Microbiological Risk Assessment of Fresh Produce Grown in Canterbury, New Zealand

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Abstract

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A recent trend to consume healthy foods has led to greater preference for natural food and raw vegetables. At the same time, concerns have increased about the microbiological risks of fresh produce. This thesis focusses on the microbiological risk assessment of fresh produce grown in organic and conventional farms and those sold by different retail shops in Christchurch. The incidence of foodborne outbreaks for fresh produce is estimated as 22.8% of the total foodborne illnesses in the USA and approximately 10% of cases in NZ. To achieve microbiological food safety of produce, it is important to identify the current microbiological status of fresh produce. Many people prefere organic produce these days but there may be some risks associated with organic produce because many organic growers use animal faeces (manure) as an alternative to chemical fertilizer. Organic farms use different types of soil enrichment such as compost, manure and crop-livestock rotation farming. Crop-livestock rotation farms rotate land for crop farming and livestock farming every three to four years and do not require chemical fertilizers. There is little data about microbiological assessment comparing these different types of soil enrichments. Recent trends and factors affecting the microbiological contamination of fresh produce are reviewed in the first chapter, focussing on: 1) microbial contamination of fresh produce; 2) frequency of fresh produce related foodborne outbreaks; and 3) factors affecting microbial status of fresh produce. The second and third chapters investigate two main objectives: microbiological risk assessment of fresh produce grown in organic and conventional farms with different soil enrichment systems in Canterbury, New Zealand and microbiological risk assessment of fresh produce from different retail shops in Christchurch. The results of farms in this study showed that over a six week

sampling period, there were significant differences ($p<0.05$) between the conventional farm vegetable samples and those of the organic farms for the average APC, coliform, yeast and mould and *Staph. aureus*, between the compost farm vegetable samples and those of the conventional farm and crop-livestock rotation farm for the average *E. coli*, and between the conventional farm silver beet samples and those of crop-livestock rotation farm for the *E. coli*. The fresh produce samples collected from different farms in Canterbury, New Zealand and acceptable levels for *E. coli* O157:H7 (not detected) but not for APC, coliform, *E. coli, Staph. aureus* or *Salmonella* spp. For APC, 88% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 33%. For coliform, 92% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 61%. For *E. coli*, 92% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 88%. For *Staph. aureus,* 21% samples had satisfactory levels for the organic farms and that of the conventional farm was 50% based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. The yeast and mould levels (1.78 – 6.10 $\log_{10}$CFU/ml) were similar to studies previously reported for samples purchased in retail shops. Most *Staphylococcus aureus* and *Salmonella* spp. positive strains from farms and retail shops showed higher resistance to β-lactams. Similarly, the results of retail shops showed that over a six week sampling period, ppercent of unsatisfactory levels as per the guidelines given by FSANZ (2001) or PHLS (2000) varied within a retail shop. For APC, 75-87.5% samples had unsatisfactory levels and for coliform, 75-91.7% samples had unsatisfactory levels. For *E. coli*, 80-100% samples had unsatisfactory levels, and for *Staph. aureus,* 17.4-21.7% samples had unsatisfactory levels. However, these results and the yeast and mould (0-5.23 lpg_{10}CFU/ml) were similar to studies previously for samples purchased in retail shops, except for *E. coli* (83.3-100% of samples had *E. coli*). Vegetables from supermarket were less contaminated with *E. coli* than samples from other retail shops. However, retail shop B had the highest unsatisfactory levels of APC (87.5%), coliform (91.7%), *E. coli* (100%) and *Staph. aureus*

(21.7%) based on the the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. Eight percent of samples from four different retail shops were positive for *Salmonella* spp. but no *E.coli* O157 H7 was detected. In conclusion, the extent of microbiological safety differed between shops. In addition, there was a tendency that the potential risks of fresh produce in some retail shops were identified. Between farms, the fresh produce collected from the organic farms was more contaminated with some microbes than produce from the conventional farm.

Keywords: food safety, microbiological risk, fresh produce, bacterial culture and colony counting, organic produce, soil enrichment, antibiotic resistance

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Microorganisms

Aerobic Plates Count	APC
Escherchia coli	*E. coli*
Escherchia coli O157:H7	*E. coli* O157:H7
Salmonella typhimurium	*S. typhimurium*
Salmonella newport	*S. newport*
Salmonella typhi	*S. typhi*
Staphylococcus aureus	*Staph. aureus*
Staphylococcus epidermidis	*Staph. epidermidis*
Listeria monocytogenes	*L. monocytogenes*
Yersinia enterocolitica	*Y. enterocolitica*
Yersinia pseudotuberculosis	*Y. pseudotuberculosis*
Clostridium botulinum	*C. botulinum*
Clostridium perfringens	*C. perfringens*
Clostridium sporogenes	*C. sporogenes*

Units

degree	^{o}C
volume/volume	v/v
hour	h
minute	min
second	s
kilohertz	kHz
litre	L
micro	µ
parts per million	ppm
gram	g
miligram	mg
kilojoule	kj
metre	m
millimetre	mm
colony forming unit	CFU
potential hydrogen	pH

General

polymerase chain reaction	PCR
percent	%
European Union	EU
United States of America	USA
calcium oxide	CaO
plus	+

List of Publications and Presentations

Conference Abstract

1. Wadamori, Y., Fam, J., Gooneratne, R. and Hussain, M.A. (2015) Microbiological risk assessment of fresh produce grown in organic and conventional farms in New Zealand. International Journal of Food Science and Technology Conference 2015, held on February 17-19, 2015 at Lincoln University, New Zealand.

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Manuscripts Ready for Submission

1. Wadamori, Y., Fam, J., Gooneratne, R. and Hussain, M.A. Microbiological contamination of fresh produce (Trends in Food Science and Technology)
2. Wadamori, Y., Fam, J., Gooneratne, R. and Hussain, M.A. Microbiological risk assessment of fresh produce from different soil enrichment systems in Canterbury, New Zealand. (Food Control)
3. Wadamori, Y., Fam, J., Gooneratne, R. and Hussain, M.A**.** Microbiological risk assessment of fresh produce from different retail shops in Christchurch, New Zealand. (Journal of Food Safety)

Chapter 1 Introduction

1.1. Background

Food safety is considered indispensable for food commodoties from domestic and international sources. This is a global concern and many researchers are investigating and discussing food safety. Published reports clearly show that in spite of stricter controls, the number of foodborne outbreaks have not declined because the global food trade has become increasingly complex. Recent food safety scares, such as Fonterra's supposed botulinum incidence shows that New Zealand needs to be proactive to maintain its well-perceived 'clean and green' image (Ministry for the Environment, 2001).

Pathogens such as *Salmonella*, *Escherichia coli*, *Campylobacter, Listeria monocytogene* and *E. coli* O157:H7 are commonly associated with food and food products. There are several methods to detect foodborne pathogens. For example, conventional culture and colony counting methods are still widely used in food microbiology. Polymerase chain reaction (PCR) methods have been developed to detect pathogens more precisely. A recent approach is to employ a combination of different types of PCR, conventional microbiological and other methods (Velusamy et al., 2010, Joseph A O and Carlos G L-V, 2012, Jay et al., 2004). In terms of rapid and novel detection methods, immunology based detection, biosensors and metabolomics have been developed to detect pathogens directly, quickly and precisely. However, from the point of view of cost, these novel techniques are still somewhat expensive for many food industries to adopt.

Contamination can occur at any point in the food supply chain, from the farm to the plate. According to the World Health Organization (WHO) (2008), hazards can exist in production systems, post-harvest practices, water, local environment, fertilizer, worker health and hygiene, and consumption patterns and practices. In addition, recently among fresh produce many consumers have become concerned about the use of chemicals such as fertilizer and pesticides and many people now tend to eat organic products. However,

organic fresh produce, may risk food poisoning even more than conventional fresh produce due contamination from manure. Therefore, the desire to improve food safety specifically for organically grown fresh produce has been rising (Marsha Laux, 2013). However, limited studies have investigated microbiological risks associated with fresh produce sold through different retail systems. Fresh produce is sold through different retailing systems such as supermarkets, street markets and growers markets and each system could be contaminated with microbes in the supply chain. This study focused on microbiological risk assessment of fresh produce grown in different conditions (organic and conventional farming systems) and sold by different types of vegetable retail shops.

1.2. Importance of the study

This study was conducted to obtain information on the food safety status of the fresh produce grown and sold in the Canterbury region of New Zealand. The microbiological risk assessment of fresh produce grown at organic farms in New Zealand is not well studied. In addition, there have been little research conducted comparing the microbiological safety status of organic farming and conventional farming systems. This research is important to provide an assessment of the current situation of fresh produce contamination in selected Canterbury farms.

In this research, samples were collected from three different types of farms, conventional farms, organic farm fertilised using manure and organic farms fertilised through Crop-Livestock integration. There have been no reports on microbiological risk assessment of fresh produce grown in farms using the Crop-Livestock integration (considered one of the most sustainable farming systems). This research also investigated fresh produce contamination levels in the food supply chain (retail shops). It is expected that the results of this research will help to design future research that will reduce the risks of food poisoning associated with fresh produce.

1.3. Aims and objectives of study

1. Identify the potential risks of microbial contaminations in selected fresh produce samples in Canterbury, New Zealand;
2. Clarify whether organic farming has more risks of pathogen contamination compared to conventional farming systems in Canterbury, New Zealand; and
3. Investigate fresh produce contamination levels in the food supply chain (retail shops) in Canterbury, New Zealand.

Chapter 2 Literature Review

2.1. Summary

Natural foods and raw vegetables are the key ingredients in a healthy diet. The microbiological foodborne outbreaks associated with the consumption of fresh produce has been increasing. *Salmonella*, *E. coli*, *E. coli* O157:H7, *Campylobacter* and *Listeria* spp. are the most common pathogens that contaminate fresh produce. This chapter discusses recent foodborne outbreaks linked to fresh produce, factors that affect microbiological contamination and measures that could be adopted to reduce the incidence of food poisoning.

2.2. Microbiological safety of fresh produce

Microbiological food safety has always been a focus of the food industry and public health agencies as foodborne pathogens cause many illnesses and deaths throughout the world. According to Eurosurveillance editorial team (2013), a total of 5,048 outbreaks of foodborne illnesses occured in the European Union (EU) in 2011; the most common pathogens responsible were *Campylobacter* (220,209 cases) followed by *Salmonella* (95,548), *E. coli* (9,485) and *Echinococcus multilocularis* (781). New food consumption trends indicate that people are interested in fresh produce because of the availability of many essential nutrients for health and wellbeing. Vegetable and fruit production have been increasing and their average consumption per capita has risen from 45 to 54% from 1976 to 2009 in the USA (Cook, 2011) and this trend has continued.

The numbers of the foodborne outbreaks linked to the consumption of fresh produce have also increased, with an estimated 14.8% in 1998 to 22.8% in 2007 in the USA (Wijnands *et al*., 2014). Table 2.1 lists some of the recent foodborne outbreaks associated with fresh produce. It is interesting to note that the type of pathogen responsible for each outbreak was different (table 2.1). Outbreaks of *Yersinia pseudotuberculosis* in New Zealand in 2014 and *Cyclospora cayetanensis* in the USA in 2013 and 2014 were two of the most significant outbreaks associated with fresh produce.

Contamination can occur at any point, from the farm to the plate. According to World Health Organization (WHO, 2008), a hazard can exist in production systems due to several factors: post-harvest practices, water, local environment, fertilizer, worker health and hygiene, and consumption patterns and practices. As fresh produce is normally consumed raw or with minimum proccesing, it is important to keep the microbial load of fresh produce as low as possible to prevent foodborne illnesses. This review chapter discusses the contamination types of fresh produce, frequency of foodborne outbreaks and factors affecting the microbial food safety status of fresh produce, as well as future implications of microbiological contamination of fresh produce.

2.3. Microbial contamination of fresh produce

According to Westrell *et al*. (2009), *Salmonella* poisoning caused 151,995 cases of foodborne outbreaks, the second most prevalent in the 27 EU Member States and the four European Free Trade Association (EFTA) countries. In 1995, there was a large international outbreak of *Salmonella* poisoning in the USA and Finland (Mahon *et al*., 1997), originating from the seeds of alfalfa sprouts. Similarly, Mohle-Boetani *et al*. (2009) reported outbreaks of *Salmonella* poisoning in the USA in 2000 and seven outbreaks of *Salmonella* poisoning during the period from 2000 to 2002, all related to the consumption of raw mung bean sprouts. Greene *et al*. (2008) reported a *Salmonella* poisoning outbreak (510 cases in 2002) from tomato caused by *S. newport*, a rare strain of *Salmonella*.

Table 2.1 Recent foodborne outbreaks associated with fresh produce

Year/Country	Produce involved	Pathogen/cases	Source
2014/Canterbury, New Zealand	Fresh vegetables (exact source remains unknown)	*Yersinia pseudotuberculosis* (334 cases, 65 hospitalised)	MPI (MPI, 2014)
2014/England	Lettuce, cucumber	Enteroinvasive *E. coli* O96 (50 cases)	Public Health England (Public Health England (PHE), 2014)
2014/England	Salads	*Salmonella singapore* (4 cases)	PHE (PHE, 2014)
2014/USA	Raw clover sprouts	*E. coli O121* (19 cases, 8 hospitalised)	CDC (Centers for Disease Control and Prevention (CDC), 2013)
2014/USA	Coriander	*Cyclospora cayetanensis* (304 cases, 7 of 183 hospitalised)	CDC (CDC, 2013)
2013/USA	Bean sprouts	*S. enteritidis* (87 cases, 27 hospitalised)	CDC (CDC, 2013)
2013/USA	Imported cucumber	*Salmonella* (84 cases, 17 hospitalised)	CDC (CDC, 2013)
2013/USA	Imported pomegranate seeds	Hepatitis A virus (165 cases, 69 hospitalised)	CDC (CDC, 2013)
2013/USA	Salad mix	*Cyclospora cayetanensis* (631 cases, 50 hospitalised)	CDC (CDC, 2013)
2013/USA	Imported cucumber	*E. coli O157:H7* (33 cases, 11 hospitalised)	CDC (CDC, 2013)
2013/England	Watercress	Vero cytotoxin-producing *E. coli* O157	PHE (PHE, 2014)

Recently, the incidence of food poisoning caused by *E. coli* O157:H7 have been on the increase. For example, in 1995 *E. coli* O157:H7 was detected in 40 patients (13 people hospitalized) in the USA, and 70% of these patients reported having consumed leaf lettuce (Ackers *et al*., 1998). In Japan, in 1996 *E. coli* O157:H7 poisoning likely occurred from white radish sprouts. In this outbreak 7,996 people became ill, with 398 people hospitalized and three deaths occurring (Michino *et al*., 1999). *E. coli* O157:H7 has also been traced to sprouts (Watanabe *et al*., 1999), cantaloupes, apples and leaf lettuce (Ackers *et al*., 1998, Hilborn *et al*., 1999). Wachtel and Charkowski (2002) reported that 72 cases of *E. coli* O157:H7 poisoning occurred in the USA in 1999 were from shredded iceberg lettuce. Westrell *et al*. (2009) reported a total of 2,905 cases of *E. coli* O157:H7 poisoning in the 27 EU Member States and the four European Free Trade Association (EFTA) countries.

Staph. aureus is also a major cause of foodborne diseases, generally existing on nasal mucosa, skin and hair of animals and sometimes produce enterotoxins (Loir *et al*. 2003). Of the total food poisoning cases in the United Kingdom, 1 to 6% between 1969 and 1981 and 0.5 to 1% between 1982 and 1990 were caused by *Staph. aureus* (Wienekea *et al*., 1993). The most common origin of *Staph. aureus* poisoning is from meat products, but any product can be contaminated by *S. aureus* because it can be passed on to produce from the skin of workers (Wienekea *et al*., 1993). It is commonly detected in fresh vegetables, with 56.9% of salad vegetables purchased from street vendors in India (Viswanathan and Kaur, 2001) and all vegetables purchased from five different shops in Bangladesh (Rahman and Noor, 2012) contaminated with *Staph. aureus*.

L. monocytogenes is a well-known pathogen for which mortality is sometimes higher than for other pathogens (Cook, 2011). According to Lim *et al*. (2010), 23 listeriosis notifications were reported in New Zealand in 2010, of which 31 people were hospitalised and seven died. Eighty-five percent of the listeriosis illnesses were estimated to be

foodborne and origin of 50% of cases were from ready-to-eat meat. In 2010, the number of New Zealand listeriosis cases was higher in females, the most vulnerable being women over 70 years old (all death cases were over 70 years old) (Lim *et al*., 2010). The most dangerous factor was underlying illness (68.8%) or receiving immunosuppressive drugs (52.9%). The trend of the number of the outbreaks has fluctuated. Lim *et al*. (2010) reported that *L. monocytogenes* isolates were confirmed from 22 people in 2010 in New Zealand. In 2011, during a multi-state outbreak of listeriosis caused by *L. monocytogenes* in cantaloupe, 99% of patients were hospitalized and 33 patients (22%) died in the USA (McCollum *et al*., 2013).

Figure 2.1 shows the contribution of the different of pathogens associated with foodborne outbreaks of fresh produce in the USA between 1998 and 2012 (Centers for Disease Control and Prevention (CDC), 2013).

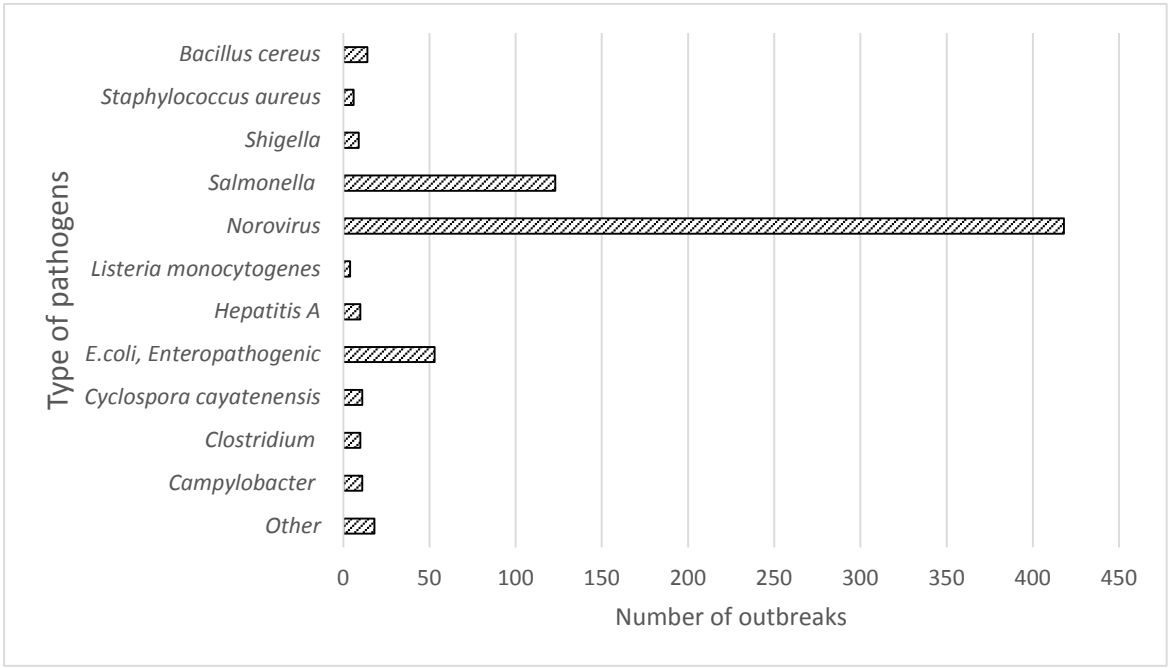


Figure 2.1 Foodborne pathogens linked to fresh produce poisoning outbreaks in the USA between 1998 and 2012. Source: CDC (2013)

According to the CDC (2013), in the USA between 1998 and 2012, 889 outbreaks out of 16,576 were related to fresh produce. Of these, pathogen type was identified in 699

cases. As seen in figure 2.1, norovirus was the most common pathogen, followed by *Salmonella* and Enteropathogenic *E. coli*. Table 2.2 lists some selected studies on microbial contamination of fresh produce or cut vegetables. All these studies showed similar results for the level of contamination with APC and the presence of pathogens.

