloths.

The effects of immersing contaminated household cloths in boiling water are shown in Table 7.1 together with other physical treatments (47). As shown in this table, a considerable reduction of bacterial counts in sponges or cloths can also be achieved by heating in a microwave (47), but as this apparatus is intended to prepare ready-to-eat foods, other alternative heat treatments are preferable.

Table 7.1. Reduction of bacterial counts in household sponges[a]

Treatment	N	Pre-treatment Counts	Post-treatment Counts	Range of Post-treatment Counts	Bacterial reduction (%)[b]
Laundering without bleach or detergent [c]	3	2.9×10^8	2.8×10^7	8.8×10^6 - 6.0×10^7	90.3
Laundering without bleach [c]	2	2.2×10^7	6.0×10^5	4.2×10^5 -7.7×10^5	97.3
Laundering with detergent and bleach [c]	5	3.6×10^8	6.7×10^3	1.9×10^2 -1.4×10^4	99.998
Dishwashing with heat cycle - without detergent	10	3.0×10^8	12.0×10^5	8.2×10^2 - 1.2×10^6	99.93
Dishwashing with detergent [c] - without heat cycle	2	4.9×10^9	2.4×10^4	6.7×10^3 - 4.2×10^4	99.95
Dishwashing with detergent and heat cycle	10	5.8×10^8	6.7×10^2	2.0×10^1 - 1.4×10^3	99.9999
Boiling	5	1.5×10^9	1.9×10^1	< 10-40	99.999999
Microwave oven	5	1.6×10^9	1.5×10^2	< 10 -360	99.999

[a] Unit of measure is CFU/sponge

[b] Based on average counts, determined by: (Average post-treatment counts - average pre-treatment counts)/ Average pre-treatment counts x 100

[c] At room temperature or max. $40^{o}C$

Source : Ikawa and Rossen (47)

Significance of competitive microorganisms in cleaning cloths

Members of Enterobacteriaceae are frequently found as microflora of household cleaning cloths or sponges, including *E. cloacae* and *E. aerogenes* (114, 117). In the present study *E. cloacae* was isolated in 53% of 55 household cloths and sponges. This is in agreement with the results found by Speirs et al. (117) who recovered *E. cloacae* from 52.2% of 46 household samples, more than double that isolated by Scott et al. (114). Next to *E. cloacae, E. aerogenes* and *E. sakazakii* were isolated in 5% and 9% of dishcloths, respectively.

The competitive microorganisms in sponges, including *Enterobacter,* are likely more tolerant than *Salmonella* against antibacterial dishwashing liquid and hypochlorite treatment (Chapters 3 and 4). Krieg and Holt (61) also indicated that *E. cloacae* was less susceptible to chlorination than *E. coli*. Furthermore, survival experiments in kitchen sponges under different relative humidities revealed that *E. cloacae* survived better than *E. coli* (Figure 7.1). At a relative humidity of 65%, *E. coli* decreased with 3 log-units, whereas the *E. cloacae* number was relatively constant at a high level (10^6 CFU/sponge) during a week of observation.

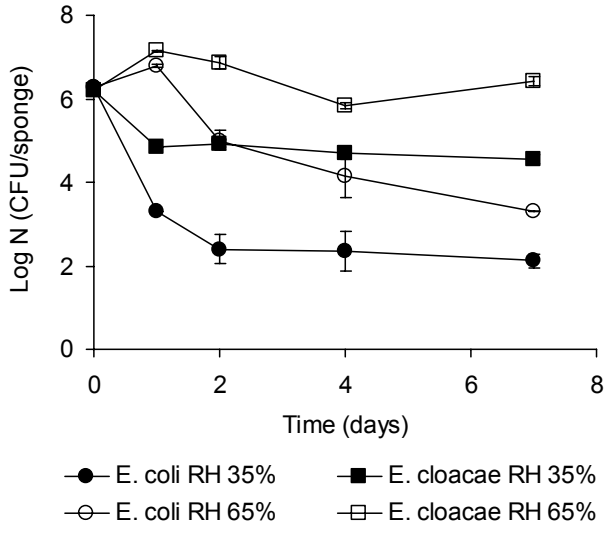

Figure 7.1. Effect of relative humidity on the counts of *E. coli* and *E. cloacae* in artificially contaminated sponges (Kusumaningrum, unpublished)

At a relative humidity of 35% *E. cloacae* decreased with 1 log-unit while *E. coli* was reduced with 4 log-units. This confirms observations that *Enterobacter* species show a better survival than other non-spore forming bacteria and become the predominant microflora in used sponges at room temperature. Even in presence of food residues, the pathogens never became the predominant microflora in the used sponges.

Fortunately there is no evidence that *E. cloacae* can cause foodborne human infections. Only in people with a precarious immune system they become opportunistic pathogens, causing bacteraemia, especially in hospitals where it is mainly acquired through cross-transmission when infection control is poor, including poor hand washing (92).

Over the last few years there has been a growing concern about foodborne infection caused by an *Enterobacter* species namely *Enterobacter sakazakii*. *E. sakazakii* was called "yellow-pigmented *E. cloacae*" until 1980 when it was renamed *E. sakazakii* (28). The majority of cases of *E. sakazakii* infections reported in the literature occurred with neonates, with sepsis, meningitis, or narcotising enterocolitis (83, 87) as symptoms, and the case-fatality rate among infected neonates has been reported to be as high as 33%. The pathogen is also a rare cause of bacteraemia in adults (87). While the reservoir of *E. sakazakii* is unknown, a growing number of reports suggest a role for powdered milk-based infant formulas as a vehicle for infection (83, 84, 87, 88). However, this species may be ubiquitous since it is also found in different processing plants and household environments. *E. sakazakii* was isolated in 9% of household cloths, as mentioned previously. Continued research will improve our understanding of the behaviour of this bacterium in the kitchen environment.

Microbial adaptation in kitchen environments

Adverse changes in the microbial environment may cause bacteria to experience stress. Although the stress kills many bacteria, some may survive, due to their elaborative systems to adapt and change. An unfortunate consequence of this process, however, is the development of increased tolerance or resistance to environmental stresses, including to antibiotics, food preservation agents and disinfection processes. Studies have shown that cells encountered in a stress condition may develop a cross protection against another stress (57, 70, 79, 105). Increased tolerance of *S.* Enteritidis to sodium hypochlorite after exposure to reduced water activity surfaces was observed (Chapter 5). Chlorine resistance also progressively increased through the starvation period (73). It is likely that stress response mechanisms play a significant role in determining bacterial survival in the environment, in food preservation hurdles as well as in exposure to disinfectants (110).

Some bacteria attach to surfaces as their predominant form of survival in nature and in man-made ecosystems (72). In many types of environments, microorganisms produce extracellular polysaccharides following attachment to surfaces, resulting in the formation of a biofilm. It has been found that microorganisms in biofilms can find protection from the effect of disinfectants, including chlorine (66). Such biofilms are also found in kitchen sponges (Chapter 3). Theoretically, the topography of a surface affects the ability of a disinfectant to approach the cell. A freely suspended (planktonic) organism is susceptible to a disinfectant from all sides and at all angles, while an organism attached to a surface is susceptible from only one side (11). However, this is certainly not the only reason why microorganisms are more resistant in biofilms than in suspension. Understanding the physiological state of the

pathogens used in challenge studies is critical to proper evaluation of their survival in foods or environments.

Implications for hygiene procedures

A hygiene procedure is a procedure that is applied to reduce the number of viable organisms on an object or product to a level that is considered safe for its intended use. This may be achieved by a process of removal of the microbes, or by inactivation in situ using heat or a disinfectant. A combination of both processes may also be used (45). Cleaning is the removal of food residues (organic matters), dirt, grease or other objectionable matter (43), but does not have bactericidal, sporicidal, virucidal, or fungicidal activity and does not necessarily reduce the level of microbial contamination (45).

Comprehensive discussions have been raised to determine what level of hygienic cleaning will be satisfactory from the point of view of public health. The main purpose in applying hygiene procedures in the home kitchen is to reduce microorganisms to a level that is not harmful to health, but it is not intended to achieve sterility. Some concerns must be carefully addressed in promoting suitable hygiene procedures in the household kitchen. There is the suggestion that some exposure to microorganisms is beneficial to maintain a healthy immune system against infection (96, 102). Other concerns are the possible toxicity effects of chemical disinfectants released into the environments and the identified links between resistance to antibiotics and some biocides, particularly in view of the current trend of adding antibacterial agents to household cleaning products and tools. However, in recent research no evidence was found to link the use of biocide to development of antibiotic resistance (7). Furthermore, confident measures to control or reduce the microorganisms in the domestic environment should also take into consideration the dynamic nature of microorganisms in ecosystems. During transmission, microorganism often experience environmental stresses, such as temperature variation, nutrient starvation, or fluctuations in osmotic pressure, which will activate their intrinsic adaptive mechanisms.

Cross contamination and exposure assessment

We are entering the era of risk management, a time in which we recognize that life is full of risks. Good data and risk analyses play a critical role in the decisions made as individuals and collectively. Success in this era depends on the ability to effectively manage variability and uncertainty, and on a good understanding of how the systems work (124). Large degrees of uncertainty require that decisions be made with great caution; however, there is no excuse for not making the best decision on the basis of available information. Many gains in decision support can be achieved through model-based risk assessment (65).

Before there can be a risk assessment, there must be an exposure assessment. The ultimate goal of exposure assessment is to evaluate the level of microorganisms or microbial toxins in the food at the time of consumption. An ideal exposure assessment needs

information including the presence of the pathogens in raw materials; the effects that food processing, distribution, handling and preparation steps have on the pathogens; and consumption patterns, e.g. portion size. Because a specific pathogen tends to be heterogeneously distributed in food, both the frequency and the level of contamination are needed (135). The level of pathogens on foods can be estimated by using literature data, sampling or by using predictive microbiology, since that level will depend on the occurrence in the raw materials, survival, re-contamination and growth (140).