Table 2.2 Selected reports of microbial contamination of fresh produce

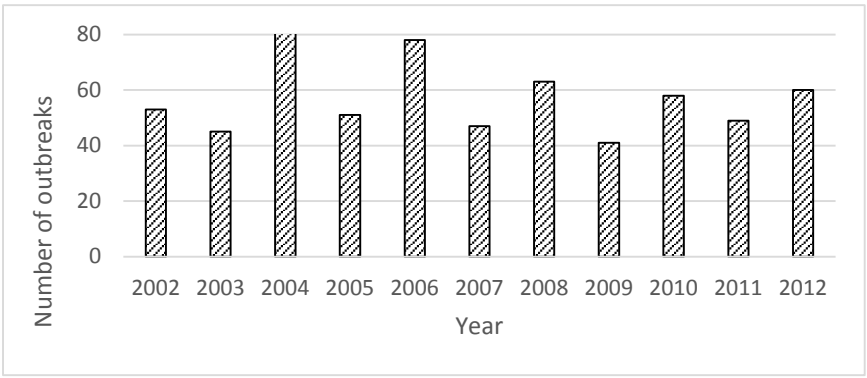
Produce	Microbial contamination level	References
Mix salad, ready-to-eat (RTE) salads and sprout samples	APC 10^7-10^8 CFU/g; *E. coli* 40%; *Salmonella* 1.3%; and *L. monocytogenes* 0.7%. No *Y. enterocolitica*; *E. coli* O157:H7; and *Campylobacter.*	Abadias *et al*. (2008)
Whole vegetables	APC 0–7.4 $\log_{10}$CFU/g; *E. coli* 0–3.8CFU/g.	Aycicek *et al*. (2006)
Fruits and vegetables, packed RTE vegetables	APC 10^0-10^{10} CFU/g; coliform 10^0-10^{10} CFU /g; yeast and mould 10^1-10^9 CFU/g.	Badosa *et al*. (2008)
Whole vegetables, RTE vegetables, mixed salads and mixed lettuce	*Salmonella* 0.75% of the whole vegetables; *L. monocytogenes* 1.61% of the whole vegetables; and 0.29% in RTE samples. No *E.coli* O157:H7.	De Giusti *et al*. (2010)
Whole vegetables, herbs and fruits	APC 10^4-10^8 CFU/g; *L. monocytogenes* 0.64%. No *Salmonella*; *Shigella*; or *E. coli* O157:H7.	Johnston *et al*. (2005)
Whole vegetables	*E. coli* 8.94%; *L. monocytogenes* only in organic 1.12%. No *E. coli* O157:H7; *Salmonella.*	Loncarevic *et al*. (2005)
Whole vegetables	APC 10^6-10^7CFU/g; *E. coli* 41.5%- organic and 40%-conventional. No *Salmonella.*	Maffei *et al*. (2013)
Mix vegetables and whole vegetables	*Aerininas* 34%. No *E. coli; E.coli* O157:H7; *Salmonella; Listeria; or Campylobacter.*	McMahon and Wilson (2001)
Whole vegetables	APC 10^5CFU/g conventional, 10^6 CFU/g organic; *E. coli* 12.9% conventional and 22.2% organic. No *E.coli* O157:H7; *L. monocytogenes; or Salmonella.*	Oliveira *et al*. (2010)
Whole vegetables	Not satisfactory *E. coli; Listeria.* No *Campylobacter; E. coli* O157:H7; *or Salmonella.*	Sagoo *et al*. (2001)
Whole vegetables and fruits	APC 10^1-10^9 CFU/g. No *E. coli*; or *Salmonella.*	Seow *et al*. (2012)
Whole vegetables	APC 10^5-10^6CFU/g.	Sirsat and Neal (2013)
Whole vegetables, sprouts and fruits	APC 10^9-10^{10} CFU /g; *E. coli* 39.2%; *Staph. aureus* 58.3%; and *Salmonella* 28.3%.	Viswanathan and Kaur (2001)

2.4. Frequency of fresh produce related foodborne outbreaks

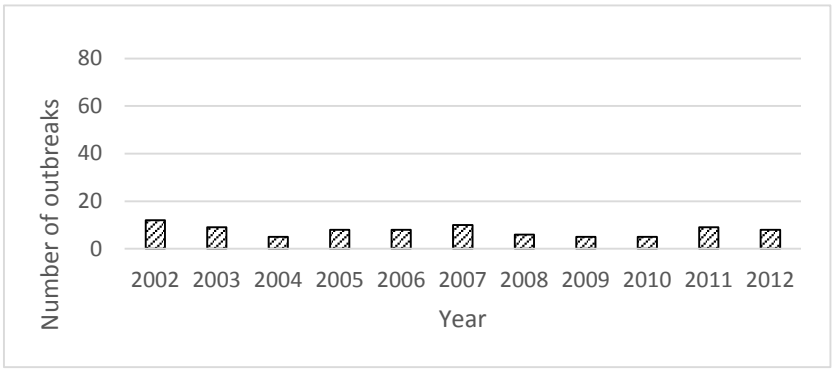
According to the Center for Science in the Public Interest (CSPI) (2009), of 4,638 outbreaks (117,136 cases) of foodborne illnesses in the USA from 1998 to 2007, 57 to 70% of them could not be traced to the contamination source. The most frequent source was seafood (838 outbreaks) followed by fresh produce (684 outbreaks), poultry (538 outbreaks) and pork (200 outbreaks) (CSPI, 2009). Vegetables contributed to 33% (228 outbreaks) of the

produce category and about 50% (345 outbreaks) to dishes of produce including salads (CSPI, 2009). In New Zealand, 716 food poisoning outbreaks occurred in 2012, 13.3% of which were from leafy vegetables, followed by root vegetables (10%), fruits and nuts (6.7%) and stalk vegetables (3.3%) (The Institute of Environmental Science and Research Ltd (ESR), 2012). The most common pathogen was Norovirus (27%) followed by *Salmonella* spp. (20%) and *Campylobacter* spp. (17%) (ESR, 2012). Figure 2.2 shows the frequency of the foodborne outbreaks linked to fresh produce contamination between 2002 and 2012.

In the USA, the number of fresh produce outbreaks have fluctuated but not declined (Figure 2.2.A). On average, the USA had 57 outbreaks due to fresh produce contamination each year. In Japan the frequency of foodborne outbreaks (bacteria, viruses and chemicals) related to fresh produce declined between 2002 and 2012 (Ministry of Health Labour and Wealfare, 2014). There was a 33% decrease in the total number of outbreaks between 2002 and 2012 in Japan (figure 2.2.B). Figure 2.2.C presents the data for fresh produce (vegetables, fruits and nuts) in New Zealand (ESR, 2014). No specific trend can be seen in the incidence of outbreaks between 2002 and 2012. However, in 2012 there were five times as many outbreaks than a decade ago. It is apparent from these examples that fresh produce is a considerable contributor towards foodborne outbreaks throughout the world.



(A)



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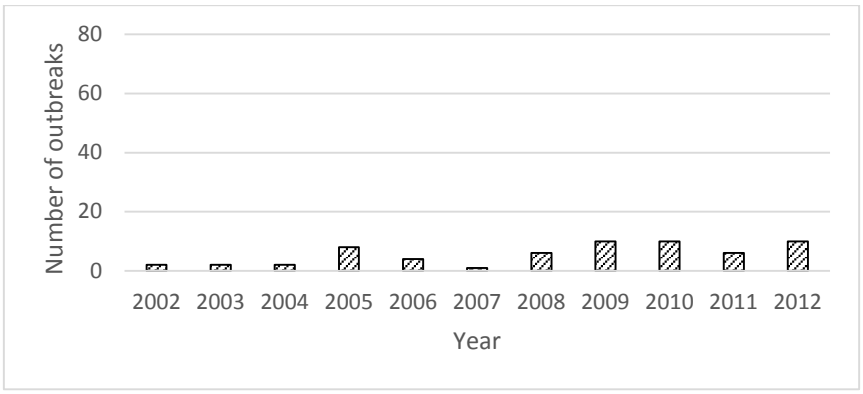


(C)

Figure 2.2 Frequency of foodborne outbreaks linked to fresh produce contamination between 2002 and 2012: A, USA (CDC, 2013); B, Japan (Ministry of Health Labour and Wealfare, 2014); C, New Zealand (ESR, 2014)

Figure 2.3 shows the number of *Salmonella* positive samples in fresh produce in the European Union (EU) member countries from 2011 to 2012 (European Food Safety Authority and 2014). The number of *Salmonella* positive samples differed between countries, probably because each country sets its own rules to manage food safety of fresh produce or used different types of detecting methods. In 2011, only three countries in the EU had *Salmonella* positive samples, but in 2012, eight countries had *Salmonella* positive samples. This indicates management of food safety was not effective in the EU member countries. An effective management system for fresh produce could be set up to correct this.

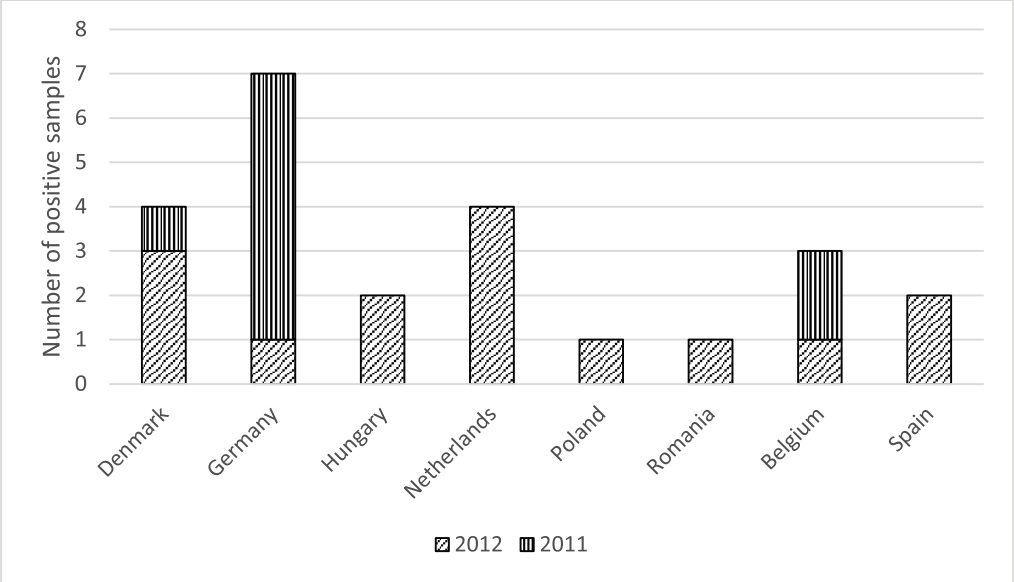


Figure 2.3 Number of *Salmonella* positive samples in fresh produce in the European Union countries from 2011 to 2012. (EFSA, 2014)

2.5. Factors that affect microbial contamination of fresh produce

There are many intrinsic and extrinsic factors that favour microbial growth. Firstly, the moisture content in fresh produce is approximately a_w = 0.97 – 1.00, which is favourable for the growth of microbes. The optimum moisture content for *Salmonella* spp., enterohemorrhagic *E. coli* and *Campylobacter* spp. is 0.99; that of *Staph. aureus* is 0.98

and the minimum moisture content for *L. monocytogenes* is 0.92 (U.S. Food and Drug Administration, 2013). The problem is that it is difficult to reduce the moisture content of fresh produce unless it is processed. Secondly, pH is an important factor because each microbe type has an optimal pH level for its growth. According to Koutsoumanis and Sofos (2004), *E. coli* O157:H7 and *S. typhimurium* can tolerate even acidic pH (to pH 5.0), while *L. monocytogenes* grows only to pH 6.0. According to the FDA (2013), pH 4.6 is about the minimal growth limit pH for most pathogens. However, there are some pathogens, such as *C. botulinum*, that can withstand acidic pH as low as pH 4.2. Several researchers, including Kim and Hung (2012), used electrolyzed oxidizing water to change the surface pH of blueberries and this was effective in reducing the microbial load from fresh produce. A third factor is the different types of nutrients in food, such as proteins, fat, minerals and vitamins. Sugars and proteins are the most common energy source for pathogens. However, some pathogens require special nutrients to grow. For example, *Staph. aureus* requires biotin for its growth (Mah *et al*., 1967), while the growth of *Salmonella* increases in the presence of iron (compared to a non-iron environment) (Kortman *et al*., 2012). As each vegetable has a different composition of nutrients, analysis of nutrients for optimal pathogen growth on different types of vegetables should be studied further.

According to Fernández *et al*. (2013), shape or structure of foods such as 'the stomata of lettuce, the convolutions of strawberry surfaces and the walls of the eukaryotic cells of potato tissue' protect *Salmonella* from inactivation by gas plasma (cold ionized gases). Injury to vegetables or fruits can increase the chances of contamination by pathogens. For example, Eblen *et al*. (2004) reported that holes in the peel of an orange could allow *E. coli* O157: H7 and *Salmonella* to enter the produce easily. Internalization of pathogens can occur not only from damage to fresh produce but also from the roots. According to

Solomon *et al*. (2002), *E. coli* O157:H7 can pass from soil and water to the inside of plants through their root systems. However, according to Trevor (2011) the risk of internalization from roots is negligible. Since leafy vegetables have many places for pathogens to conceal, different interventions would be required to suit each group of fresh produce to manage food safety.

Oxidation-reduction (or redox) potential (the ability of material to oxidize or reduce) also influences the growth of pathogens. According to Snyder (2008), oxidation-reduction potential varies between food types and has a profound effect on the growth of some microorganisms, such as *C. botulinum* (Hambleton, 1994). The redox potential of a food relates to many factors, such as pH, packaging and types of ingredients. Normally the redox potential of fresh produce is low (FDA, 2013). Guentzel *et al*. (2008) analyzed the effect of redox potential on *E. coli*, *S. typhimurium*, *Staph. aureus*, *L. monocytogenes* and *Enterococcus faecalis* using electrolyzed oxidizing water at a near-neutral pH condition and reported that all microbial counts were reduced effectively. This method has no side effects and therefore is a good way to maintain food safety of fresh produce without application of chemicals or antimicrobial agents.

Antimicrobial agents which originate from natural or artificial materials have a strong impact on pathogen growth. Sirsat and Neal (2013) used vinegar (2.5% acetic acid) on *E. coli* and *Salmonella* and reported that it significantly reduced microbial count (2 to 3 $\log_{10}$CFU/g). Similarly, Medina *et al*. (2007) reported that the effect of vinegar, olive oil and several beverages including wine were effective in controlling the growth of certain pathogens (*S. enteritidis* and *L. monocytogenes*). According to Gálvez *et al*. (2007), many lactic acid bacteria can produce bacteriocin through lactic fermentation, which can act as

an antimicrobial agent. Vidhyasagar and Jeevaratnam (2013) reported that the bacteriocin produced from one of the lactic acid bacterial species (*Pediococcus pentosaceus* VJ13) has antimicrobial effects on *Mycobacterium smegmatis*, *Klebsiella pneumonia*, *C. perfringens* and *Staph. epidermidis*. Since bacteriocins are effective and produce few side effects, investigations into applying it to fresh produce would be helpful. In addition, the FDA (2013) reported that there are many kinds of artificial antimicrobial agents (preservatives or additives) such as nitrates and nitrites, sulfur dioxide, acetic acid and nisin that could be used to control or eliminate foodborne pathogens in fresh produce. For example, sulfur dioxide, acetic acid, sorbic acid, benzoic acid, and BHA and BHT are effective for vegetable products as well as fruits products (FDA, 2013).

Processing conditions also affect the growth of pathogens. Firstly, the packaging or product environment has a significant impact on pathogen growth. According to Sun *et al*. (2014), chlorine dioxide gas significantly reduces the total bacterial count, *E. coli*, yeast and mould concentration on fresh blueberries. Simon et al. (2004) reported that an optimum concentration of gases (low oxygen and high carbon dioxide concentrations) could be determined for each product to minimize microbial growth. Thompson (2010) reported that oxgen, nitrogen and carbon dioxide are the most important gases in a modified atmosphere for pathogen control in fresh produce. Nitrogen has antimicrobial properties, oxygen is effective in controlling anaerobic microbes and carbon dioxide, which can reduce pH if dissolved in the liquid portion of a food, has an inherent antimicrobial activity. The recommended percentage of these three gases differ for each produce type and is an area that requires further investigation.

Temperature is the most important factor for bacterial growth. Koseki and Isobe (2005) conducted an experiment on the growth of *E. coli* O157:H7 and *Salmonella* spp. in lettuce at different times (0 h to 120 h) and exposure temperatures (5, 10, 15, 20, and 25^{o}C). Both

bacteria increased with time and temperature. The optimal temperature for each of the following pathogens are: *S. typhi* - 35 to 37^{o}C (ESR, 2010e), *E. coli* O157:H7 - 37^{o}C (ESR, 2010c), *Campylobacter* - 42^{o}C (ESR, 2010a), *Staph. aureus* - 37^{o}C and *L. monocytogens* - 37^{o}C (ESR, 2010d). There is one exception as ESR (2010b) reported that the optimal temperature of some strains of *C. botulinum* (those that are proteolytic, mesophilic and produce toxins A, B or F (group 1)) is 35-40^{o}C, but for those strains that are non-proteolytic, mesophilic and produce B, E or F type toxins (group 2), it is 18-25°C. In addition, the minimum temperature at which *C. botulinum* group 2 strains can grow is only 3°C, whereas the minimum growth for group 1 strains is 10°C (ESR, 2010b). *C. botulinum* is an anerobic bacterium and botulism (which can be fatal) tends to occur in those who consume canned products stored in a cold place because inside of the canned product is oxygen free environment. Date *et al*. (2011) reported three outbreaks of botulinum poisoning caused by consumption of canned vegetables produced at home in the USA. According to Date *et al*. (2011), 38% of botulinum poisoning between 1999 and 2008 in the USA were from canned products produced at home. Commercial canned products have also caused botulinum poisoning (Date *et al*., 2011). In 2007, an outbreak of botulinum poisoning in the USA was caused by canned hotdog chili sauce (Juliao *et al*., 2013). In order to achieve better food safety, optimal temperature of processing for different kinds of packaging and/or products should be set.

According to Shalini and Singh (2014), factors that inhibit pathogen growth are called ‘hurdles’. ‘Hurdle technology’ (a combination of ‘hurdles’) should be used to prevent pathogen growth. Hurdles include the factors mentioned above, such as different kinds of processing methods. For fresh produce such as lettuce, radish sprouts and apples, a combination of calcium oxide and sonication was effective in lowering the pathogen load (*E. coli* O157:H7, *L. monocytogenes* and *S. typhimurium*) more than sonication or calcium

oxide alone (Yoon *et al*., 2013). Similarly, Brown *et al*. (2011) used a combination of chlorine and lactic acid bacteria to inhibit *E. coli* O157:H7 and *C. sporogenes* in ready-to-eat spinach. It was reported that the hurdles were effective in reducing the pathogen load, except in *C. sporogenes* which has ability to grow at low (3.3^{o}C) temperature (Brown *et al*., 2011). In contrast, Ganesh *et al*. (2012) used 'natural agents' as hurdles, such as malic, tartaric and lactic acids and grape seed extract as an electrostatical spray on spinach and lettuce to reduce *E. coli* O157:H7 load, and found that all agents except tartaric acid were effective. As there is the possibility of using a combination of hurdles, more research could be directed to investigate the most effective hurdle combination for each fresh produce.

2.6. Future implications

Numbers of food poisoning outbreaks have been variable in spite of the many measures taken to reduce the incidence. There is no trend in food poisoning related to fresh produce, although outbreaks have varied between different countries. Recent trends show that people prefer healthy produced foods. As fresh produce is normally eaten raw, more research is required to reduce pathogen load on fresh produce. Currently, not many countries have regulations to monitor pathogens in fresh produce. Therefore, guidelines to manage fresh produce to prevent contamination of pathogens should be set and monitored regularly. In addition, effective technologies to manage fresh produce with materials produced from natural agents need to be tested. The list of recommended strategies to reduce bacterial pathogens contamination levels in fresh produce is shown in table 2.3. Although some studies report the use of natural agents as hurdles, there is little information on the combined effects of these agents (Gupta *et al.*, 2012, Gastélum *et al*., 2012). Other possible hurdles reported include use of electrolyzed oxidizing water (Ding *et al*., 2011a), bacteriocins (Mitić-Ćulafić *et al*., 2014), modified atmosphere (Gomes *et al*., 2012), bacteriophage spray (Viazis *et al*., 2011) and strict management of temperature and storage times. To keep fresh produce safe, further studies are required

to determine how to manage pathogens on fresh produce with natural agents instead of using chemicals.