In order to estimate the levels of *Salmonella* and *Campylobacter* on foods after a food preparation process where cross-contamination occurred, a model of cross-contamination of *Salmonella* and *Campylobacter* on domestic kitchen surfaces is described (Chapter 6). This study illustrates how the cross-contamination during food preparation in the domestic kitchen can be modelled by linking currently available data and experimental data. The models have not been validated for the condition to which they are applied, and hold assumptions that are disputable. Nevertheless with this model and data, we have been able to include several sources of variability and to predict the probability and the extent of *Salmonella* and *Campylobacter* presence in a salad vegetable after household preparation. However, cross-contamination in domestic environment does not always result in infectious disease. The risk of infections is highly variable and depends on several factors, including the pathogenicity of microorganisms, the infective dose and the susceptibility of the host. These factors are characterised in the other step in risks assessment, i.e. hazard characterisation. In a complete risks assessment the exposure and dose-response assessment are integrated to give an overall probability of occurrence and severity of health effects in a given population (135).

Microbiological risk assessment dealing with exposure assessment should also cover the implications of the ecology of the microorganisms in question in relevant habitats, be that in the environment or in the production systems. New understanding in microbial ecology, including stress response survival and biofilm ecology, can significantly improve risk reduction efforts. In some instances, it may be necessary to limit the scope to be able to address the questions by making them more specific or alternatively, to develop more than one assessment. The exposure assessment should be made as simple as possible while still including the important sources of risk (135).

The take-home message

Foodborne infectious diseases present old and new challenges. No matter how sophisticated and complex a measure and control system is, it will be never finished nor complete, because change is constant (46). Changing life styles, population demographic, and global food trade are a few examples of factors that relate with the occurrence of foodborne disease. As foodborne disease occurrence continues over time, prevention and control measures must be managed on a continuous basis. Continued research will improve our understanding of the complex factors that cause foodborne disease.

Accompanying microbial evolution that has always happened and will continue to occur, human foodborne disease surveillance will continue to be very important. Data on the incidents of foodborne infectious disease can provide a measure of the magnitude of the disease problems and knowledge on the cause of disease to learn how to prevent future similar outbreaks from occurring. Information enables assessment of trends in prevalence of outbreaks caused by specific etiologic agents and in vehicles of transmission (20, 120). Pathogens for which the transmission is well understood may ultimately be controlled. Others may emerge through mutation, or may move into a new niche in the food chain. As a result, the frequency of specific infections can change substantially, reflecting a balance between the ecologies that support bacterial populations contaminate food, and the cultural habits as well as technologies that limit or prevent occurrence of contaminations (121).

Moreover, householder behaviour continues to play a significant role in exposure to foodborne disease. Consumers are sometimes unaware of or inattentive to their personal ability to reduce the risk of foodborne disease. Research indicates that people consider themselves fairly knowledgeable about hygienic guidelines, and for the most part they are. However, knowledge and awareness does not always translate into behaviour changes (100). Relatively few consumers perceive food safety problems due to actions in the home – the final stage of the food safety system. It is important to consider potential contributions from all segments of the food chain, including the household kitchen. It is important to remember, that when it comes to food safety, everyone is consumer. Scientist, food manufacturers and public health officials are also consumers and share the desire for safe foods. The preventive activities should work on all sides of the food chain: from decreasing the level of infection in reservoirs until decreasing the risk of transmission by informing consumers on food and kitchen hygiene. Considering the measures in the home to prevent foodborne disease most important issues are prevention of cross-contamination, the combination of proper hygiene and sanitation related to food handling and preparation, appropriate methods of refrigeration and freezing and thorough cooking of food. Much of the cross infection that occurs in the home arises from colonised persons who handle the food and can be controlled only by changes in behaviour. If people do not recognise and accept their role in the transmission of foodborne disease problems, change in behaviour is unlikely. Prevention and control measures of transmission of foodborne diseases are a complex combination of factors, including the pathogens, the host and the environment in which they exist and interact as well as the behaviour of the householders.

Concluding remarks

Domestic kitchen environments can potentially spread pathogenic bacteria, including *S.* Enteritidis, *C. jejuni* and *S. aureus*. However, foodborne disease outbreaks related with this environment are more often associated with specific incidents and practices rather than continuously present large populations of foodborne pathogens in the kitchen. As foodborne

disease occurrence continues over time, prevention and control measures must be managed on a continuous basis. Hygiene procedures in the kitchen should be considered as a purpose to reduce microorganisms to a level that is not harmful to health, but they are not intended to achieve sterility.

It is also apparent that public awareness of hygiene alone may not be enough – what is needed is increased understanding leading to improved practices. As with many issues of health, education is an important part of the fight against the spread of foodborne diseases. By learning what threats are posed by foodborne diseases and by changing behaviour people can reduce the risk. It is important to remember that each individual can play a critical role in preventing and controlling illness. Basic personal and kitchen hygiene can greatly help to defend against harmful microorganisms.

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Summary

Epidemiological data indicate that a considerable number of foodborne diseases are attributable to improper preparation practices in the home including cross-contamination. Studies have shown that although raw material is possibly the main source of contamination in the kitchen, the areas surrounding the kitchen and the person who handle the food could also act as sources of harmful microorganisms. Dishcloths and sponges, for example, have been recognized as potential vehicles in the spread of microorganisms, and it has been observed that bacteria persist in these sites. The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. In order to reduce bacterial contaminations in the kitchen and therefore the impact of pathogens on human health, rational decisions about the kind of interventions need a scientific-based approach. Quantitative microbiological risk assessment, which can facilitate estimation of the probability and severity of a health disturbance as a result of consumption of food, is increasingly recognised as a tool for decision-making with regard to control of the spread of foodborne diseases. However, as relevant and accurate data are often lacking in the exposure assessment, systematic studies are still needed to provide quantitative data for risks management efforts in domestic environment. The aims of this research were to gain a quantitative understanding of survival, cross-contamination and stress response of foodborne pathogens in household kitchen environments. The results, particularly from the cross-contamination study, were included in a development of an exposure assessment model that in the later phase can be incorporated in a more extensive microbiological risk assessment.

As an example of survival in a household kitchen environment, survival of *Salmonella* Enteritidis, *Campylobacter jejuni* and *Staphylococcus aureus* against air-drying on surfaces was quantified and described in Chapter 2. Even after a single contamination event, these pathogens survive on stainless steel surfaces for hours or days, depending on the species, initial counts and the presence of food residues. *S. aureus* is the most tolerant against air-drying on surfaces, followed by *S.* Enteritidis and *C. jejuni*. These pathogens are readily transmitted from the wet sponges to stainless steel surfaces and from these surfaces to cucumber and chicken fillet slices. Therefore, effective cleaning and/or sanitizing of food preparation surfaces is apparently important to prevent the cross-contamination.

Tolerance of *S.* Enteritidis and *S. aureus* to surface cleaning by wiping using regular, micro-fiber and antibacterial-treated cloths was investigated and described in Chapter 3. Wiping of surfaces using clean cleaning cloths results in a considerable reduction of microorganisms from surfaces of 3 to 5 log units, despite the fact that it was more difficult to remove *S. aureus* than *S.* Enteritidis. Subsequent air-drying of the surfaces or application of

disposable antibacterial-impregnated napkins results in additional reduction of microorganisms on surfaces. However, the use of these napkins are often only intended for specific purposes instead of for daily cleaning activities in the kitchen because of the higher costs as compared to the regular cloths. An important observation is that the wiping practice tends to transfer microorganisms from the surface to cleaning cloth when a clean cloth is used. Directly after a re-useable cloth is used to clean the contaminated surfaces, the cloth becomes highly contaminated with bacteria. As bacteria are readily spread from these contaminated cleaning cloths or sponges to surfaces and subsequently from surfaces to foods, as described previously, keeping the cloth clean is obviously important to prevent the spreading of microorganisms. Household bleach (sodium hypochlorite) is often applied to reduce microorganisms in these sites. Study on tolerance of *S.* Enteritidis and *S. aureus* to sodium hypochlorite indicates that *S. aureus* shows a better tolerance against sodium hypochlorite than *S.* Enteritidis. Inactivation of microorganisms in cloths requires a higher concentration of sodium hypochlorite than is needed in the suspension test. Presence of food debris in the cloths, which occurs regularly in practical situations, decreases the effectiveness of sodium hypochlorite, leading to the requirement of higher concentrations of these compounds. Food residues interfere with the hypochlorite, and possibly protect the microorganisms from direct contact with the sanitizers.

In Chapter 4 the survival of *Escherichia coli, Salmonella* Enteritidis, *Staphylococcus aureus* and *Bacillus cereus* on kitchen sponges was investigated. Following a single contamination and air-drying at room temperatures, pathogens remain on cleaning sponges for days, with or without the presence of food residues. With food residues present in the sponges the survival is prolonged. Study on the effect of antibacterial products on the microflora in sponges indicates that the antibacterial dishwashing liquid was effective in reducing pathogens only in the suspension test but not in the used sponges. Over a 24-h period, the antibacterial dishwashing liquid did not significantly reduce these organisms in used sponges in which food residues were present. Application of this product under practical situations in households did not make a significant difference as compared to a regular product.

The survival of microorganisms on dry(ing) surfaces, resulting in exposure to low water activity (a_w), may affect the morphology and physiological activity of the cells. In Chapter 5 the morphological changes and cell viability of *S.* Enteritidis challenged to low a_w surfaces were analysed. Viability was determined using the Live/Dead *Bac*Light bacterial viability kit with epifluorescence microscopy and flow cytometry. The results indicate that exposure to reduced a_w surfaces induces filamentation of the cells, up to 90% of the total number of cells, some of which reach a size of 50 μm or more. Filamentous cells maintain their membrane integrity after exposure to low a_w surfaces for a long period of time and are able to split in single cells under favourable conditions, resulting in the instantaneous appearance of a large number of viable cells. Furthermore, both short and elongated cells pre-challenged to low a_w surfaces demonstrate better tolerance against sodium hypochlorite than control cells. These tolerant cells are able to survive disinfection and therefore could be a

source of contamination of foods coming in contact with surfaces. This result indicates the need of increased attention in the disinfection procedures of surfaces in processing plants or household environments.