Table 2.3 Key recommendations to reduce pathogen load on fresh produce

Produce type	Recommended strategies	References
Leafy greens	Bacteriophage cocktail, BEC8 (10^6 CFU/leaf with the essential oil trans-cinnameldehyde (0.5% v/v).	Viazis *et al*. (2011)
Fresh produce (lettuce, radish sprout and apple)	Calcium oxide (2% CaO) and sonication (10min at a frequency of 20 kHz).	Yoon *et al*. (2013)
Lettuce	Wash with calcinated calcium (the heated scallop shell powder).	Kim *et al*. (2011)
Carrots	Mildly heated (45°C), slightly acidic electrolyzed water with low available chlorine (23 mg/L).	Koide *et al*. (2011)
Cauliflower	8% Salt; 0.3% Citric acid; 300 ppm Potassium metabisulphite; and 300 ppm Sodium benzoate. Store at 5-7°C.	Sinha *et al*. (2013)
Broccoli	Irradiation with UV-C light (8 kJ m^{-2}) and then heating (45°C, 3h in air oven) .	Lemoine *et al*. (2008)
Paprika	Wash in 1% calcium chloride and 6% calcium ascorbate combined with 50°C water temperature for 2 min.	Das *et al*. (2010)
Cantaloupe	Hot water (75°C, 1min); and gaseous ozone (10,000 ppm, 30min).	Selma *et al*. (2008)
Tomato	Humidified flow of ozone-enriched air (4 ± 0.5 μL^{-1} of ozone for 30 min every 3 h).	Aguayo *et al*. (2006)
General	Conducting research in fresh produce safety; Implementation of food safety programs; Outreach education for farmers, shippers and processors; Promote the safe and careful handling of fresh produce.	Alliance for Food and Farming (2010)

Chapter 3 Microbiological risk assessment of fresh produce from different soil enrichment systems in Canterbury, New Zealand

3.1. Summary

The microbiological status of fresh produce from different farms in Canterbury, New Zealand was acceptable for *E. coli* O157:H7 (not detected) but not for APC, coliform, *E. coli, Staph. aureus* or *Salmonella* spp. For APC, 88% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 33%. For coliforms, 92% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 61%. For E. coli, 92% samples had unsatisfactory levels for the organic farms and that of the conventional farm was 88%. For *Staph. aureus,* 21% samples had satisfactory levels for the organic farms and that of the conventional farm was 50% based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. The yeast and mould (1.78 – 6.10 $\log_{10}$CFU/ml) were similar to studies previously reported for samples purchased in retail shops (Sirsat and Neal, 2013, Maffei et al., 2013, Seow et al., 2012). *Staph. aureus* and *Salmonella* spp. showed higher resistance to some antibiotics. There were significant differences ($p<0.05$) between the conventional farm vegetable samples and those of the organic farms for the average of APC, coliform, yeast and mould and *Staph. aureus.* In addition there were were significant differences ($p<0.05$) between the compost farm vegetable samples and those of the other farms for the average of *E. coli*, and between the conventional farm silver beet samples and those of crop-livestock rotation farm for the of *E. coli*. The fresh produce collected from the organic farms was more contaminated with a wider range of pathogens than produce from the conventional farm. Since the consumer perception of organic produce is because of its 'chemical free' status with little regard to the microbial status, it is prudent to conduct further studies to determine the

type(s) of natural agents that would reduce the microbial load to make organic produce safer for human consumption.

3.2. Introduction

Microbiological food safety is a grobal concern. As global food chain becomes increasingly complex, extra challenges confront food safety. Nowadays, people eat more fresh produce such as raw vegetables and fruits because these are perceived as healthy food choices. In addition, many consumers are concerned about chemicals used in food production such as fertilizer and pesticides. Hence the number of people who purchase organically grown food products are increasing. Organic farming is considered as a sustainable, humane and non-polluting method of producing food products without the use of toxic chemicals and / or genetically modified organisms (Food and Agriculture Organization of the United Nations (FAO), 1998). In Europe, approximately 5.4% of the agricultural production land is used for organic farming (European Union, 2013) compared to 0.6% and 1.6% in the USA and New Zealand (FiBL and IFOAM, 2013). There are some drawbacks in organic farming. Firstly, production per hectare is less than from conventional farms. Anwar *et al*. (2005) found that the yield and quality of farm products is the highest in conventional farms than the farms that use a combination of organic manure and fertilizer. Secondly, risk of food poisoning from organic fresh produce is probably more than from conventional fresh produce because of the use of manure and other types of soil enrichments. Pell (1997) suggested a higher risk of pathogen contamination of products because manure which is animal faeces contain in excess of 10^{10} bacteria/g. Therefore, the concern for the food safety in organically grown fresh produce has been growing. According to Jiang *et al*. (2002), *Escherichia coli* O157:H7 is able to survive in the soil with manure for 231 days at 21^0C . However, Johannessen *et al*. (2004) found no significant difference in the bacteriological quality of organic produce farmed in soils with or without *E.coli* O157:H7. Most studies did not find *E. coli* O157:H7 or *Salmonella* in the fresh organic produce (McMahon and Wilson, 2001, Oliveira *et al*.,

2010, Sagoo *et al*., 2001) and there was no consistent trend of the existence of pathogens (Maffei *et al*., 2013) or when found were within the acceptable limit compared to guidelines in UK (PHLS, 2000) for *E.coli* and *L. monocytogenes,* and the microbiological guidelines in Norway (The Norwegian Food Safety Authority, 2000) for *E.coli* O157:H7and *Salmonella* spp. (Loncarevic *et al*., 2005). This maybe because the pathogens are killed by high temperature generated during aging of manure. Himathongkham *et al*. (1999) reported that destruction speed of pathogens in manure was the highest at 37^0C compared to 4^0C and 20^0C. Therefore, appropriate heat treatment of manure minimises pathogen contamination of food produce. However, there have been several outbreaks of *E. coli* O157:H7 poisonings related to consumption of produce from manure applied farms (Chapman *et al*., 1997, Jiang *et al*., 2002). Pathogens such as *E. coli* O157:H7 in the soil-manure mix (Solomon *et al*., 2002) are 'internalized' which means pathogens are able to enter the plant tissues from the roots. In contrast, Gu *et al*. (2013) reported that organic soil prevents internalization of *Salmonella* in the tomato plants. Threshold for the number of *Salmonella* to internalize into produce is high and therefore, risk of microbial contamination due to internalization of from the roots is negligible (Trevor, 2011). Even when internalized, *Salmonella* in the upper part of plant is less and also inactive than in the lower part (Ge *et al*., 2013).

In organic farming, conventionally, compost is used widely instead of fertilizer because of its rich nutritional matrix (Fang *et al*., 1998). Many types of organic waste such as bark, leaf mould, and treated animal manure, have been used in compost making (Raviv *et al*., 1986). *E. coli* and *S. enteritidis* in cow manure are destroyed by composting at 45^0C (Lung *et al*., 2001). Composting of plant wastes also effectively eliminates fungi because of the higher temperatures generated during the process (Suarez-Estrella *et al*., 2003). Crop-Livestock rotation farming is a sustainable effective conventional organic farming method with rotational cropping and livestock production on the same land and offers many

benefits (Vilrla *et al*., 2003). For example, cropping improves pasture growth for livestock and supplies forage even in winter or dry season for livestock and livestock improves soil structure with their organic material and pasture can work as a cover for soil (Vilrla *et al*., 2003). As a result, both crop and livestock production can be increased with lower production cost. However, Hilimire (2011) reported concerns about food safety in the crop-livestock rotation farming. *E. coli* O157:H7 and *Campylobacter* spp. were not detected in crop-livestock rotation systems but *Salmonella* spp. were found in the soils (Hilimire, 2011). There have been no reports of microbiological assessment of fresh produce in the crop-livestock rotation farming system. This study is on microbiological risk assessment (APC, Coliform, *E. coli*, *E.coli* O157:H7, yeast and mould, *Salmonella* spp. and *Staph.aureus*) of fresh produce collected from different farming systems (compost farm, crop-livestock rotation farm and a conventional farm) in New Zealand using conventional and advanced microbiological methods. To confirm *E.coli* O157:H7 identity, a novel molecular detection system (3M, USA) was used.

3.3. Materials and methodsSelection, transport and handling of samples

Samples were obtained from three different farming systems. These included two organic farms and one conventional farm in Lincoln, Canterbury, New Zealand. One organic farm used compost (which did not include animal waste), the other used a crop-livestock rotation system (using sheep with rotation every four years). The conventional farm used fertilizer. The details of the sample plan and the number of samples are shown in Appendix (Appendix: 4 and 6). The collection of vegetable, soil and water samples was conducted from May 2014 to October 2014 which is winter time in New Zealand. The types of vegetables collected were the ones that were growing at that time in each farm. The priority was to collect vegetables that are normally eaten without cooking. The collected vegetables were: spinach, coriander, carrot, parsley, cabbage, silver beet, leek and spring onion from the compost farm, parsley, green cabbage, pac choi, spinach, silver beet and lettuce from the crop-livestock rotation farm, and spring onion, leek, radish, pac

choi (two types) and silver beet from the conventional farm. In addition, a total twelve soil samples and six water samples were collected from each farm on three different occasions a month apart. Samples were packed in individual plastic bags and transported to the laboratory. Samples were placed in the refrigerator (at 4°C) following transport to the laboratory. Samples were cut into small pieces (about three square centimetres) and placed in a sterile plastic bag for sample processing. The collection continued one time in a week for six weeks. The details of the sample plan and the number of samples are shown in Appendix (Tables 6-2 and 6-4).

3.3.2. Sample preparation

For APC, coliform, *E. coli*, *E.coli* O157:H7, *Staph. aureus* and yeast and mould, 25 g of sample was placed in a sterile stomacher bag with 225 g of 0.1% peptone water and mechanically homogenised for three min using a stomacher.

3.3.3. Enumeration of microorganisms

Plate Count agar plates were used for enumerating APC (ISO 4833:2003). The plates were placed in a 37°C incubator for 24 h. MacConkey agar plates were used for enumerating *E. coli* and coliforms (ISO 21567); plates were incubated at 37°C for 24 h. Yeast and mould agar plates were used for enumerating yeast and mould (ISO 7954:1987); plates were incubated at 30°C for 48 h. For *Staph. aureus*, Baird-Parker agar plates were used for eumerating (ISO 6888-2:1999); plates were incubated at 37 °C for 24h. The results were expressed as colony-forming units per gram (CFU/g).

3.3.4. Detection of *S. typhimurium*

For *Salmonella* spp., a commercial kit (Reveal® 2.0 for *Salmonella*, Neogen Corporation, USA) was used for the detection. 25 grams of sample was placed into a sterile stomacher bag with 200 g of steriled-purified water preheated to 42°C and mixed with supplied reagents (Reveal® 2.0 item 9705, Neogen Corporation, USA). After that, the sample was mixed by hand by grasping through the stomacher bag and incubated at 37 °C for 4h. One bottle of reconstitute 2×RV (Reveal® 2.0 item 9715, Neogen Corporation, USA) was

dissoleved into 200 g of sterilised, purified water preheated to 37 °C and mixed with the prepared samples, then samples were mixed gently and incubated at 42 °C for 24 h. Eight drops of samples were transferred into the Reveal sample cup (Reveal® 2.0 item, Neogen Corporation, USA) and the Reveal 2.0 for *Salmonella* device (Reveal® 2.0 item, Neogen Corporation, USA) was put into the cup. Results were recorded after 15 min.

3.3.5. Confirmation of *S. typhimurium*

The samples used for the detection of *Salmonella* spp. were confirmed by using *Salmonella* test kit based on the latex agglutination test (Oxoid, UK). One drop of sample prepared as above (4.3.4) was transferred onto the reaction card using a micropipet. One drop of *Salmonela* latex agent (Oxoid, UK) was added to the sample and mixed. Agglutination was observed after 2 min and compared with negative and positive controls.

3.3.6. Enumeration and confirmation of *E. coli* O157:H7

Sorbitol MacConkey agar with supplements (potassium tellurite and cefixime) was used for the detection of *E. coli* O157:H7 as described in McMahon and Wilson (2001) and plates were incubated, after the preparation described in 3.3.2, at 37 °C for 24h. 25 grams of sample was placed into a sterile stomacher bag with 225 g of bufferd peptone water (3M, USA) and incubated at 42°C for 24 h. 20 microlitres of the sample was then transferred into a lysis tube (3M, USA) and mixed. The lysis tube was heated in a heating block (100°C, 3M, USA) for 15 min and after cooling on a chill block (3M, USA) for 10 min and at the room temperature for 5 min, 20 µL of each sample lysate was transferred into reagent tubes and mixed with 20 µL of NC lysate (3M, USA). The sample was then transferred onto a speed loader tray (3M, USA) and placed in the instrument (3M™ Molecular Detection System) (3M, USA) for the assay. The detailed procedure, vegetables and soil sample test outputs are shown in Appendix (Appendix: 8, 9, 10 and 11).

3.3.7. Antibiotic resistance profiling of selected microbial isolates

Disc diffusion tests were conducted on Mueller-Hinton agar plates (Neogen, 2011) as noted in the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012). The antibiotic discs used (Oxoid, UK) were: ampicillin (10 µg)(AMP), tetracycline (30 µg)(TE), gentamycin (30 µg)(GN), erythromycin (15 µg)(E), trimethoprim–sulfamethoxazole (25 µg)(SXT), nalidixic acid (30 µg)(NA), chloramphenicol (30 µg)(C), ciprofloxacin(5 µg)(CIP), penicillin(10 µg)(P), kanamycin (30 µg)(K), streptomycin (30 µg)(S) and vancomycin (30 µg)(VA). Strains were classified as susceptible, intermediate or resistant according to the size of the inhibition zones surrounding the colony susceptibility of antibiotic resistance.

3.3.8. Statistical analyses

Statistical analysis was conducted using Excel 2013 and SPSS. Geometric means, standard deviations, ranges and medians were calculated using Microsoft Office Excel 2013 (Microsoft, USA) and one-way ANOVA tests with Tukey comparisons were used to derive statistical differences ($p<0.05$) of microbial levels by using SPSS version 21 (IBM, USA).

3.4. Results

3.4.1. Microbial quality

A total of 91 samples were analysed. The counts of APC, coliform, *E. coli*, yeast, mould and *Staph. aureus* in vegetables, soil and water samples are shown in tables 3.1 and 3.2 respectively.

Table 3.1 Levels (log_{10}CFU/g) of APC, coliform, *E. coli*, yeast, mould and *Staph.aureus* in vegetables from different farming systems; Crop-Livestock Rotation = C-L

	Samples	Compost	Samples	C-L	Samples	Conventional
APC	Spinach	6.22 -7.06	Parsley	5.30-7.08	Spring onion	0.00-5.45
	Coriander	5.45-6.65	Cabbage	3.70-6.35	Leek	4.38-5.64
	Carrot	0.00-7.12	Pac choi	5.44-6.19	Radish	0.00-4.70
	Parsley	4.69-6.20	Spinach	4.55-6.35	Silver beet	5.35-5.61
	Cabbage	5.64-6.73	Lettuce	5.64-6.60	Pac choi	4.38-5.95
			Silver beet	6.32-6.52		
Coliform	Spinach	5.75-6.54	Parsley	5.54-6.75	Spring onion	2.39-5.46
	Coriander	4.82-6.76	Cabbage	4.47-6.02	Leek	3.78-6.49
	Carrot	2.37-5.20	Pac choi	5.43-6.01	Radish	2.65-6.41
	Parsley	5.03-6.86	Spinach	4.55-6.04	Silver beet	3.99-4.97
	Cabbage	5.61-6.76	Lettuce	4.26-7.78	Pac choi	3.09-6.21
			Silver beet	5.83-6.53		
E. coli	Spinach	3.16-5.27	Parsley	3.31-5.76	Spring onion	0.00-3.49
	Coriander	3.77-6.11	Cabbage	0.00-3.59	Leek	2.16-4.04
	Carrot	1.97-4.93	Pac choi	0.00-5.71	Radish	1.99-4.93
	Parsley	2.77-6.11	Spinach	2.85-5.24	Silver beet	3.67-3.99
	Cabbage	2.40-5.68	Lettuce	3.41-5.06	Pac choi	1.98-5.36
			Silver beet	5.83-6.06		
Yeast and mould	Spinach	3.77-5.80	Parsley	3.68-6.39	Spring onion	2.46-4.13
	Coriander	3.23-5.83	Cabbage	3.54-5.80	Leek	3.21-5.50
	Carrot	1.87-4.11	Pac choi	4.00-5.50	Radish	1.78-4.01
	Parsley	5.04-5.76	Spinach	3.37-5.36	Silver beet	4.05-5.09
	Cabbage	4.61-6.10	Lettuce	2.48-4.16	Pac choi	3.83-5.03
			Silver beet	4.65-4.84		
Staph. aureus	Spinach	2.93-4.16	Parsley	1.70-4.31	Spring onion	0.00-3.35
	Coriander	2.45-4.15	Cabbage	1.90-5.58	Leek	0.00-4.37
	Carrot	0.00-1.67	Pac choi	1.68-5.16	Radish	0.00-4.17
	Parsley	2.29-3.51	Spinach	1.47-4.19	Silver beet	0.99-1.67
	Cabbage	1.46-3.84	Lettuce	2.21-2.21	Pac choi	0.00-3.51
			Silver beet	2.21-2.64		

Table 3.2 Levels (log_{10}CFU/g) of APC, coliform, *E. coli*, yeast, mould and *Staph. aureus* in soil and water from different farming systems

		Compost	Crop-Livestock	Conventional
Soil	APC	5.35-6.62	5.57-6.49	5.49-6.67
	Coliform	5.62-7.16	5.34-7.27	3.72-6.83
	E. coli	3.67-6.73	4.87-6.38	0.00-4.49
	Yeast and Mould	4.58-5.81	3.91-4.96	4.04-4.99
	Staph. aureus	1.97-3.51	0.00-5.22	0.00-3.00
Water	APC	0.00-0.00	0.00-0.00	1.48-3.72
	Coliform	0.00-0.00	0.00-0.00	0.00-4.34
	E. coli	0.00-0.00	0.00-0.00	0.00-2.18
	Yeast and Mould	0.00-0.00	0.00-0.00	0.00-3.76
	Staph. aureus	0.00-0.00	0.00-0.00	0.00-0.00

For *Salmonella* spp. detection, 79 vegetable and eight soil samples were analysed. Nine samples from all farms were positive (table 3.3).

Table 3.3 Occurrence of *Salmonella* spp. in vegetables

Salmonella	Positive	Negative	Total	%
Conventional	2	25	27	7.4
Compost	3	23	26	11.5
Crop-Livestock Rotation	4	22	26	15.4
Total	9	70	79	11.4

Salmonella spp. was present in radish, pak choi (conventional farm), spinach, parsley (compost farm) and pak choi, parsley and lettuce (crop-livestock rotation farm). Between the soil samples, two from the conventional farm and three each from the compost and crop-livestock rotation farms were contaminated with *Salmonella* spp. Nine samples were positive for *Salmonella* spp. based on the confirmation kit (Oxoid, UK).

Twenty five samples positive for *E. coli* were analysed for O157:H7 using a novel molecular detection system (3M, USA), but no *E. coli* O157:H7 isolates were present (Appendix: 11).

3.4.2. Antibiotic resistance profiles of *Salmonella* and *S. aureus* isolates

The bacterial isolates were classified as susceptible (≥20mm, S), intermediate (15–19mm, I) or resistant (≤14mm, R) according to the size of the inhibition zones at 24 h. All *Salmonella* spp. positive vegetable samples showed resistance to at least three antibiotics and all *Salmonella* spp. positive soil samples showed resistance to at least two antibiotics. 81% of *Staph. aureus* positive vegetable and 60% of soil samples showed resistance to at least one antibiotic. All *Salmonella* spp. positive vegetable samples showed resistance to vancomycin, ampicillin and penicillin. All soil samples showed resistance to penicillin and more than 80% of samples showed resistance to ampicillin. Similarly, more than 80% of *Staph. aureus* positive vegetable samples showed resistance to penicillin. In the soil *Staph. aureus* positive samples, less percentage (less than 40%) of resistance to antibiotics was observed. Individual percentages of antibiotic resistance for each farms are shown in

table 3.4 (vegetables for *Salmonella* spp.), table 3.5 (vegetables for *Staph. aureus*), table 3.6 (soils for *Salmonella* spp.) and table 3.7 (soils for *Staph. aureus*).