In the last part of this research, a quantitative analysis was carried out to estimate the probability of contamination and the levels of *Salmonella* and *Campylobacter* on salad as a result of cross-contamination from contaminated chicken carcasses via kitchen surfaces (Chapter 6). This study illustrates how the cross-contamination during food preparation in the domestic kitchen can be modelled by linking experimental data and currently available data. The models need to be validated for the condition to which it is applied, and hold assumptions that are disputable. Nevertheless, this study has shown that it is realistic to expect that a fraction of the human exposure to, particularly, *Campylobacter* originates from cross-contamination in private kitchens during food handling. The probability that salads become contaminated with *Campylobacter* is higher than that with *Salmonella* since both the prevalence and the levels of *Campylobacter* on chicken carcass are higher than those of *Salmonella*. While the reduction of concentration of *Campylobacter* on the positive chicken carcasses is important in relation to reduce the risk of becoming infected, it is obviously important to use separate surfaces or to properly wash the surfaces between preparation of raw and cooked foods or ready to eat foods to cut the cross-contamination routes.

In conclusion, domestic kitchen environments can spread pathogenic bacteria, including *Salmonella*, *Campylobacter* and *S. aureus*. However, foodborne disease outbreaks related with this environment are more often associated with specific incidents and practices rather than the continuously presence of large populations of foodborne pathogens in the kitchen. As foodborne disease occurrence continues over time, prevention and control measures must be managed on a continuous basis. Each individual plays a critical role in preventing and controlling illness. Basic personal and kitchen hygiene can greatly help to defend against harmful microorganisms.

Samenvatting

Uit epidemiologische gegevens is gebleken dat onjuiste voedselbereiding in de huishoudelijke keuken, inclusief kruisbesmetting, een significante bijdrage levert aan het voorkomen van voedselinfecties. Wetenschappelijke studies hebben aangetoond dat naast rauwe producten, zoals vlees en groenten, ook de keukenomgeving en de personen die in aanraking komen met het voedsel tijdens bereiding belangrijke bronnen kunnen zijn voor de verspreiding van pathogene bacteriën. Vaatdoeken en schoonmaaksponsjes, waarin zich hoge aantalen micro-organismen kunnen bevinden, zijn geïdentificeerd als potentiële vectoren van bacteriën. Wanneer deze bacteriën zich hechten aan oppervlakken die in contact komen met levensmiddelen en daar overleven is er een kans op kruisbesmetting. Voorkomen of, beter gezegd, het reduceren van microbiële besmetting in de keuken is mogelijk door te werken volgens een hygiënecode, waarin staat hoe in bepaalde risicovolle situaties, gehandeld moet worden. Zo moeten oppervlakken die in contact zijn geweest met (rauwe) levensmiddelen, gereinigd worden. Daarna moeten ze zo snel mogelijk gedroogd worden, waardoor minder cellen overleven en de groei van overlevende micro-organismen wordt geremd.

Het onderzoek dat in dit proefschrift wordt beschreven, is uitgevoerd omdat er weinig kwantitatieve gegevens zijn over het gedrag van pathogene bacteriën in huishoudelijke keukens. In eerste instantie is voor een aantal belangrijke ziekteverwekkers (*Salmonella* Enteritidis, *Campylobacter jejuni* en *Staphylococcus aureus*) gevonden, dat deze tot enkele dagen kunnen overleven op kunstmatige besmette roestvrijstalen oppervlakken. *S. aureus* overleeft het langst, gevolgd door *S.* Enteritidis en *C. jejuni*. Een hoge beginbesmetting en aanwezigheid van voedselresten verlengen de overleving van alle onderzochte soorten (Hoofdstuk 2).

Vaatdoekjes, zogenaamde wonderdoekjes (microvezeldoek) en antibacteriële doekjes zijn gebruikt om oppervlakken, die kunstmatig besmet werden met *S.* Enteritidis en *S. aureus*, te reinigen. De resultaten van dit onderzoek zijn vermeld in Hoofdstuk 3. Het afvegen van een besmet oppervlak met een schone doek resulteerde in een reductie van de toetsorganismen met een logfactor 3-5 per 100 cm^2. Daaropvolgend drogen aan de lucht voor 15 minuten verlaagde de aantallen van *S.* Enteritidis in het algemeen tot onder de detectie grens (0.6 log KVE/100 cm^2). Tientallen cellen van *S. aureus* konden dit korte drogen nog overleven. Gebruik van microvezeldoekje leverde dezelfde resultaten als gewone vaatdoeken. Gebruik van wegwerpdoekjes met antibacteriële stoffen resulteerde in een grotere reductie (logfactor 5) of tot onder de detectie grens. Een belangrijke observatie tijdens deze studie is dat het vegen van een oppervlak leidt tot een overdracht van micro-organismen van het oppervlak naar de doekjes, vooral als een schone doek wordt gebruikt. Na het schoonmaken van een besmet oppervlak bevat de doek veel micro-organismen. Herhaald gebruik van de doek zal minder effect hebben in reductie en zelfs tot verspreiding van micro-organismen kunnen leiden.

Fabrikanten van reinigingsmiddelen raden soms aan bleekmiddel of een antibacterieel reinigingsmiddel te gebruiken om de kiemgetallen in doekjes en/of sponsjes laag te houden. In dit onderzoek is aangetoond dat voor het reduceren van bacteriën in een vaatdoek die dagelijks wordt gebruikt is een hoge concentratie van bleekmiddel nodig, 800 ppm tot 2400 ppm. Dit wordt veroorzaakt vooral door de aanwezigheid van voedselrestanten in de doek die de activiteit van het bleekmiddel vermindert (Hoofdstuk 3).