Table 3.4 Antimicrobial susceptibility for *Salmonella*

		VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Compost	Parsley	R	S	S	S	S	I	S	S	S	R	R	S
Compost	Parsley	R	S	S	I	S	S	S	S	S	R	R	S
C-L	Lettuce	R	S	S	R	S	R	S	S	S	R	R	S
C-L	Parsley	R	R	S	R	S	R	S	S	S	R	R	S

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), streptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT).

** Zone Diameter (mm) S=Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14.

Table 3.5 Levels of antimicrobial susceptibility for *Staph. aureus*

	%	VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Total	S	38	94	94	31	69	31	69	100	50	13	56	94
	I	44	0	0	56	0	6	6	0	31	6	31	0
	R	19	6	6	13	31	63	25	0	19	81	13	6
Conventional	S	50	100	100	25	50	50	50	100	50	25	50	100
	I	50	0	0	50	0	0	25	0	0	25	25	0
	R	0	0	0	25	50	50	25	0	50	50	25	0
Compost	S	40	80	80	60	80	20	80	100	60	20	40	80
	I	20	0	0	40	0	0	0	0	20	0	40	0
	R	40	20	20	0	20	80	20	0	20	80	20	20
C-L	S	29	100	100	14	71	29	71	100	43	0	71	100
	I	57	0	0	71	0	14	0	0	57	0	29	0
	R	14	0	0	14	29	57	29	0	0	100	0	0

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), streptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT).

** Zone Diameter (mm) S=Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14.

Table 3.6 Antimicrobial susceptibility for *Salmonella* spp. positive soil samples

	VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
C-L	R	S	S	I	S	R	R	S	S	R	R	S
C-L	S	S	S	S	S	I	S	S	S	R	R	S
Compost	S	S	S	S	S	S	S	S	S	R	R	S
Compost	I	S	S	S	S	I	S	S	S	R	R	S
Conventional	R	S	S	I	S	I	S	S	S	R	I	S
Conventional	R	S	S	S	S	R	S	I	R	R	R	S

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), streptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT).

** Zone Diameter (mm) S=Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14.

Table 3.7 Antimicrobial susceptibility for *Staph. aureus* positive soil samples

	VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Compost	I	S	S	I	R	R	R	S	I	R	I	S
Compost	I	S	S	R	R	R	R	S	S	R	I	S
C-L	S	S	S	S	S	I	S	S	I	S	S	R
C-L	S	S	S	I	S	S	S	S	S	S	S	S
Conventional	S	S	S	S	S	S	S	S	S	S	S	S

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), streptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT).

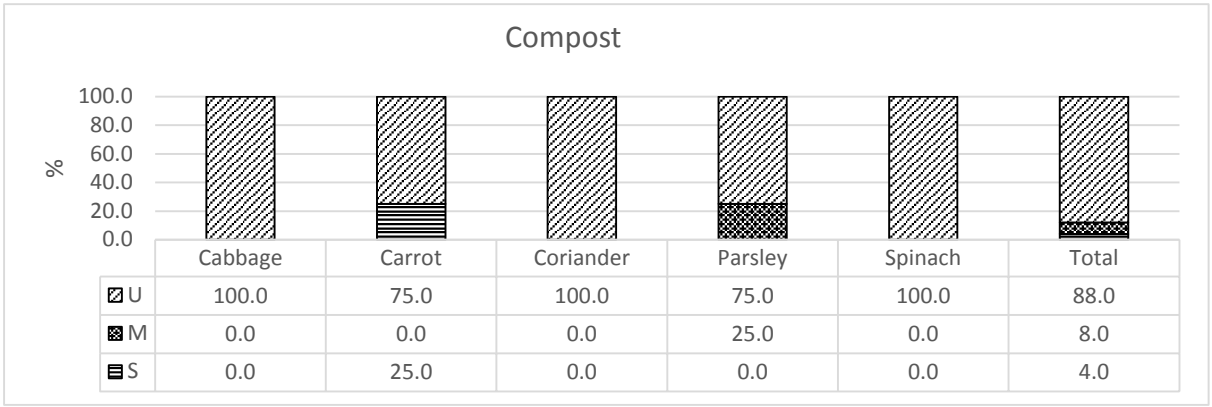
** Zone Diameter (mm) S=Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14.

3.5. Discussion

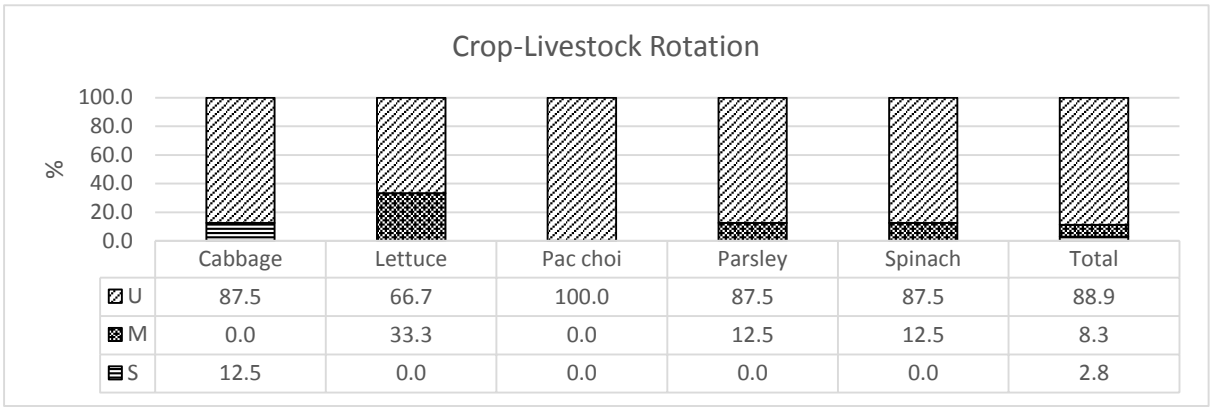
3.5.1. Microbial quality of produce

Few studies have compared the microbial quality of fresh produce from different farming systems. Halablab *et al*. (2011) reported that APC of 63 samples from several riverside farms in Lebanon ranged from 4.3-10.4 $\log_{10}$CFU/g. Oliveira *et al*. (2010) showed that the aerobic mesophilic count (AMC) of organic and conventional lettuce collected from farms in Spain varied from 0.69-6.35 $\log_{10}$CFU/g and 0.80-5.67 $\log_{10}$CFU/g, respectively. Many leafy vegetables have a large, folded surface and higher APC are often observed (Aycicek *et al*., 2006), due to the fact that splashing of soil during watering and rain events is more

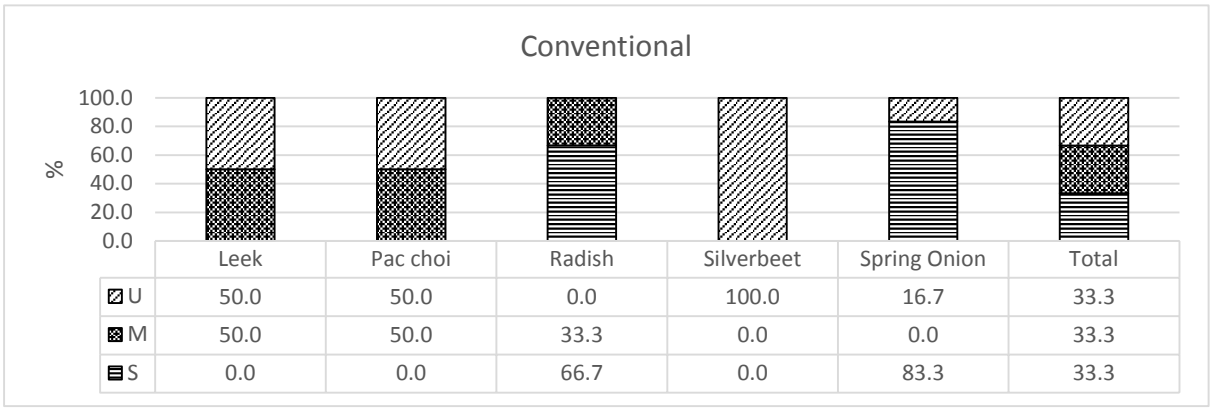
likely to contaminate leafy vegetables (Abadias *et al*., 2008). Although no specific standard has been set for New Zealand fresh produce, according to FSANZ (2001), <4 log_{10}CFU/g of APC is considered satisfactory, <5 log_{10}CFU/g of APC marginal and >5 log_{10}CFU/g of APC is unsatisfactory for ready-to-eat foods. In general, microbial counts increase more during harvesting and processing than while still at the farm (Sirsat and Neal, 2013). In this study, due to different vegetables being collected because of non-availability from all farms, a select group of vegetables were used to compare the results between the different farms, including pac choi and silver beet between the conventional and crop-livestock rotation farms and cabbage, parsley and spinach between the compost and crop-livestock rotation farms. As shown in figure 3.1, more than 88 percent of the samples from the organic farms had higher unsatisfactory levels of APC based on FSANZ (2001) guidelines while that of the conventional farm was 33 percent and there was a significant difference ($p<0.05$) in the average of APC (log_{10}CFU/ml) between organic farms and the conventional farm (figure 3.2).



	Cabbage	Carrot	Coriander	Parsley	Spinach	Total
U	100.0	75.0	100.0	75.0	100.0	88.0
M	0.0	0.0	0.0	25.0	0.0	8.0
S	0.0	25.0	0.0	0.0	0.0	4.0



	Cabbage	Lettuce	Pac choi	Parsley	Spinach	Total
U	87.5	66.7	100.0	87.5	87.5	88.9
M	0.0	33.3	0.0	12.5	12.5	8.3
S	12.5	0.0	0.0	0.0	0.0	2.8



	Leek	Pac choi	Radish	Silverbeet	Spring Onion	Total
U	50.0	50.0	0.0	100.0	16.7	33.3
M	50.0	50.0	33.3	0.0	0.0	33.3
S	0.0	0.0	66.7	0.0	83.3	33.3

Figure 3.1 Percentage of unsatisfactory (U), marginal (M) and satisfactory (S) of APC compared with FSANZ (2001) guidelines

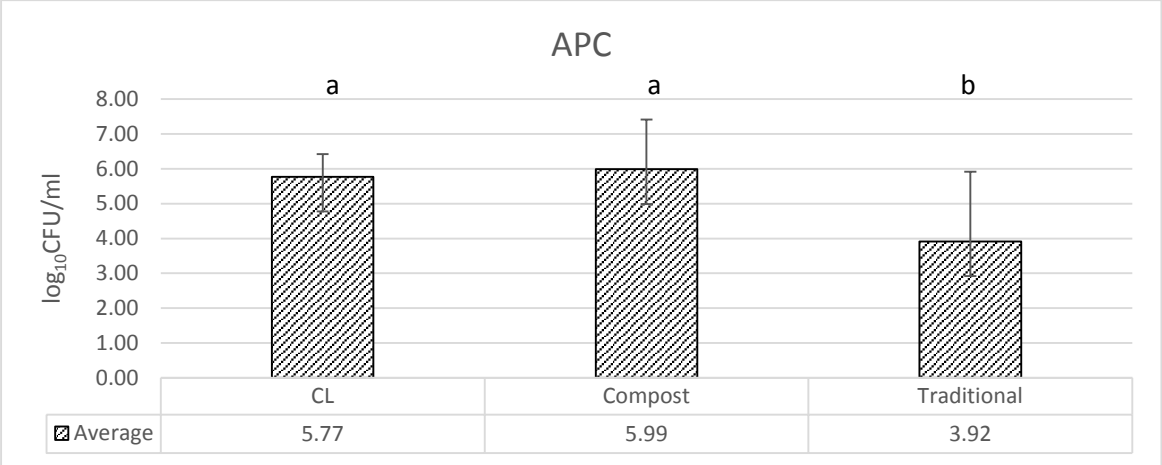


Figure 3.2 Average of log_{10}CFU/ml of APC from three farms (crop-livestock rotation farm, compost farm and conventional farm)

Different letters on bars indicate significant differences between farms ($p<0.05$).

In general, total coliform count is not a good indicator of harm to human health (BC Centre for Disease Control (BCCDC), 2013). However, among the total coliforms, *E. coli*, one of the faecal coliforms, is pathogenic to humans (BC Centre for Disease Control (BCCDC) (2013). For coliforms, FSANZ (2001) states that <2 log_{10}CFU/g is satisfactory, 2-4 log_{10}CFU/g is marginal and >4 log_{10}CFU/g is unsatisfactory. Figure 3.3 shows the percentage of satisfactory, marginal and unsatisfactory coliform in the samples based on FSANZ (2001) guidelines. As shown in figure 3.3, more than 90 percent of the samples from the organic farms had higher unsatisfactory levels of coliform based on FSANZ (2001) guidelines while that of the conventional farm was 61 percent and there was a significant difference ($p<0.05$) in the average coliform (log_{10}CFU/ml) between organic farms and the conventional farm (figure 3.4).

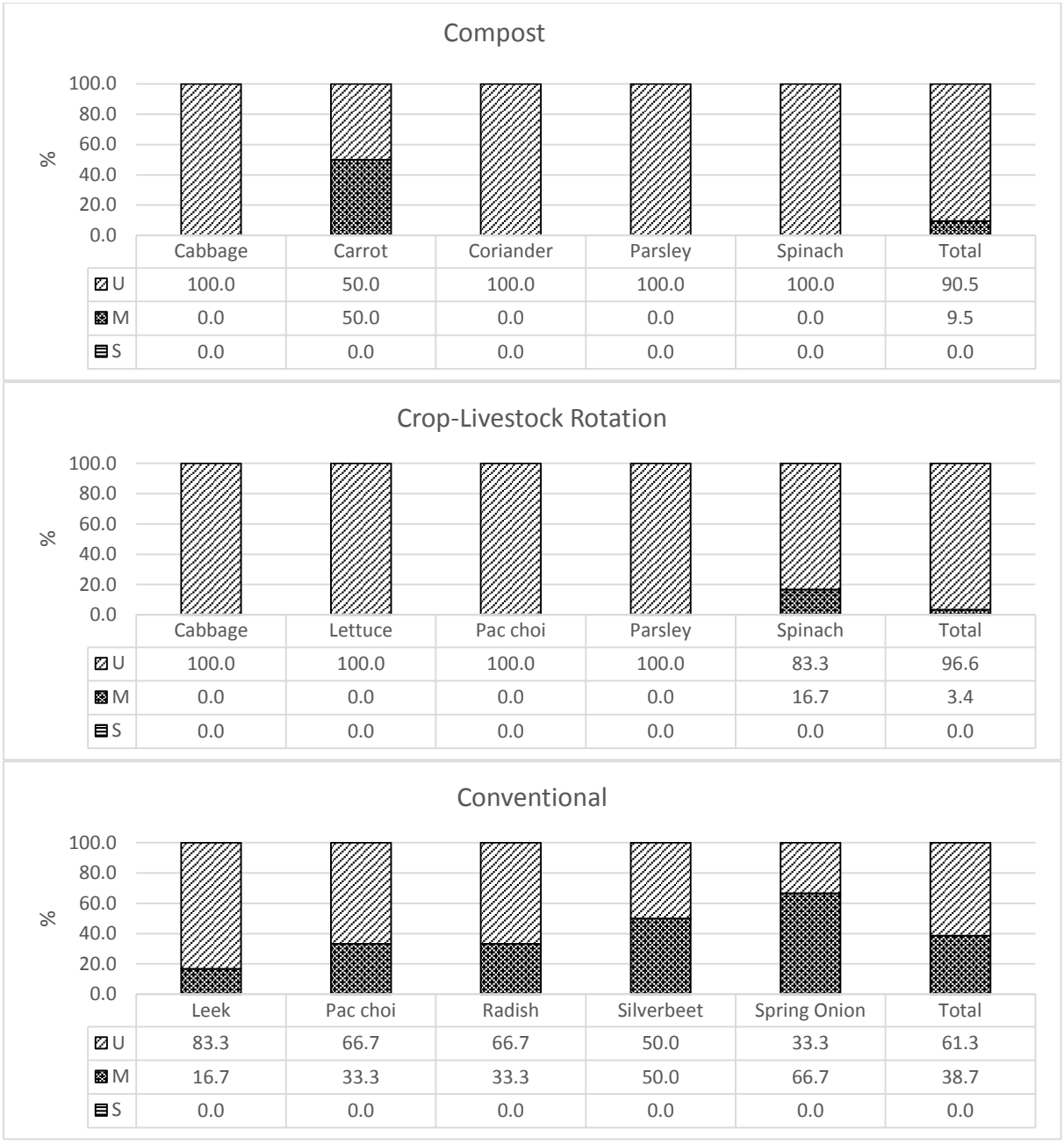


Figure 3.3 Percentage of unsatisfactory (U), marginal (M) and satisfactory (S) coliform compared with FSANZ (2001) guidelines

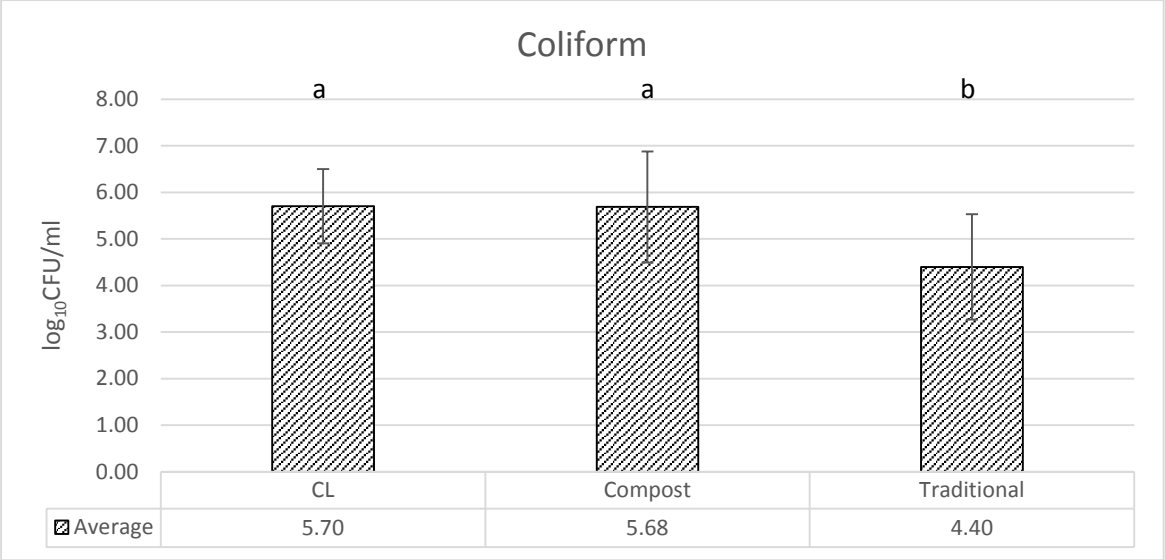


Figure 3.4 Average of log_{10}CFU/ml of coliform from three farms (crop-livestock rotation farm, compost farm and conventional farm)

Different letters on bars indicate significant differences between farms ($p<0.05$).

E. coli reported in vegetables in the literature are highly variable. Halablab *et al*. (2011) reported that 1-8.8% of vegetable samples collected from farms in Lebanon contained *E. coli*. Loncarevic *et al*. (2005) reported similar results (up to 8.9%) for organic lettuce collected in Norway, while Oliveira *et al*. (2010) reported values of 22.2% for organic lettuce and 12.5% for lettuce farmed conventionally in Spain. In contrast, Sagoo *et al*. (2001) found that less than 1.5% of organic vegetables collected from retail shops in the UK contained *E. coli*. Sirsat and Neal (2013) reported that both conventional lettuce and organic lettuce collected in the USA contained 2-3.5 log_{10}CFU/g of *E. coli*, both of which are unsatisfactory. Ideally levels should be zero, but <0.48 log_{10}CFU/g is considered satisfactory while >2 log_{10}CFU/g is unacceptable (FSANZ, 2001). In the current study, 93.8% of vegetables samples contained *E. coli* (92% from conventional farm, 96% from the compost farm and 93% from the crop-livestock rotation farm). Among the soil samples, 83% contained *E. coli* (50% from the conventional farm, 100% from the compost farm and 100% from the crop-livestock rotation farm). These results are much higher than in previous reports. As shown in figure 3.5, more than 92 percent samples from the compost and the crop-livestock rotation farms showed unsatisfactory *E. coli* based on

FSANZ (2001) guidelines while that of the conventional farm was 88 percent. There was a significant difference ($p<0.05$) in the average of *E. coli* (log_{10}CFU/ml) between the compost farm and the other farms (figure 3.6). *E. coli* in different vegetables were not significantly different ($p<0.05$) except for *E. coli* in silver beet from the conventional farm and the crop-livestock rotation farm (figure 3.7).

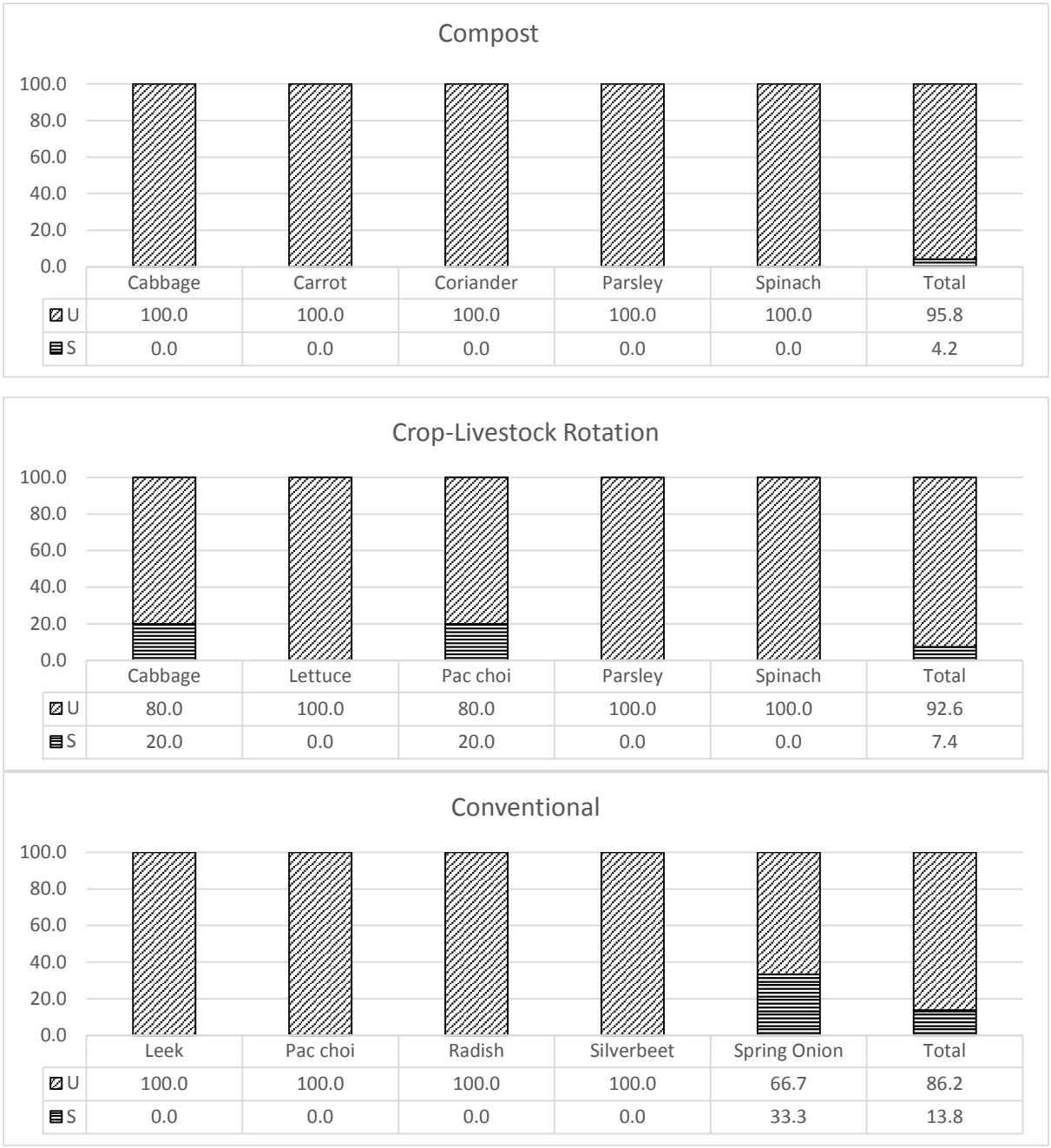


Figure 3.5 Percentage of unsatisfactory (U), and satisfactory (S) of *E. coli* based on FSANZ (2001) guidelines

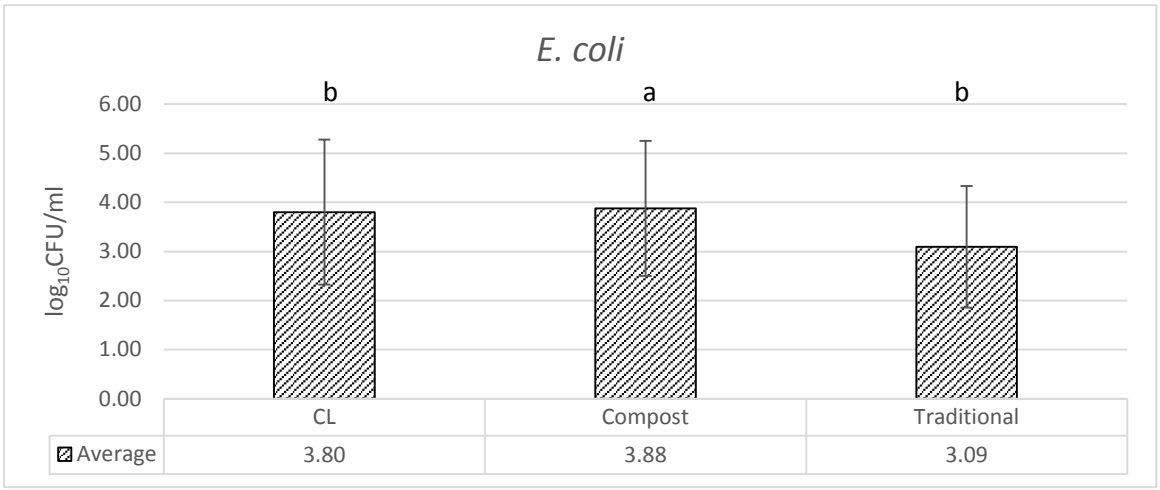


Figure 3.6 Average of log_{10}CFU/ml of *E. coli* from three farms (crop-livestock rotation farm, compost farm and conventional farm)

Different letters on bars indicate significant differences between farms ($p<0.05$).

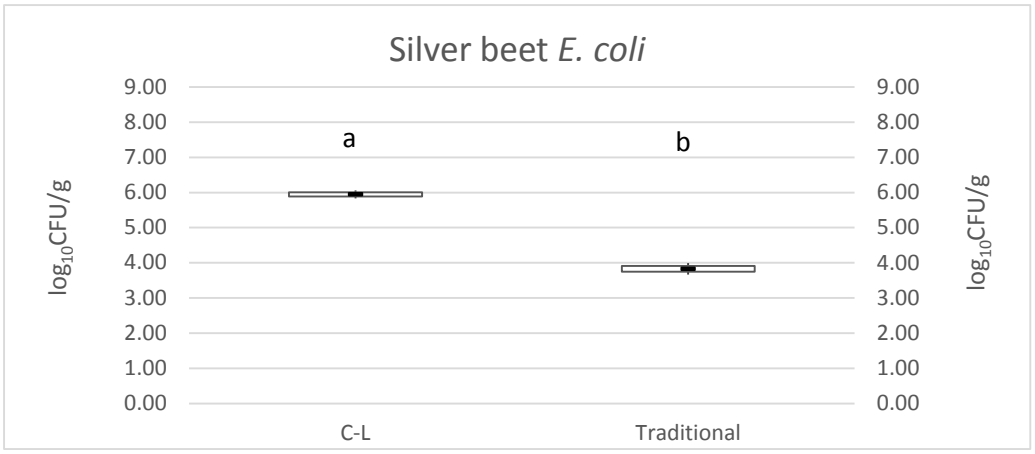


Figure 3.7 *E. coli* (log_{10}CFU/g) in silver beet from a conventional and a crop-livestock rotation farm

Different letters (a, b) indicate significant differences between the median value ($p<0.05$).

Yeast exists naturally in the environment and on rare occasions higher concentrations can contaminate and cause vegetable spoilage as well as change the colour of vegetables (Corato, 2012) or produce volatile metabolites that can lower the quality of vegetables (Ragaert *et al*., 2006). Moulds cause more harm to human health as some can produce harmful mycotoxins (Kovacs, 2004). Mycotoxins, such as aflatoxins can cause carcinogenesis, immunosuppression and developmental abnormalities in reproductive and nervous systems. The yeast and mould concentrations in different vegetables and

between the different farming systems were not significantly different ($p>0.05$). There is a lack of yeast and mould contamination data from different vegetable farming systems in New Zealand. In previous studies, yeast and mould were variable in fresh produce purchased from retail shops. Sirsat and Neal (2013) reported 5.5 and 5 $\log_{10}$CFU/g of yeast in conventional and organic lettuce collected in the USA, respectively and Nguz *et al*. (2005) reported 1.6-3.8 $\log_{10}$CFU/g for moulds in mixed cut vegetables collected in Zambia. Similarly, Oliveira *et al*. (2010) reported yeast and mould at 3.91-5.57 $\log_{10}$cfu/g and 3.25-5. 17 $\log_{10}$cfu/g for organic and conventional lettuce collected in Spain, respectively. Erkan and Vural (2008) reported 2.7-6.3 $\log_{10}$CFU/g of yeast and 2.3-4.2 $\log_{10}$CFU/g of mould in whole vegetables collected in Turkey. Tournas (2005) reported 2-8.6 $\log_{10}$CFU/g of yeast and 2-4.6 $\log_{10}$CFU/g of mould in cut and whole vegetables collected from retail shops in the USA. Other studies have reported combined yeast and mould. Maffei *et al*. (2013) reported a value of 4-7 $\log_{10}$CFU/g (Brazil), Abadias *et al*. (2008) reported it as 2-2.6 $\log_{10}$CFU/g (Spain), Badosa *et al*. (2008) reported it as 4-7 $\log_{10}$CFU/g (Spain), and Seow *et al*. (2012) reported it as 4-7 $\log_{10}$CFU/g for whole vegetables purchased from markets in Singapore. Mohammad et al. (2012) reported 5.68 $\log_{10}$CFU/g for cut vegetables and 5.78 $\log_{10}$CFU/g for herbs collected in Iran. In the current study, the level of yeast and mould was similar. There was a significant difference ($p<0.05$) in the average of yeast and mould ($\log_{10}$CFU/ml) between organic farms and the conventional farm (figure 3.8). As there is a lack of data on the yeast and mould of fresh produce collected from farms, further study is needed.

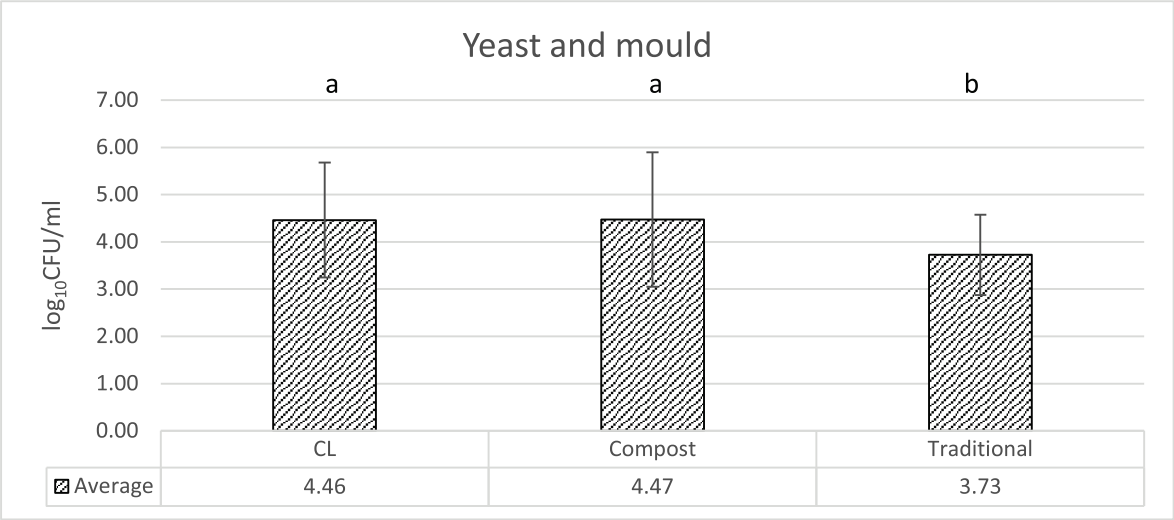


Figure 3.8 Average of log_{10}CFU/ml of yeast and mould from three farms (crop-livestock rotation farm, compost farm and conventional farm)

Different letters on bars indicate significant differences between farms ($p<0.05$).

3.5.2. Microbial quality of soil and water

According to Johannessen *et al*. (2004), microbial quality of soil varies significantly between soil enrichment types but no significant difference was observed in the lettuce grown in the different soils in Norway at harvest. For example, in the soil with no added fertilizer APC were 6.16-6.24 log_{10}CFU/g and *E. coli* were less than 1-2.14 log_{10}CFU/g, but in soils with added inorganic fertilizer, APC were 5.83-6.16 log_{10}CFU/g and *E. coli* less than 1-1.89 log_{10}CFU/g (Johannessen et al., 2004). In soils with added compost fertilizer, APC were 6.53-7.06 log_{10}CFU/g and *E. coli* 1-2.07 log_{10}CFU/g (Johannessen et al., 2004). In soils with added manure, APC were 6.17-6.73 log_{10}CFU/g and *E.coli* were 1-2.44 log_{10}CFU/g (Johannessen et al., 2004). In the current study, the range of APC for each farm were less than those reported by Johannessen *et al*. (2004) but *E. coli* were higher. In soil samples, *E. coli* (log_{10}CFU/g) were significantly different ($P<0.05$) between the conventional and compost farms, and also between the compost and crop-livestock rotation farms (figure 3.9).

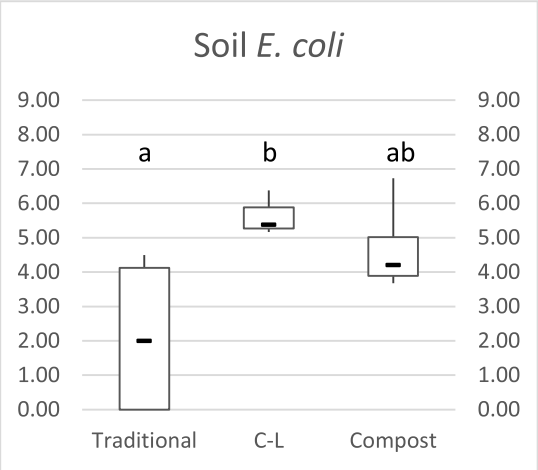


Figure 3.9 *E. coli* (log_{10}CFU/g) in soils from the three farms

Different letters (a, b) indicate significant differences between the median value ($p<0.05$).

The APC, coliform, yeast, mould and *Staph. aureus* (not shown) in vegetables from the different farms were not significantly different ($p<0.05$). As there have been few studies comparing pathogen contamination of soils to soil enrichment types, further studies are required before any firm conclusions can be made.

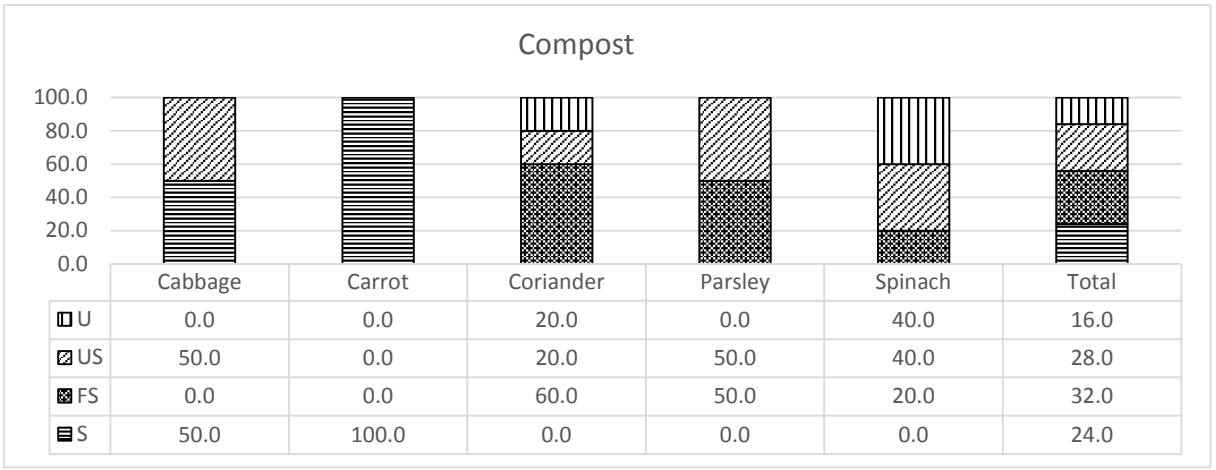
Most of the water samples showed no microbial contamination. Two water samples from the conventional farm were significantly ($p<0.05$) contaminated with APC, coliform, *E. coli* and yeast. It was suspected in this study that the contamination may have been from the water hose. The water collected from the compost farm and crop-livestock rotation farm was taken from tap water without hoses but in the conventional farm water was collected from a long hose. As the water collected from each farm was used for washing the harvested vegetables before distribution, it is important to check the pathogen contamination of the hoses, it is likely that this is rarely practiced.

3.5.3. Pathogen detection and confirmation in farm produce, soil and water

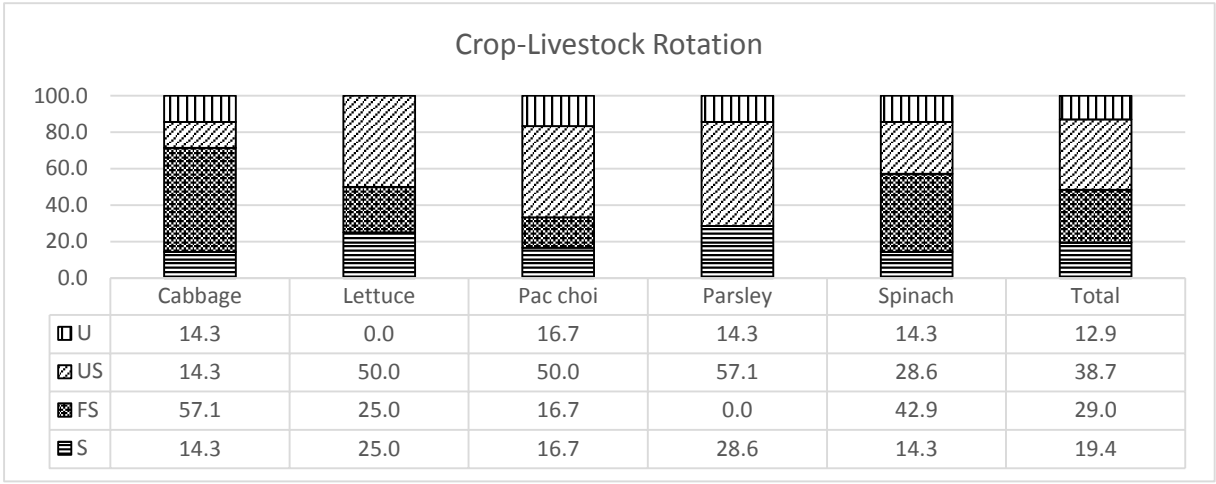
3.5.3.1. *Staphylococcus aureus*

Staph. aureus usually exists in the mucosa of nasal passages of animals and humans. In most instances it is not harmful to human health because it is vulnerable to 'hurdles,' to reduce microbial load (Maistro *et al*., 2012). The existence of *Staph. aureus* in farm produce, soil or water indicates lack of proper hygienic conditions. Halablab *et al*. (2011)

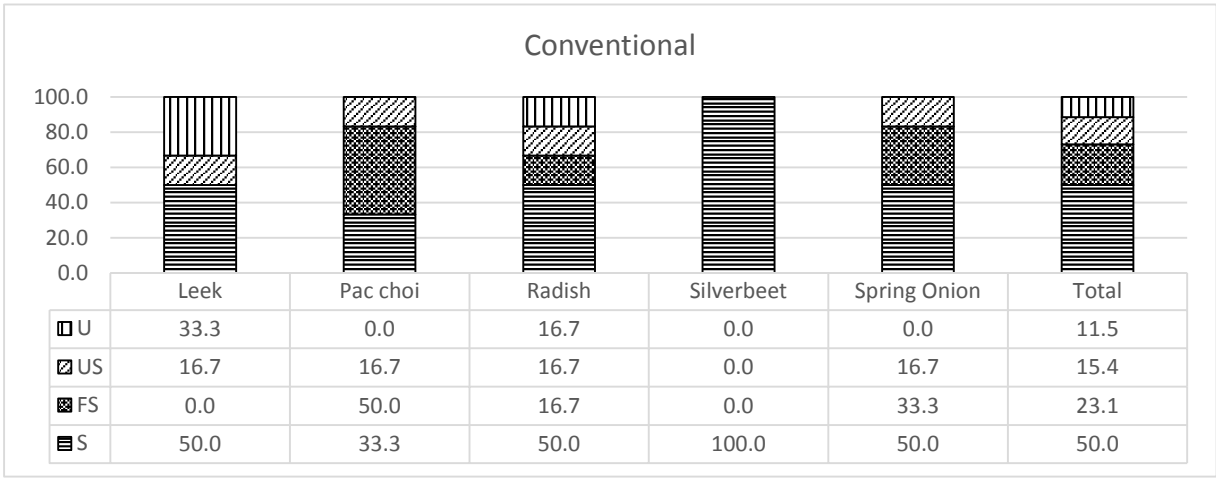
reported *Staph. aureus* in whole vegetables collected in Lebanon as 1.47-8.77 $\log_{10}$CFU/g. According to PHLS (2000), *Staph. aureus* of contamination are graded as satisfactory (<2 $\log_{10}$CFU/g), fairly satisfactory (2-3), unsatisfactory (3-4) and unacceptable (>4). The grading of the samples in this study based on PHLS (2000) guidelines is shown in figure 3.10. Approximately 20 percent of the vegetable samples from organic farms were satisfactory levels of *Staph. aureus* contamination while that of the conventional farm was 50 percent based on PHLS (2000) guidelines. There was a significant difference ($p<0.05$) in the average of *Staph. aureus* ($\log_{10}$CFU/ml) between organic farms and the conventional farm (figure 3.11). The comparison of *Staph. aureus* between different types of vegetables, same calculation was conducted with that of APC of the samples. However, no significant differences ($p<0.05$) were observed for *Staph. aureus* presence in vegetables (data not shown).