In Hoofdstuk vier is beschreven dat, ondanks het gebruik van een middel met antibacteriële componenten, (pathogene) micro-organismen dagenlang in sponsjes kunnen overleven. In aanwezigheid van voedselresten is zelfs uitgroei mogelijk.

De overleving van micro-organismen op droge oppervlakken, waar een lage water activiteit heerst, kan aanleiding geven tot verandering van morfologie en fysiologische activiteit van de cellen. Circa 90% van de *Salmonella*-cellen die op een oppervlak met lage wateractiviteit worden gebracht, strekken zich, soms tot lengtes van 50 µm of meer. Deze lange cellen kunnen dagenlang overleven tijdens de periode van lage wateractiviteit. Worden ze overgebracht in een gunstige omgeving, dan splitsen ze zich in relatief korte tijd (enkele uren) in vele korte (normale) cellen. Zowel korte als lange cellen, die blootgesteld zijn geweest aan een periode met lage wateractiviteit zijn beter bestand tegen huishoudelijk bleekmiddel dan gewone (controle) cellen (Hoofdstuk 5).

In het laatste deel van dit proefschrift (Hoofdstuk 6) is de overdracht gekwantificeerd van salmonella's en campylobacters op natuurlijk besmette kip, via een snijplank naar een salade. Hoewel het model nog gevalideerd moet worden, blijkt uit deze studie (Hoofdstuk 6) dat, gebruikmakend van experimentele gegevens uit dit proefschrift en gegevens uit de literatuur, het aannemelijk is dat een deel van de *Campylobacter*-infecties wordt veroorzaakt door kruisbesmetting tijdens voedselbereiding in de keuken. De kans dat salade wordt besmet met campylobacters is groter dan een besmetting met salmonella's door het hogere besmettingsniveau van kip met campylobacters. Reductie van die aantallen zal de kans op overdracht verminderen, maar het gebruik van schone snijplanken en toepassen van strikte hygiëneregels heeft een groter effect.

Dit proefschrift levert een belangrijke bijdrage aan de kennis over het gedrag van pathogene micro-organismen in de huishoudelijke keuken. Ziekteverwekkers, zoals *Salmonella*, *Campylobacter* en *S. aureus* kunnen zich daar langere tijd handhaven. Voedselinfecties veroorzaakt door deze bacteriën hebben meestal te maken met onjuist hygiënisch handelen. Daarom is het van het grootste belang nodig dat iedereen, zo jong mogelijk, vertrouwd raakt met persoonlijke hygiëne en keukenhygiëne.

Ringkasan

Data epidemiologi menunjukkan bahwa pengolahan makanan yang tidak sempurna dan kontaminasi silang memberi kontribusi yang cukup besar terhadap penyebaran penyakit yang disebabkan oleh keracunan dan infeksi makanan. Bakteri penyebab penyakit dapat memasuki lingkungan tempat pengolahan melalui bahan mentah, misalnya daging dan sayuran. Selain bahan mentah, lingkungan pengolahan dan pengolah makanan juga merupakan sumber penting penyebaran bakteri penyebab penyakit. Bakteri sering ditemukan pada sabut pencuci dan lap pengering peralatan pengolahan bahkan pada permukaan peralatan pengolah. Keberadaan mikroorganisme pada permukaan peralatan tersebut meningkatkan risiko kontaminasi silang pada saat peralatan tersebut digunakan untuk mengolah makanan.

Penurunan derajat cemaran mikrobiologis di lingkungan pengolahan dapat dilakukan dengan penerapan kode-kode higiene. Berbagai studi terhadap status kebersihan lingkungan pengolahan pada umumnya menampilkan data mikrobiologis yang bersifat kualitatif, sementara data-data kuantitatif masih sangat kurang. Sebagai usaha untuk menurunkan angka keracunan dan infeksi makanan, dalam pengambilan keputusan kebijakan intervensi diperlukan suatu pendekatan yang bersifat 'scientific-based' dimana kebutuhan terhadap data-data kuantitatif sangat diperlukan. Sehubungan dengan masih sangat diperlukannya studi secara sistematis untuk mengumpulkan data-data kuantitatif, penelitian ini disusun dengan tujuan utama melakukan kuantifikasi terhadap ketahanan, kontaminasi silang dan respons bakteri penyebab penyakit di lingkungan dan terhadap lingkungan pengolahan rumahtangga.

Pada Bab 2 disajikan ketahanan *Salmonella* Enteritidis, *Campylobacter jejuni* dan *Staphylococcus aureus* pada permukaan anti karat. Meskipun hanya dengan kontaminasi tunggal, bakteri penyebab penyakit tersebut dapat bertahan hidup dalam beberapa jam atau beberapa hari pada permukaan bahan tersebut, tergantung dari jenis bakteri, jumlah awal bakteri dan keberadaan residu makanan. *S. aureus* merupakan bakteri yang paling tahan terhadap kondisi kering pada permukaan, disusul oleh *S.* Enteritidis dan *C. jejuni*. Bakteri tersebut dengan mudah dipindahkan dari lap basah ke permukaan bahan anti karat dan dari permukaan tersebut ke bahan makanan.