	Cabbage	Carrot	Coriander	Parsley	Spinach	Total
U	0.0	0.0	20.0	0.0	40.0	16.0
US	50.0	0.0	20.0	50.0	40.0	28.0
FS	0.0	0.0	60.0	50.0	20.0	32.0
S	50.0	100.0	0.0	0.0	0.0	24.0



	Cabbage	Lettuce	Pac choi	Parsley	Spinach	Total
U	14.3	0.0	16.7	14.3	14.3	12.9
US	14.3	50.0	50.0	57.1	28.6	38.7
FS	57.1	25.0	16.7	0.0	42.9	29.0
S	14.3	25.0	16.7	28.6	14.3	19.4



	Leek	Pac choi	Radish	Silverbeet	Spring Onion	Total
U	33.3	0.0	16.7	0.0	0.0	11.5
US	16.7	16.7	16.7	0.0	16.7	15.4
FS	0.0	50.0	16.7	0.0	33.3	23.1
S	50.0	33.3	50.0	100.0	50.0	50.0

Figure 3.10 Percentage of unacceptable (U), unsatisfactory (US), fairy satisfactory (FS) and satisfactory (S) *Staph. aureus* of vegetables based on PHLS (2000) guidelines and a comparison between different farm types

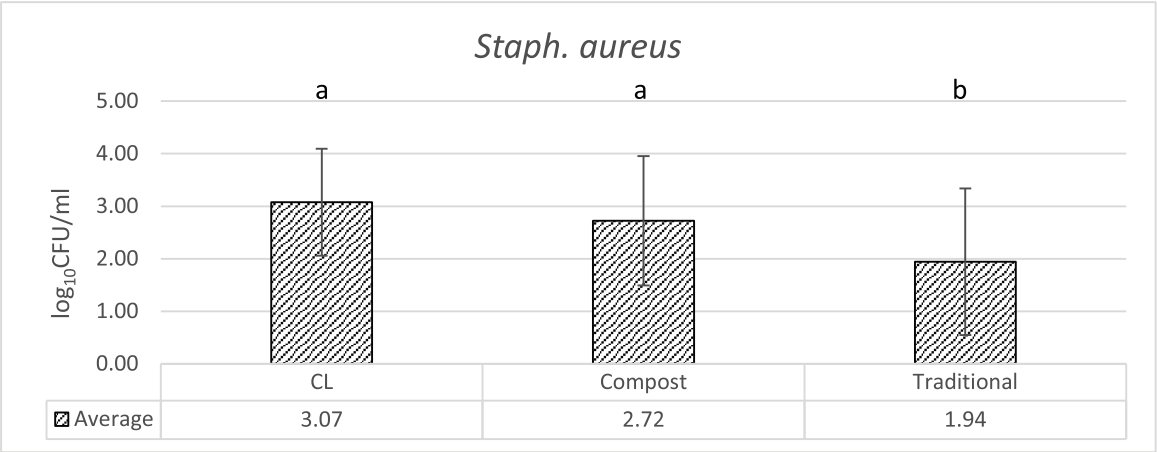


Figure 3.11 Average of log_{10}CFU/ml of *Staph. aureus* from three farms (crop-livestock rotation farm, compost farm and conventional farm)

Different letters on bars indicate significant differences between farms ($p<0.05$).

3.5.3.2. *Escherichia coli* O157:H7

E. coli O157:H7 is normally found in milk or faeces of cattle (Himathongkham *et al*., 1999) and contamination of vegetables is not common. No *E. coli* O157:H7 was detected in 3,200 whole organic vegetables collected in the UK (Sagoo *et al*., 2001), 400 whole vegetables collected in the USA (Johnston *et al*., 2005) and 44 lettuce samples collected in University restaurants in Spain (Soriano *et al*., 2000). However, there is potential for contamination of vegetables by *E. coli* O157:H7 during application of manure, irrigation or from 'contaminated surface runoff' (Ackers *et al*., 1998, Hilborn *et al*., 1999). *E. coli* O157:H7 is able to survive in manure for long periods depending on the environmental conditions (Wang *et al*., 1996, Kudva *et al*., 1998). Reduction times of *E. coli* O157:H7 in cow faeces depend on a variety of conditions. One hundred and five days at 4°C or 45 days at 37°C is required to achieve a more than 5 log_{10}CFU/g reduction (Himathongkham *et al*., 1999). In contrast, it was reported that *E. coli* O157:H7 survived for 77 days at 5°C, 226 days at 15°C, and 231 days at 21°C in manure-amended autoclaved soil (Jiang *et al*., 2002). Similarly, it was reported that *E. coli* O157:H7 survived for 21 months at 23°C in air-devoid manure, and for 100 days at -200°C and at 4 or 10°C in bovine and ovine manure,

respectively (Kudva *et al*., 1998). Similarly, according to Wang *et al*. (1996) 3 $\log_{10}$CFU/g *of E. coli* O157:H7 could survive for 42 days at 37^{o}C, 63 days at 5^{o}C and 49 days at 22^{o}C, and 5 $\log_{10}$CFU/g for 49 days at 37^{o}C, 70 days at 5^{o}C and 56 days at 22^{o}C. *E. coli* O157:H7 in raw cow faeces is 2-5 $\log_{10}$CFU/g (but lower inside than in the outer layers) and levels could potentially decline by 5 $\log_{10}$CFU/g in 15 days at 4^{o}C (Himathongkham et al., 1999). According to Buck. J. W et al. (2003), composting or aging is therefore important if manure is to be used as fertilizer and animals should not come in contact with fresh vegetable produce at any time during its production. According to FSANZ (2001), *E. coli* O157:H7 should not be present in ready-to-eat foods. In the current study, though many *E. coli* O157:H7 suspected colonies were observed in the MacConkey agar with sorbitol, cefixime and tellurite plates (all 52 samples), confirmation using a novel molecular detection system (3M, USA) showed negative results in 25 samples from fresh vegetables and soils from the three different farm. This could be due to the limitation of the MacConkey agar with sorbitol, cefixime and tellurite plates (HAEDY Diagnostics, 1996). It has been reported that sometimes sorbitol negative colonies are produced underneath the sorbitol positive colonies (which are the majority of colonies) and therefore it is difficult to obtain an accurate estimation of the exact number of colonies (HAEDY Diagnostics, 1996). Therefore, it is recommended to test the sample using biochemical and serological tests also. Furthermore, according to Lauri and Mariani (2009), the molecular detection system sometimes may give false positive results because of contamination from the environment and/or equipment, a PCR inhibitor in the sample, or the DNA of the vegetable and soil itself because of its size (larger than bacteria). As the results obtained were all negative, this limitation of the technique is not applicable to this study.

3.5.3.3. *Salmonella* spp.

Most previous studies have shown that there were no *Salmonella* detected or confirmed in organic fresh produce. For example, no *Salmonella* spp. have been detected in any

organic produce collected in Spain, Norway and the UK (Oliveira *et al*., 2010, Loncarevic *et al*., 2005, Sagoo *et al*., 2001), including 86 organic vegetable samples from retail shops and farms in the UK (McMahon and Wilson, 2001). Even when present, composting reduces the concentration from 7 $\log_{10}$CFU/g *S. enteritidis* to zero within 48 h (Lung *et al*., 2001). According to van Diepeningen *et al*. (2006), soil in organic farms contains less nitrate and total soluble nitrogen and more non-pathogenic bacteria than conventional farms. It is suggested that these differences affect the 'resistance to colonization by microorganisms' (Gu *et al*., 2013). Organic soil has a greater capacity to suppress the growth of *S. typhimurium* than conventional soil (He *et al*., 2010), probably by bacteria acting synergistically to protect plants from pathogens (Mendes *et al*., 2011) because of microbial diversity (Wetzel *et al*., 2010) and because the population (Gu *et al*., 2013) was higher in organic than conventional farming systems, with an inverse relationship between microbial diversity and *S. typhimurium* populations (Klerks *et al*., 2007). According to FSANZ (2001) guidelines, no *Salmonella* spp. should be present in ready-to-eat foods. In the current study, even though *Salmonella* spp. were confirmed in all soil samples, only 11.4% of vegetable samples were contaminated. Between the three farms, there were no significant differences ($P<0.05$, not shown) in *S. typhimurium*. According to Joseph and Carlos (2012), the Reveal kit, based on lateral flow immunoassays, sometimes gives false positive results because antibodies that are used for the detection may connect with denatured, captured antibodies which may be produced during the reaction. Therefore, the result obtained in this study may have some positive results.

3.5.4. Bacterial resistance to antibiotics

Bacterial resistance to antibiotics is a major concern worldwide. In the current study, all *Salmonella* spp. positive strains from vegetable samples and most of the strains from soil samples showed resistance to vancomycin, ampicillin and penicillin, but a bacterium from some *Staph. aureus* positive strain from vegetable samples and soil samples were not resistant to ampicillin and vancomycin while *Staph. aureus* positive strains from

vegetables were resistant to penicillin. The results for *Salmonella* positive strains were similar to the study by Yildirim *et al*. (2011). This is also in agreement with Gündoğan *et al*. (2006) who reported resistance of *Staph. aureus* to β-lactam such as penicillin and ampicillin, and Yildirim *et al*. (2011) who reported resistance of *Salmonella* spp. from raw chicken carcasses to penicillin, oxacillin, clindamycin, vancomycin, erythromycin and ampicillin. Similar resistance to antibiotics has been reported for *Salmonella* spp. from retail chicken and beef to nalidixic acid, tetracycline, trimethoprim and streptomycin (Dallal *et al*., 2010). Further studies are urgently required to fully understand the extent of antibiotic resistance of bacteria in fresh farm produce and it is important to develop strategies to prevent this.

3.6. Conclusion

In conclusion, based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods, the microbiological status of fresh produce from different farms in Canterbury, New Zealand was acceptable for *E. coli* O157:H7 but not for APC, coliform, *E. coli*, *Staph. aureus* or *Salmonella* spp.. The yeast and mould were similar to studies previously reported for samples purchased in retail shops in the USA, Zambia, Spain, Turkey and other countries. *Staph. aureus* and *Salmonella* spp. showed higher resistance to some antibiotics. There were significant differences ($p<0.05$) between the conventional farm vegetable samples and those of the organic farms for the average of APC, coliform, yeast and mould and *Staph. aureus,* between the compost farm vegetable samples and those of the other farms for the average *E. coli*, and between the conventional farm silver beet samples and those of crop-livestock rotation farm for the *E. coli*. The fresh produce collected from the organic farms was more contaminated with a wider range of pathogens than produce from the conventional farm. Although organically farmed produce is attractive because of its pesticide-free status, public health may be at risk from a higher microbial load. Future studies should be conducted to develop strategies to minimise microbial contamination of organic produce in Canterbury, New Zealand.

Chapter 4 Microbiological risk assessment of fresh produce sold in Christchurch, New Zealand

4.1. Summary

The objective of this study was to assess the microbiological risks of selected fresh produce sold by different retail shops in Christchurch, New Zealand using conventional and advanced detection methods. A total of 96 samples (cabbage, lettuce, carrot and cucumber) were collected from four retail shops and enumerated for APC, coliform, *E. coli*, yeast and mould, and *Staph. aureus.* A total of 64 and 22 samples were analyzed to detect *Salmonella* spp. and *E. coli* O157:H7, respectively. Four *Salmonella* spp. positive samples and 13 *Staph. aureus* positive samples were further screened for resistance to 12 antibiotics. Overall, In conclusion, the microbiological quality of fresh produce for APC ranged from 0.00-7.21 $\log_{10}$ CFU/g; coliforms from 0.00-7.07 $\log_{10}$ CFU/g; *E. coli* from 0.00-6.10 $\log_{10}$ CFU/g; *Staph. aureus* from 0.00-5.11 $\log_{10}$ CFU/g; and yeast and mould from 0.00-5.23 $\log_{10}$ CFU/g. No *E. coli* O157:H7 was detected from the samples tested using a novel molecular detection system (3M, USA). However, 7.8% of samples contained *Salmonella* spp.. Percentage of unsatisfactory to the guideline by FSANZ (2001) or PHLS (2000) varied within a retail shop, such as 75-87.5% samples had unsatisfactory for APC, 75-91.7% samples had unsatisfactory for coliform, 80-100% samples had unsatisfactory for *E. coli*, and 17.4-21.7% samples had unsatisfactory for *Staph. aureus*. However, these results and the yeast and mould (0-5.23 lpg_{10}CFU/ml) were similar to studies previously for samples purchased in retail shops in the USA, Zambia, Spain, Turkey and other countries, except for *E. coli* (83.3-100% of samples had *E. coli*). In addition, *Staph. aureus* and *Salmonella* spp. showed resistance to β-lactam antibiotics. There were significant differences ($p<0.05$) between the retail shops for of APC, coliform, *E. coli* and *Staph. aureus.* In addition*, E. coli* in carrots between the retail shops tested also showed significant difference ($p<0.05$). Furthermore, the levels ($\log_{10}$ CFU/g) of APC between the

vegetables obtained from retail shop A, and the average (log_{10} CFU/g) of *E. coli* between retail shop A and other shops showed significant difference ($p<0.05$) and retail shop B had the highest unsatisfactory of APC (87.5%), coliform (91.7%), *E. coli* (100%) and *Staph. aureus* (21.7%) based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. In order to keep fresh produce safe, it is important for retail shops to take steps necessary to minimise microbiological contamination of their produce, and some shops are already taking such measures as washing with chlorine containing water.

4.2. Introduction

Continuous efforts have been made to reduce food poisoning incidents. Though food safety has improved and knowledge of food hazards has increased, food poisonings still occur. In fact, there were over five thousand foodborne outbreaks in the European Union in 2011 (Eurosurveillance editorial team, 2013). Recently, consumption of fresh produce has increased because fruits and vegetables are considered to improve human health. In fact, the production and consumption of vegetables and fruits has risen in most countries (FAO, 2007). The main source of food poisoning is meat or dairy products, with fresh produce accounting for approximately 20% of total food poisoning outbreakes in the United States (Wijnands *et al*., 2014). Therefore, food poisoning related to fresh produce is now regarded as a serious problem in the food industry.

There are many kinds of microbes that cause food poisoning related to fresh produce; there have been many reports published about food borne illnesses related fresh produce. For example, *E. coli* O157: H7 outbreaks from fresh produce have been reported by many researchers (Ackers *et al*., 1998, Hilborn *et al*., 1999, Jay *et al*., 2004, Michino *et al*., 1999, Wachtel and Charkowski, 2002, Watanabe *et al*., 1999). Similarly, *Salmonella* spp. outbreaks have also been reported (Greene *et al*., 2008, Mahon *et al*., 1997, Mohle-Boetani *et al*., 2009). *Staph. aureus* is another pathogens harmful to human health and some reports have related it to fresh produce (Rahman and Noor, 2012, Viswanathan and

Kaur, 2001). Mould which is a fungus may cause food poisoning due to its ability to produce mycotoxins. According to Kovacs (2004), mycotoxins can accumulate and cause toxic effects in humans, including carcinogenesis, developmental abnormality, affect reproduction, immune system and nervous system problems. A recent example of food poisoning in Belgium from fresh produce due to mycotoxins was reported by Van de Perre *et al*. (2014).

Foodborne outbreaks related to fresh produce are continuously reported throughout the world. In the USA, there were five outbreaks in 2013 and two in 2014 and the types of pathogens included: *S. enteritidis*, *Salmonella*, hepatitis A virus, *Cyclospora cayetanensis*, *E. coli* O157:H7, *E. coli* O121 and *C. cayetanensis* (CDC, 2013). Similarly, in England there were three outbreaks (one in 2013 and two in 2014) as follows: Verotoxin producing *E. coli* O157 in watercress in 2013 and *E. coli* O96 in lettue and cucumber and *Salmonella singapore* in salad in 2014 (Public Health England, 2014). A large outbreak of food poisoning was caused by *Y. pseudotuberculosis* in September and October, 2014 in New Zealand. By 8 October, 2014 there were 124 confirmed cases and 18 presumptive cases, and 38 people were hospitalised (MPI, 2014). According to MPI (2014), the suspected source of *Y. pseudotuberculosis* was probably ready-to-eat bagged salad, but this was not confirmed.

According to Al-Sakkaf (2012), there has been more campylobacteriosis reported in New Zealand than in any other developed country. Many different retail shops sell vegetables in New Zealand, such as supermarkets, street markets and growers markets, and there may be differences in micribiological status of fresh produce between those different retail shops. However, currently no data exists on the microbiological risk assessment of fresh produce for New Zealand. The objective of this study was to conduct a microbiological risk assessment (Aerobic Plate Count, coliform, *E. coli*, *E. coli* O157:H7, yeast and mould, *Salmonella* spp. and *Staph. aureus*) of fresh produce (cabbage, lettuce,

carrot and cucumber) sold by different types of vegetable retail shops in Canterbury, New Zealand using conventional and advanced microbiological methods.

4.3. Materials and methods

4.3.1. Selection, transport and handling of samples

Samples were obtained from four types of retail shops (A to D). These included a supermarket (retail shop A), Asian retailer (retail shop B), grower's market (retail shop C) and open market (retail shop D) located in Christchurch, New Zealand. Samples were selected based on popularity of ingredients in salads and well-known sources of microbiological contamination. Four vegetables were collected from each retail shop. The types of vegetables were green lettuce, telegraph cucumber, green cabbage and carrot, all of which were grown locally. Generally, loose vegetables were purchased but there were some exception that bulk vegetables were purchased if there were no loose vegetables sold. Samples were packed in individual plastic bags and transported to the laboratory. Samples were placed in the refrigerator (at 4^{o}C) following transport to the laboratory. Samples were cut into small pieces (about three square centimetres) and placed in a sterile plastic bag for sample processing. The collection continued one time in a week for six weeks. The details of the sample plan and the number of samples are shown in Appendix (Appendix: 5 and 7).

4.3.2. Sample preparation

For APC, coliform, *E. coli*, *E.coli* O157:H7, *Staph. aureus,* yeast and mould, 25 g of sample was placed in a sterile stomacher bag with 225 g of 0.1% peptone water and mechanically homogenised for 3 min using a stomacher.