Toleransi dari *S.* Enteritidis dan *S. aureus* terhadap perlakuan sanitasi peralatan menggunakan alat pembersih biasa, lap yang tersusun dari serat-serat mikro (micro-fiber) dan lap sekali pakai yang mengandung bahan antibakteri di sajikan di Bab 3. Pembersihan permukaan dengan menggunakan bahan biasa dapat menurunkan derajat kontaminasi bakteri dengan 3-5 log uint koloni/100cm^2. Reduksi sampai dengan batas limit pengukuran dapat dicapai dengan membiarkan permukaan mengering atau dengan mengunakan lap sekali pakai yang mengandung bahan antibakteri. Satu observasi yang penting yang diamati pada saat penelitian adalah pada saat pembersihan permukaan bakteri dipindahkan dari permukaan ke lap pembersih. Untuk menghindari penyebaran mikro organisma selanjutnya dari lap pembersih tersebut ke perlatan lain sanitasi terhadap alat pembersih sering diterapkan

menggunakan garam hipokhlorit sebagai bahan disinfeksi. Studi lanjut terhadap pengaruh bahan tersebut terhadap *S.* Enteritidis dan *S. aureus* menunjukkan bahwa *S. aureus* lebih tahan terhadap garam hipokhlorit dibandingkan dengan *S.* Enteritidis. Inaktivasi bakteri dalam lap pembersih memerlukan konsentrasi garam hipokhlorit yang lebih tinggi dibandingkan jika bakteri berada dalam suatu supensi. Keberadaan residu makanan yang sering ditemukan pada kondisi sehari-hari, dapat menurunkan efektivitas dari garam hipokhlorit. Bahan organik dapat berinterferensi dengan garam hipokhlorit atau dapat mencegah terjadinya kontak langsung antara bakteri dengan hipokhlorit.

Pada Bab 4 disajikan hasil studi tentang survival dari *E.coli*, *S.* Enteritidis, *S.aureus* dan *B. cereus* pada spons pembersih dapur yang mendapat perlakuan dengan bahan pencuci komersial yang mengandung bahan antibakteri. Bakteri tersebut dapat bertahan hidup sampai bebrapa hari, dengan atau tanpa adanya sumber makanan. Produk antibakteri berpengaruh pada survival bakteri jika pengujian dilakukan dalam uji suspensi sedangkan jika pengujian dilakukan pada kondisi sehari-hari hasilnya tidak nyata.

Pada Bab 5 disajikan respons dari *S.* Enteritidis terhadap kondisi dengan aktivitas air (Aw) rendah. Paparan terhadap permukaan dengan Aw rendah menyebabkan perubahan morfologis dan aktifitas fisiologi dari sel bakteri tersebut. Pada Aw tertentu filamentasi (perpanjangan sel sebagai hasil dari pembelahan sel yang tidak sempurna) terjadi pada sel-sel, sampai dengan 90% dari populasi, dengan panjang sel sampai dengan 50 μm atau lebih. Viabilitas sel diuji menggunakan Live/Dead *Bac*light bacterial viability kit diamati dengan epifluoresnce mikroskop dan flow cytometri. Sel yang mengalami filamentasi mempertahankan viabilitasnya sampai beberapa hari, dan dapat membelah kembali dan membentuk sel tunggal dengan jumlah yang cukup besar jika berada pada kondisi yang normal. Selanjutnya, pengujian terhadappengaruh garam hipokhlorit menunjukkan bahwa sel yang telah mengalami paparan terhadap Aw rendah mempunyai ketahanan yang lebih baik dibandingkan dengan sel kontrol.

Pada bagian terakhir dari penelitian ini dilakukan kuantifikasi untuk mengestimasi probabilitas dan tingkat cemaran *Salmonella* dan *Campylobacter* pada makanan sebagai akibat dari kontaminasi silang yang berasal dari ayam yang tercemar secara alami melalui kontak dengan permukaan alat pengolah (Bab 6). Study tersebut menunjukkan bahwa meskipun model matematik yang digunakan masih perlu divalidasi, dengan menggabungkan antara data-data percobaan dengan data pustaka, secara rational dapat diperkirakan bahwa sebagian dari infeksi *Campylobacter* terjadi karena kontaminasi silang pada saat pengolahan. Probabilitas makanan tercemar oleh *Campylobacter* lebih tinggi dibandingkan dengan tercemar oleh *Salmonella,* berdasarkan temuan bahwa tingkat cemaran *Campylobacter* pada daging ayam lebih tinggi dibandingkan dengan *Salmonella*. Meskipun upaya untuk menurunkan tingkat cemaran *Campylobacter* pada daging ayam dapat mengurangi risiko kontaminasi silang pada makanan, selama konsumen tidak mengetahui kualitas mikrobiologi dari produk ayam, penggunaan permukaan pengolah yang berbeda untuk mengolah bahan mentah dan bahan yang siap saji atau penerapan sanitasi dapat memutus jalur kontaminasi.

Studi yang telah dilakukan menunjukkan bahwa lingkungan dapur rumah tangga merupakan tempat yang potensial untuk penyebaran bakteri penyebab penyakit, termasuk *Salmonella*, *Campylobacter* dan *S. aureus*. Selama infeksi makanan akan selalu berlangsung terus-menerus, pencegahan dan pengendalian harus berdasarkan dengan sistim pengendalian yang berkesinambungan. Setiap individu mempunyai peran yang penting dalam pencegahan dan pengendalian keracunan dan infeksi makanan. Kebersihan individu dapat merupakan pertahanan yang cukup berarti dalam melawan mikroorganisma yang berbahaya.

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During my stay, it was a coincidence that the Wageningen Agricultural University changed its name to Wageningen University, reminding me at the time when the Agricultural College (Landbouwhogeschool) of Wageningen was renamed to Wageningen Agricultural University (Landbouwuniversiteit) while I was starting my study in the period of 1986. It was a great chance to experience those historical events.

The changes that I have experienced were not only in the name and the organization of the university but also personal life of many group members. I still remember the time when my lab-mate was surprised knowing me being married with two children and yelling to the others 'ha, we have a married AIO with children in our lab', a comment that also surprised me: what is wrong with that. I recognised afterwards that their life style was different. However, in the course of the last three years there were many invitations for wedding parties I received, I cannot remember how many they were. Nor, can I remember how many 'beschuit met muisjes' I have tasted celebrating the birth of the juniors: Martine's junior, Jeroen's junior, Patrick's junior, Sonya's junior, another Jeroen's junior, just are a few of them. I enjoyed that and feel happy for them.

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Harsi Kusumaningrum, June 2003

Curriculum Vitae

Harsi Dewantari Kusumaningrum, born on May 2nd, 1964, in Yogyakarta, Indonesia, completed her secondary high school (SMA 3 Yogyakarta) in 1983. She had been studying for about 2 years at the Department of Food Technology, Faculty of Agricultural Technology, Gadjah Mada University Yogyakarta, when in 1985 she received a scholarship from The Netherlands Ministry for Development Cooperation through the collaborative Indonesian office OTO-BAPPENAS (Overseas Training Office of the State Ministry for Development Planning/National Development Planning Agency) to study in The Netherlands. After passing the Dutch language examination and 'Toelatingsexamen' at IIVO (Interfacultair Instituut voor Voortgezet Onderwijs) Utrecht, she continued her study at the Agricultural University in Wageningen and graduated in 1991 as a food microbiologist. Thereafter, she came back to Indonesia and joined the Street Food Project, a collaborative project between Bogor Agricultural University, Indonesia, Free University Amsterdam and TNO-Nutrition The Netherlands until 1992. Since 1992, she belongs to the educational staff of the Laboratory of Food Microbiology, Department of Food Technology and Human Nutrition, Bogor Agricultural University Indonesia, and was involved in different food safety activities.

In June 1999, she received a scholarship from the World Bank through the QUE-FTSP project (Quality for Undergraduate Education – Food Technology Study Program), Bogor-Indonesia, to start her PhD program at the Laboratory of Food Microbiology, Wageningen University, on 'Behaviour and cross-contamination of pathogenic bacteria in domestic environments'. In August 2002, at the 18th International ICMH (International Committee on Food Microbiology and Hygiene) Symposium - Food Micro 2002 - in Lillehammer, Norway she obtained a scholarship award for her presentation on 'Response of *Salmonella* Enteritidis to environmental stress'. In April 2003, she received from the NVvM (The Netherlands Society for Microbiology) the 'Kiem Prijs' for the best first article written by a PhD student in the category Food Microbiology.

After completing her PhD degree she will return to Indonesia continuing her task at the Laboratory of Food Microbiology, Department of Food Technology and Human Nutrition, Bogor Agricultural University Indonesia.

List of publications

Research papers

- **Beumer, R.R and H.D. Kusumaningrum.** 2003. Kitchen Hygiene in daily life. International Journal Biodeterioration and Biodegradation 51: 299-302.
- **Kusumaningrum, H.D., M.C. van Putten, F.M. Rombouts and R.R. Beumer.** 2002. Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges. Journal of Food Protection 65: 61-65.
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- **Kusumaningrum, H.D., M.H. Tempelaars, W.C. Hazeleger, J. Kieboom, T. Abee and R.R. Beumer.** 2003. Morphological changes, cell viability and development of increased tolerance to hypochlorite of *Salmonella enterica* serovar Enteritidis on surfaces with reduced water activity (Submitted for publication).
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H.D. Kusumaningrum - Behaviour and cross-contamination of pathogenic bacteria in household kitchens – relevance to exposure assessment - 2003

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Cover design: Harsi Kusumaningrum

- Background: Scanning electron micrograph (SEM) of *Staphylococcus aureus* on a stainless steel surface
- Front cover: SEM of (left to right) *Salmonella* Enteritidis cells as response to starvation in sponges, elongated cell of *Salmonella* Enteritidis as response to low water activity on surfaces, *Staphylococcus aureus* in saline solution on drying stainless steel surfaces, and elongated cell of *Campylobacter jejuni* as response to salty BHI
- Back cover: SEM of (left to right) textures of kitchen sponge, dishcloth and micro fiber cloth