4.3.3. Enumeration of microorganisms

Plate Count agar plates were used for enumerating APC (ISO 4833:2003). The plates were placed in a 37^{o}C incubator for 24 h. MacConkey agar plates were used for enumerating *E. coli* and coliforms (ISO 21567); plates were incubated at 37^{o}C for 24 h. Yeast and mould agar plates were used for enumerating yeast and mould (ISO 7954:1987); plates were

incubated at 30°C for 48 h. For *Staph. aureus*, Baird-Parker agar plates were used for eumerating (ISO 6888-2:1999); plates were incubated at 37 °C for 24h. The results were expressed as colony-forming units per gram (CFU/g).

4.3.4. Detection of *S. typhimurium*

For *Salmonella* spp., a commercial kit (Reveal® 2.0 for *Salmonella*, Neogen Corporation, USA) was used for the detection. Twenty-five grams of sample was placed into a sterile stomacher bag with 200 g of steriled-purified water preheated to 42°C and mixed with supplied reagents (Reveal® 2.0 item 9705, Neogen Corporation, USA). After that, the sample was mixed by hand by grasping through the stomacher bag and incubated at 37 °C for 4h. One bottle of reconstitute 2×RV (Reveal® 2.0 item 9715, Neogen Corporation, USA) was dissoleved into 200 g of sterilised, purified water preheated to 37 °C and mixed with the prepared samples, then samples were mixed gently and incubated at 42 °C for 24 h. Eight drops of samples were transferred into the Reveal sample cup (Reveal® 2.0 item, Neogen Corporation, USA) and the Reveal 2.0 for *Salmonella* device (Reveal® 2.0 item, Neogen Corporation, USA) was put into the cup. Results were recorded after 15 min.

4.3.5. Confirmation of *S. typhimurium*

The samples used for the detection of *Salmonella* spp. were confirmed by using *Salmonella* test kit based on the latex agglutination test (Oxoid, UK). One drop of sample prepared as dicussed in 4.3.4 was transferred onto the reaction card using a micropipet. One drop of *Salmonela* latex agent (Oxoid, UK) was added to the sample and mixed. Agglutination was observed after 2 min and compared with negative and positive controls.

4.3.6. Enumeration and confirmation of *E. coli* O157:H7

Sorbitol MacConkey agar with supplements (potassium tellurite and cefixime) was used for the detection of *E. coli* O157:H7 as described in McMahon and Wilson (2001) and plates were incubated, after the preparation described in 4.3.2, at 37 °C for 24h. 25 grams of sample was placed into a sterile stomacher bag with 225 g of bufferd peptone water

(3M, USA) and incubated at 42°C for 24 h. Twenty microlitres of the sample was then transferred into a lysis tube (3M, USA) and mixed. The lysis tube was heated in a heating block (100°C, 3M, USA) for 15 min and after cooling on a chill block (3M, USA) for 10 min and at the room temperature for 5 min, 20 µL of each sample lysate was transferred into reagent tubes and mixed with 20 µL of NC lysate (3M, USA). The sample was then transferred onto a speed loader tray (3M, USA) and placed in the instrument (3M™ Molecular Detection System)(3M, USA) for the assay. The detailed procedure, vegetables and soil sample test outputs are shown in Appendix (Appendix: 8, 9, 10 and 12).

4.3.7. Antibiotic resistance profiling of selected microbial isolates

Disc diffusion tests were conducted on Mueller-Hinton agar plates (Neogen, 2011) as noted in the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012). The antibiotic discs used (Oxoid, UK) were: ampicillin (10 µg)(AMP), tetracycline (30 µg)(TE), gentamycin (30 µg)(GN), erythromycin (15 µg)(E), trimethoprim–sulfamethoxazole (25 µg)(SXT), nalidixic acid (30 µg)(NA), chloramphenicol (30 µg)(C), ciprofloxacin(5 µg)(CIP), penicillin(10 µg)(P), kanamycin (30 µg)(K), streptomycin (30 µg)(S) and vancomycin (30 µg)(VA). Strains were classified as susceptible, intermediate or resistant according to the size of the inhibition zones surrounding the colony.

4.3.8. Statistical analyses

Statistical analysis was conducted using Excel 2013 and SPSS. Geometric means, standard deviations, ranges and medians were calculated using Microsoft Office Excel 2013 (Microsoft, USA) and one-way ANOVA tests with Tukey comparisons were used to derive statistical differences ($p<0.05$) of microbial levels by using SPSS version 21 (IBM, USA).

4.4. Results

4.4.1. Overall microbiological quality

A total of 96 samples (24 samples of each vegetable) were analysed. As seen in table 4.1, the range of APC (log_{10}CFU/g) across all retail shops were: lettuce - 4.95-6.84 log_{10}CFU/g,

cabbage - 4.74-6.52 log_{10}CFU/g, carrot - 4.30-6.53 log_{10}CFU/g and cucumber - 0.00-7.21 log_{10}CFU/g.

Table 4.1 Comparison of microbiological quality of fresh produce samples from four different retail shops (log_{10}CFU/g)

	Produce	Retail shop A	Retail shop B	Retail shop C	Retail shop D	All shops
APC	Lettuce	4.95-6.53	5.02-6.23	5.71-6.74	5.02-6.23	4.95-6.84
	Cabbage	4.74-5.90	5.09-6.38	5.28-6.52	5.35-5.63	4.74-6.52
	Carrot	5.25-6.50	4.65-6.12	4.39-6.53	4.30-6.37	4.30-6.53
	Cucumber	0.00-7.21	0.00-6.37	0.00-6.51	3.98-6.23	0.00-7.21
Coliform	Lettuce	5.19-6.68	4.84-6.99	5.17-6.94	4.84-6.99	4.84-7.07
	Cabbage	3.77-6.43	5.21-6.64	5.26-6.55	4.45-6.73	4.84-7.07
	Carrot	4.28-6.55	3.11-5.92	4.49-6.59	4.84-6.26	3.11-6.59
	Cucumber	1.97-4.40	0.00-4.81	0.00-6.10	2.74-6.33	0.00-6.33
E. coli	Lettuce	2.17-5.86	3.17-4.59	2.89-5.72	3.17-4.18	0.00-5.86
	Cabbage	2.54-5.57	0.00-4.77	0.00-4.23	2.78-4.29	0.00-5.57
	Carrot	3.12-5.90	1.99-4.03	3.48-6.10	3.28-5.66	1.99-6.10
	Cucumber	0.00-4.40	0.00-4.81	0.00-5.05	1.66-4.66	0.00-5.05
Yeast & mould	Lettuce	1.39-2.87	2.87-5.23	1.16-3.34	0.00-3.19	0.00-5.23
	Cabbage	0.00-2.12	0.00-2.87	0.00-1.60	0.00-2.87	0.00-2.87
	Carrot	0.00-0.69	0.00-0.00	0.00-2.28	0.00-2.96	0.00-2.96
	Cucumber	0.00-1.65	0.00-3.51	0.00-2.30	0.00-4.38	0.00-4.38
Staph. aureus	Lettuce	2.33-5.11	1.60-4.06	0.00-4.78	1.98-4.07	0.00-5.11
	Cabbage	0.00-3.88	0.00-4.60	1.47-4.30	1.77-4.59	0.00-4.60
	Carrot	0.00-3.60	0.00-3.46	0.00-2.65	0.00-4.16	0.00-4.16
	Cucumber	0.00-4.74	0.99-4.21	0.00-4.20	0.97-4.98	0.00-4.98

The range of coliform (log_{10}CFU/g) from all shops were: lettuce - 4.84-7.07 log_{10}CFU/g, cabbage - 4.84-7.07 log_{10}CFU/g, carrot - 3.11-6.59 log_{10}CFU/g and cucumber - 0.00-6.33 log_{10}CFU/g.

The range of *E. coli* (log_{10}CFU/g) from all shops were: lettuce 0.00-5.86 log_{10}CFU/g, cabbage 0.00-5.57 log_{10}CFU/g, carrot 1.99-6.10 log_{10}CFU/g, and cucumber 0.00-5.05 log_{10}CFU/g.

The range of yeast and mould (log_{10}CFU/g) from all shops were: 0.00-5.23 log_{10}CFU/g. Likewise, levels in cabbage were 0.00-2.87 log_{10}CFU/g, levels in carrot were 0.00-2.96 log_{10}CFU/g and levels in cucumber were 0.00-4.38 log_{10}CFU/g.

The range of *Staph. aureus* (log_{10}CFU/g) from all shops were: lettuce - 0.00-5.11 log_{10}CFU/g, cabbage - 0.00-4.60 log_{10}CFU/g, carrot - 0.00-4.16 log_{10}CFU/g and cucumber - 0.00-4.98 log_{10}CFU/g.

4.4.2. *Salmonella* spp. and *E. coli* O157:H7 prevalence

Regarding the presence of *Salmonella* spp., 64 samples were analysed with the detection kit (Reveal® 2.0 for *Salmonella*, Neogen Corporation, USA). Between them, five lettuce (retail shop A, C and D) and one cucumber (retail shop B) samples were positive (Table 4.2).

Table 4.2 Presence of *Salmonella* spp. (number)

	Positive samples (produce)	Negative	Total	%
Retail shop A	2 (lettuce)	14	16	12.5
Retail shop B	1 (cucumber)	15	16	6.3
Retail shop C	1 (lettuce)	15	16	6.3
Retail shop D	1 (lettuce)	15	16	6.3
Total	5	59	64	7.8

Between the *Salmonella* spp. positive samples (five) detected by the confirmation kit (Oxoid, UK), all samples showed positive reactions to the confirmation test using the *Salmonella* test kit. All vegetables from all retail shops (a total of 22 samples) were analysed using a novel molecular detection system (3M, USA) for the presence of *E. coli* O157:H7 and none were positive for *E. coli* O157:H7 (Appendix 12).

4.4.3. Antibiotic resistance of selected *Salmonella* spp. and *Staph. aureus*

The antibiotic resistance profile of *Salmonella* spp. *Staph. aureus* positive samples collected from retail shops to different antibiotics were analysed. The results obtained were classified as susceptible (≥20mm, S), intermediate (15–19mm, I) or resistant

(≤14mm, R) according to the area of the inhibited zones (see table 4.3 for *Salmonella* spp. and table 4.4 for *Staph. aureus*).

Table 4.3 Antimicrobial susceptibility profile of *Salmonella* isolates

	Produce	VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Retail shop A	Cucumber	R	S	S	R	S	R	S	S	S	R	R	I
Retail shop B	Lettuce	R	R	S	R	S	R	R	S	S	R	R	R
Retail shop C	Lettuce	R	R	S	I	S	I	S	S	I	R	I	S
Retail shop D	Lettuce	R	S	S	S	S	I	S	S	S	R	R	S

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), sreptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT) (** Zone Diameter (mm) S; Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14)

Table 4.4 Level of antimicrobial susceptibility (%) of *Staph. aureus* in isolates from fresh produce samples

	%	VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Overall (13 samples)	S	23	85	100	15	62	31	62	100	15	0	54	92
	I	54	15	0	62	0	0	0	0	8	0	15	8
	R	23	0	0	23	38	69	38	0	77	100	31	0
Retail shop A (3 samples)	S	0	67	100	33	67	67	67	100	0	0	67	100
	I	67	33	0	67	0	0	0	0	0	0	0	0
	R	33	0	0	0	33	33	33	0	100	100	33	0
Retail shop B (4 samples)	S	0	75	100	0	50	25	50	100	25	0	25	100
	I	75	25	0	100	0	0	0	0	0	0	25	0
	R	25	0	0	0	50	75	50	0	75	100	50	0
Retail shop C (3 samples)	S	67	100	100	0	67	0	67	100	0	0	67	100
	I	33	0	0	33	0	0	0	0	33	0	33	0
	R	0	0	0	67	33	100	33	0	67	100	0	0
Retail shop D (3 samples)	S	33	0	0	33	0	0	00	0	33	0	33	0
	I	67	100	100	0	67	0	67	100	0	0	67	100
	R	0	0	0	67	33	100	33	0	67	100	0	0

*Vancomycin30 (VA), kanamycin30 (K), gentamicyn30 (GN), sreptomycin30 (S), chloramphenicol30 (C), erythromycin15 (E), tetracycline30 (TE), coprofloxacin5 (CIP), nalidixic acid30 (NA), penicillin10 (P), ampicillin10 (AMP), and sulfamethoxazole23.75 & trimethoprim1.25 (SXT) (** Zone Diameter (mm) S; Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14)

None of the samples tested were resistant to all of the tested antibiotics. However, all *Salmonella* spp. positive samples and 86% of *Staph. aureus* positive samples showed resistance to at least three antibiotics. Most (>75%) of the *Salmonella* spp. positive samples were resistant to ampicillin. All *Salmonella* spp. positive samples were resistant to vancomycin and penicillin. Similarly, all *Staph. aureus* positive samples were resistant to penicillin. More than 65% of the *Staph. aureus* positive samples collected from retail shops in this study were resistant to erythromycin and nalidixic acid, except *Staph. aureus* positive samples from retail shop A which were resistant to erythromycin.

4.5. Discussion

4.5.1. Microbial quality of fresh produce

APC of vegetable samples studied previously have provided mixed results. Aycicek *et al*. (2006) examined whole vegetables purchased from retail shops in Turkey and reported that the range of APC were 3.3–7.4 $\log_{10}$CFU/g. Similar whole vegetable APC have been reported by Badosa *et al*. (2008) (6-8 $\log_{10}$CFU/g, Spain), Seow *et al.* (2012) (0-6.7 $\log_{10}$CFU/g, Singapore), Halablab *et al*. (2011) (4.3-10.4 $\log_{10}$CFU/g, Lebanon) and Johnston *et al*. (2005) (4.6-7.9 $\log_{10}$CFU/g, USA). Although no specific standard has been set for New Zealand fresh produce, according to FSANZ (2001), <4 $\log_{10}$CFU/g of APC is considered satisfactory, <5 $\log_{10}$CFU/g of APC marginal and >5 $\log_{10}$CFU/g of APC is unsatisfactory for ready-to-eat foods. In this study, as shown in figure 4.1, high proportion (75-87.5%) of the samples from retail shops showed unsatisfactory levels of APC (based on FSANZ 2001 guidelines) and there was a significant difference ($p<0.05$) in the unsatisfactory levels of APC. Retail shop B had the highest percentage (87.5%) of unsatisfactory levels of APC among the four retail shops.

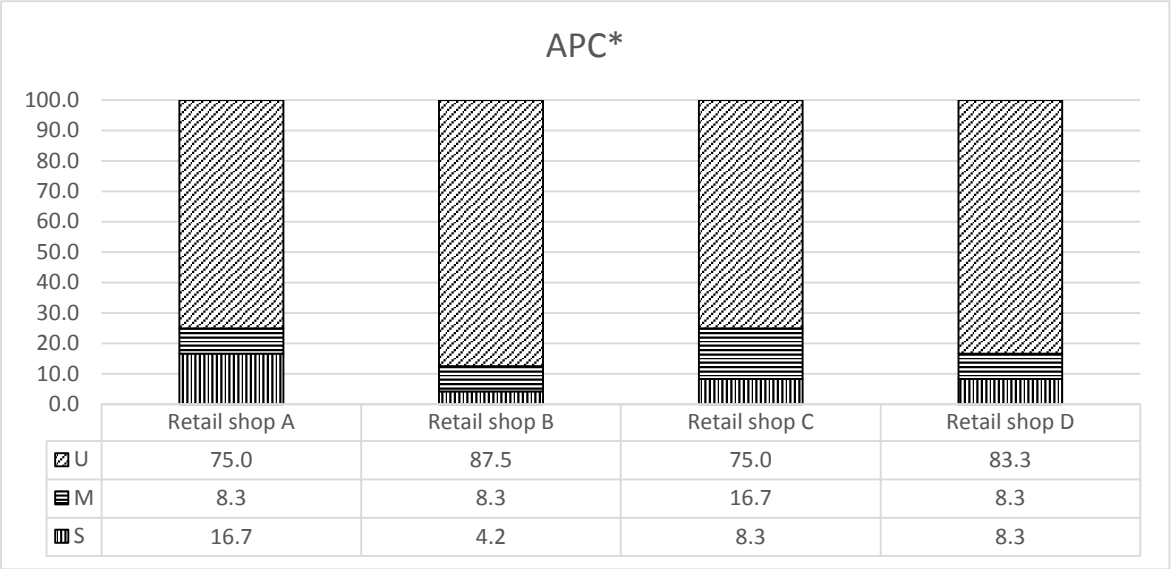


Figure 4.1 Percentage of unsatisfactory (U), marginal (M) and satisfactory (S) of APC compared with FSANZ (2001) guidelines

* Significant difference between shops ($p<0.05$).

For the differences between the types of vegetables, Aycicek *et al*. (2006) suggested that leafy vegetables are more likely to be contaminated with bacteria due to these vegetables' large and complex surface. This is because the complex surface of leafy vegetables is more likely to come in contact with soil or water from which they may become contaminated (Abadias et al., 2008). To examine the differences of microbiological quality of the vegetables from the four retail shops in the current study, the minimum (25^{th} percentile), median (75^{th} percentile) and maximum levels of $\log_{10}$CFU/g of each type of bacterium for each vegetable and the significant differences between the values of $\log_{10}$CFU/g ($p<0.05$) were calculated. Statistical differences between vegetables were shown in figure 4.2. Retail shop A only showed significant differences ($p<0.05$) between cucumber and other vegetables.

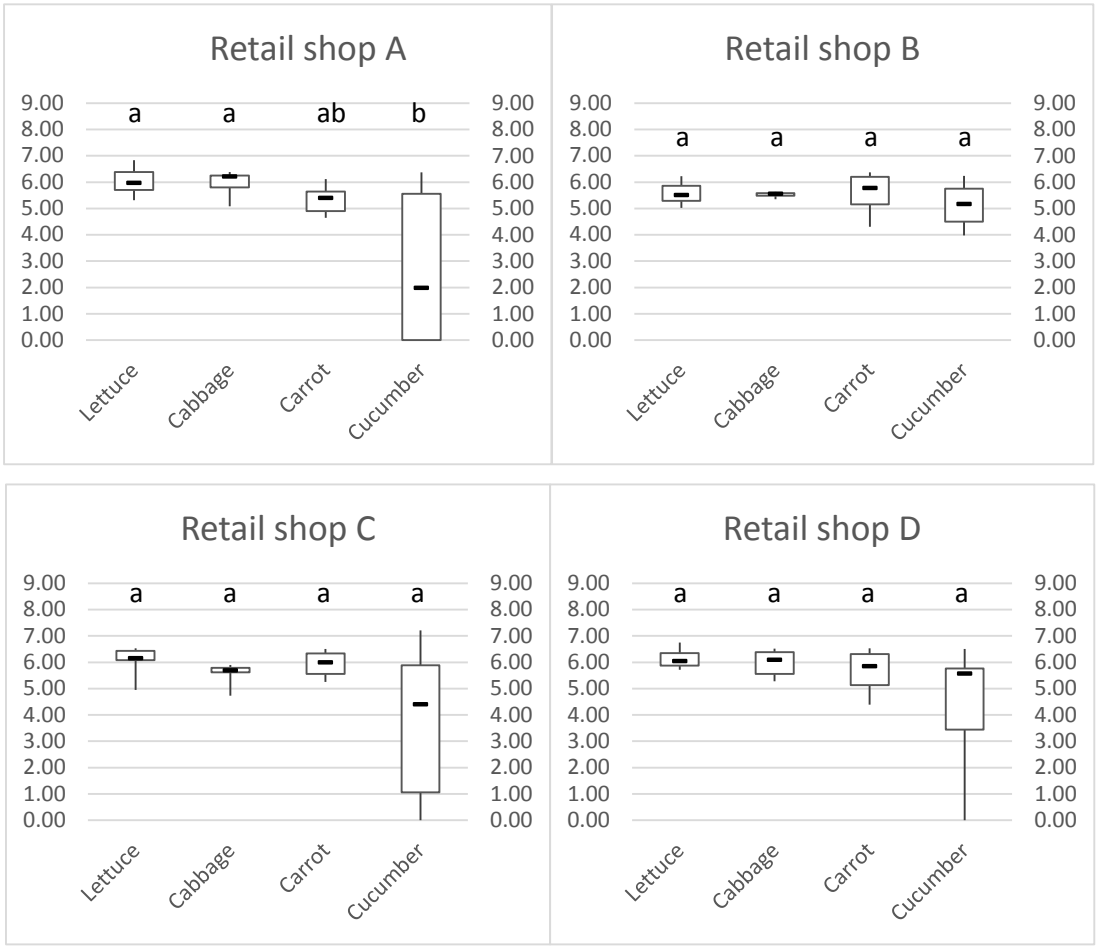


Figure 4.2 Total aerobic bacteria (log_{10}CFU/G) of all vegetables collected from retail shop A-D

The box plot indicates the minimum, 25th percentile, median, 75th percentile and maximum. Different letters indicate significant differences between the median ($p<0.05$).

In terms of total coliform count, according to BC Centre for Disease Control (BCCDC) (2013), coliforms exist in the environment but are rarely pathogenic. However, faecal coliforms, mostly originated from faeces or animal intestines, can be pathogenic. Some types of faecal coliforms are not from animal faeces but from non-faecal sources (plant and soil material) and therefore the presence of faecal coliforms may not be directly linked to faecal contamination (BCCDC, 2013). According to previous studies below, coliform can be variable. Maffei *et al*. (2013) analysed whole vegetables from markets in Brazil and reported the levels was 1-7 log_{10}CFU/g for coliforms. Similarly, Rahman and Noor (2012) reported the level was 4-6.7 log_{10}CFU/g for coliforms in whole vegetables collected from retail shops in Bangladesh and Seow *et al*. (2012) reported a range of

coliforms between 2.1 and 5.7 log_{10}CFU/g from whole vegetables collected from retail shops in Singapore. According to FSANZ (2001), < 2 log_{10}CFU/g is satisfactory, 2-4 log_{10}CFU/g is marginal and > 4 log_{10}CFU/g is unsatisfactory for coliforms. The statistical differences in coliform within each vegetable are shown in figure 4.3. None of vegetables in this study showed significant differences in the coliform between the four different retail shops. Figure 4.4 shows the percentage of satisfactory, marginal and unsatisfactory coliform in the samples based on FSANZ (2001) guidelines. As shown in figure 4.4, high proportion (75-91.7%) of the samples from retail shops showed unsatisfactory levels for APC (based on FSANZ 2001 guidelines) and there was a significant difference ($p<0.05$) in the unsatisfactory levels of coliforms (%). Retail shop B had the highest percentage (91.7%) of unsatisfactory levels of coliform in the fresh produce samples. However, these results are similar to those reported in the previous studies.

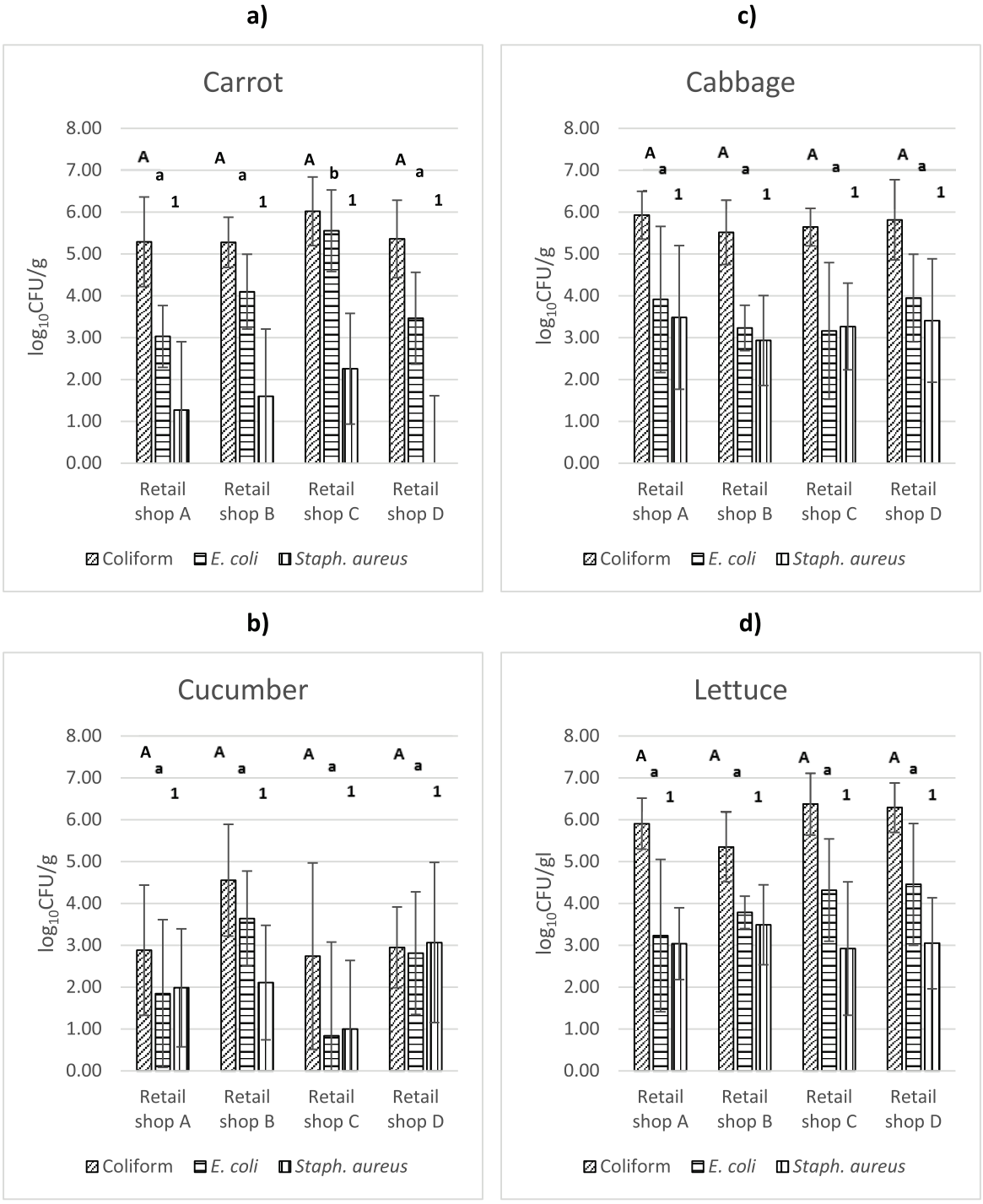
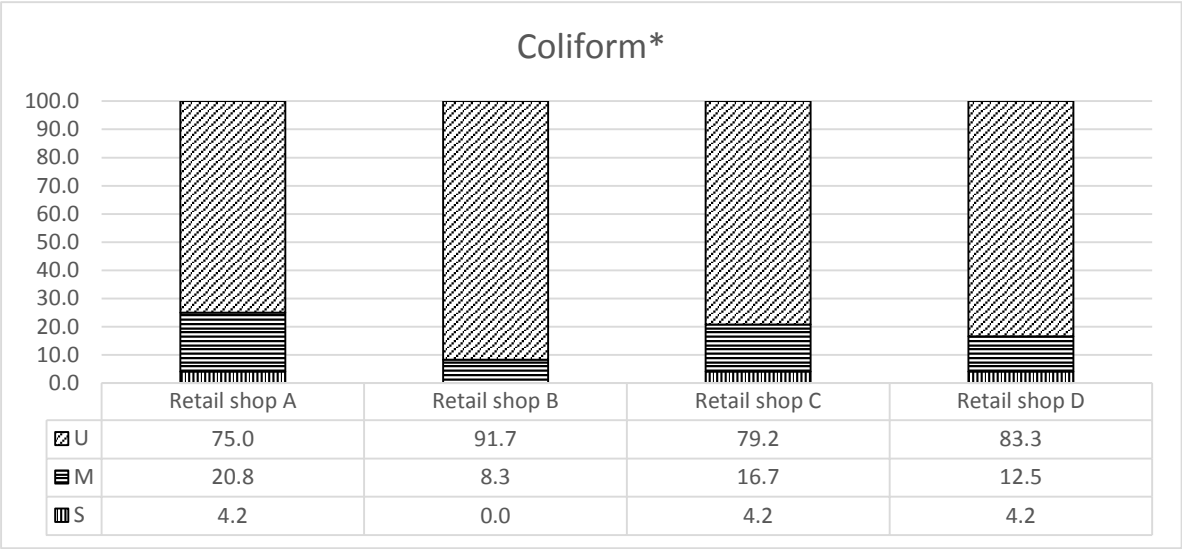


Figure 4.3 Log_{10}CFU/ml of *E. coli*, coliform and *Staph. aureus* from carrots (a), cucumber (b), cabbage (c) and lettuce (d) obtained from four retail shops (A-D) in Christchurch

Different letters within a microbial group represent significant difference at $p<0.05$.



	Retail shop A	Retail shop B	Retail shop C	Retail shop D
U	75.0	91.7	79.2	83.3
M	20.8	8.3	16.7	12.5
S	4.2	0.0	4.2	4.2

Figure 4.4 Percentage of unsatisfactory (U), marginal (M) and satisfactory (S) of coliform compared with FSANZ (2001) guidelines

* Significant difference between shops ($p<0.05$).

E. coli are bacteria that belong to the faecal coliforms and some strains of *E. coli* can cause diarrhoea in humans (BCCDC, 2013). The BCCDC (2013) suggested using *E. coli* as an indicator of the enteric pathogens. Maffei *et al*. (2013) reported that 40% of the organic and conventional vegetable samples sold in Brazil contained *E. coli*. In contrast, De Giusti *et al*. (2010) reported that 11.5-75.7% of whole vegetables collected from three different producers (processing shops) in central Italy contained *E. coli*, though that was dependent on the type of retail shops the samples were collected from. Abadias *et al*. (2008) reported that 7.1% of whole vegetable samples collected from several retail shops in Spain contained *E. coli*. Sirsat and Neal (2013) reported values in the range of 2-3.5 $\log_{10}$CFU/g for *E. coli* in lettuce collected from retail shops in the USA. Similarly, Rahman and Noor (2012) reported 4-8.7 $\log_{10}$CFU/g of *E. coli* in whole vegetables collected from retail shops in Bangladesh. The statistical differences in *E. coli* within each vegetable are shown in figure 4.3. *E. coli* in carrot were significantly different ($p<0.05$) between retail shops. It is suggested that an absence of *E. coli* is the ideal standard for food safety, but less than 0.48 $\log_{10}$CFU/g is satisfactory and more than 2 $\log_{10}$CFU/g is unacceptable (FSANZ, 2001). In the current study, the percentages of *E. coli* in samples were 83.3% for

retail shop A, 100% for retail shop B, 95.8% for retail shop C and 83.3% for retail shop D. These values are much higher than those reported in previous studies. As shown in figure 4.5, 80-100% of the samples from retail shops showed unsatisfactory *E. coli* (based on FSANZ 2001 guidelines) and there was a significant difference ($p<0.05$) in the in the unsatisfactory levels of *E. coli*. All the fresh produce samples from retail shop B had unsatisfactory levels for *E. coli*. There was also a significant difference ($p<0.05$) in the average of *E. coli* (log_{10}CFU/ml) between retail shop A (supermarket) and other retail shops (figure 4.6).

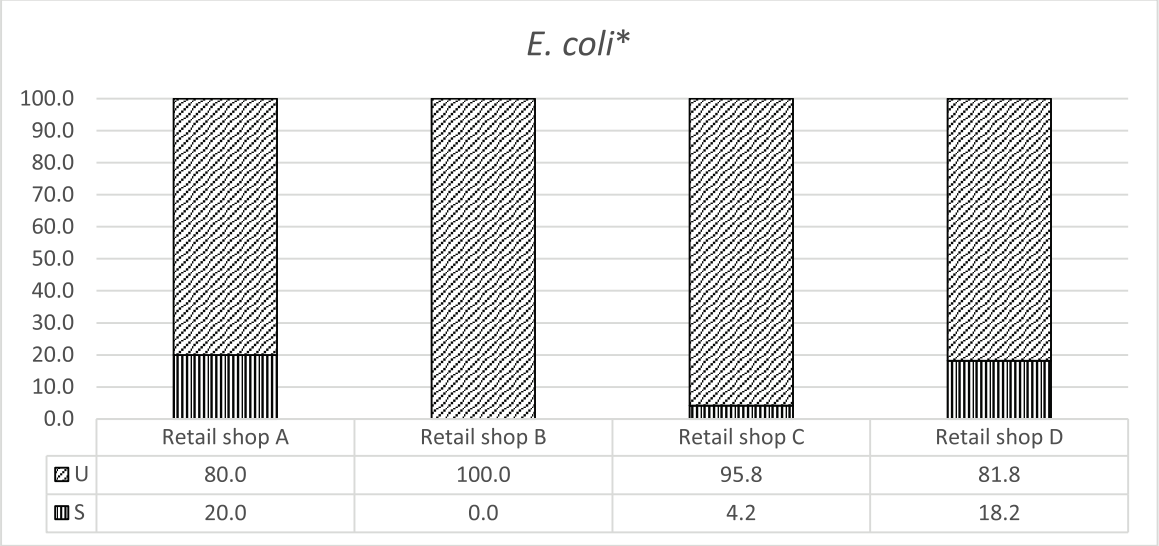


Figure 4.5 Percentage of unsatisfactory (U), and satisfactory (S) of *E. coli* compared with FSANZ (2001) guidelines

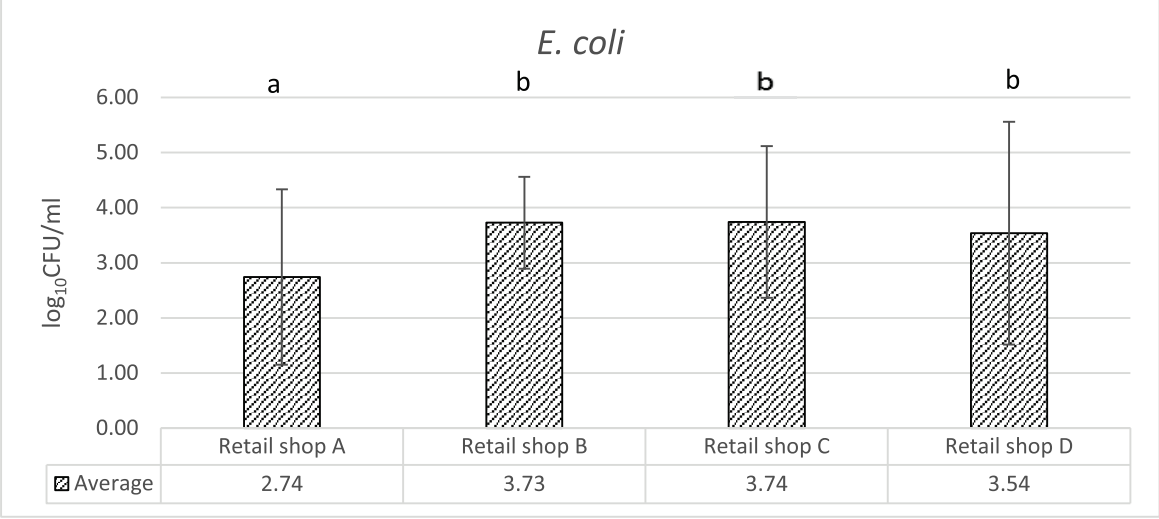
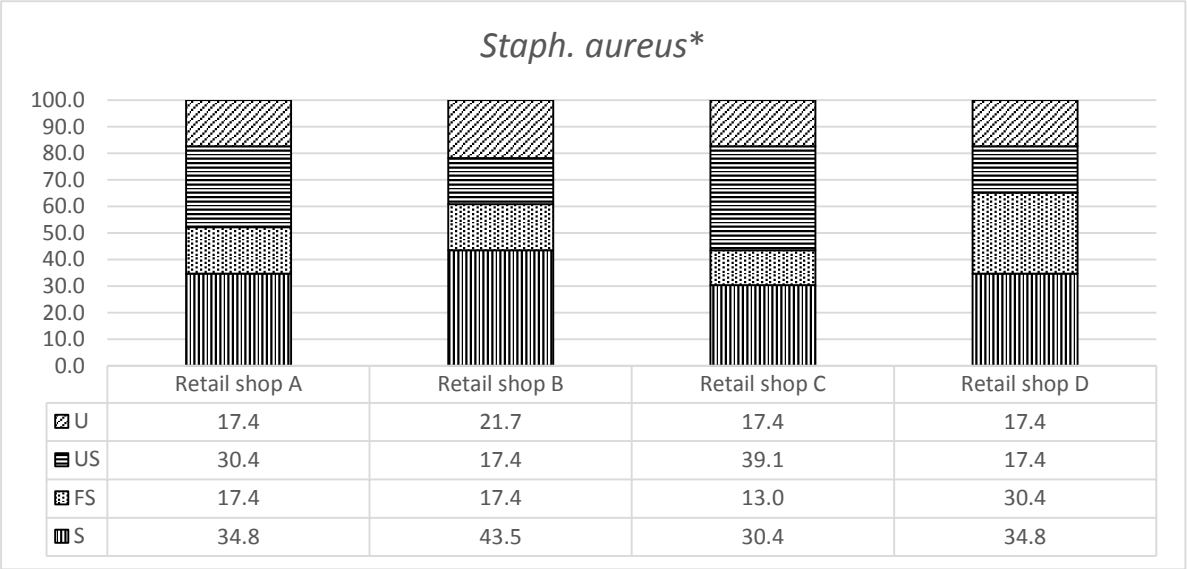


Figure 4.6 Average of log_{10}CFU/ml of *E. coli* from retail shops

Different letters on bars indicate significant differences between farms ($p<0.05$).

Staph. aureus, which normally exists in the mucosa of nasal passages, indicates a lack of proper hygiene conditions during food handling. Maistro *et al*. (2012) suggested that *Staph. aureus* is more vulnerable than more common microbes and may not be able to survive in the microbiota, and thus contamination by *Staph. aureus* may not harm human health. Mohammad et al. (2012) detected *Staph. aureus* in 94.9% of cut vegetables and herbs collected in Iran, and 23.6% of those samples were coagulase positive. Halablab *et al*. (2011) reported the levels of 1.47-8.77 $\log_{10}$CFU/g *Staph. aureus* in whole vegetables collected from retail shops in Lebanon. In contrast, Maistro *et al*. (2012) reported that no salad vegetable samples collected from retail shops in Brazil contained coagulase positive *Staph. aureus*. The statistical differences in *Staph. aureus* within each vegetable are shown in figure 4.3. None of the vegetables tested here showed significant differences ($p<0.05$) in *Staph. aureus* between retail shops (not shown). PHLS (2000) described the *Staph. aureus* contamination as follows: satisfactory, < 2 $\log_{10}$CFU/g, fairly satisfactory, 2-3 $\log_{10}$CFU/g, unsatisfactory, 3-4 $\log_{10}$CFU/g and unacceptable, > 4 $\log_{10}$CFU/g. The grading of the samples in this study based on PHLS (2000) guidelines is shown in figure 4.7. Around 20 percent (17.4-21.7%) of the samples from retail shops were unsatisfactory level of *Staph. aureus* contamination based on PHLS (2000) guidelines and there was a significant difference ($p<0.05$) in the unsatisfactory *Staph. aureus* (%). Retail shop B had the highest percentage (21.7%) of unsatisfactory levels for *Staph. aureus* in the fresh produce samples. The percentage of vegetables contaminated with *Staph. aureus* were 83.3% for retail shop A, 91.7% for retail shop B, 70.8% for retail shop C and 79.2% for retail shop D.



	Retail shop A	Retail shop B	Retail shop C	Retail shop D
U	17.4	21.7	17.4	17.4
US	30.4	17.4	39.1	17.4
FS	17.4	17.4	13.0	30.4
S	34.8	43.5	30.4	34.8

Figure 4.7 Percentage of unsatisfactory (U), marginal (M) and satisfactory (S) of *Staph. aureus* compared with PHLS (2000) guidelines

Yeast exists in the environment and is generally not harmful, though it can cause spoilage if it reaches high numbers. According to Corato (2012), some strains of yeast can change the colour of vegetables. Similarly, Ragaert *et al*. (2006) reported that some strains of yeast can produce volatile metabolites that affect the quality of vegetables. Some strains of mould can be harmful to human health because of their capacity to produce mycotoxins. Kovacs (2004) reported that some strains of mould cause carcinogen effects, immunosuppressive effects, developmental abnormalities, reproductive failure and effects on the nervous system. Yeast and mould detected in the current study are similar to those reported previously: Sirsat and Neal (2013) reported 5.5 $\log_{10}$CFU/g of yeast in lettuce collected from retail shops in the USA; Maffei *et al*. (2013) analysed whole vegetables from markets in Brazil and reported 4-7 $\log_{10}$CFU/g of yeast and mould; and Seow *et al*. (2012) reported a range of 4-7 $\log_{10}$CFU/g in whole vegetables collected from retail shops in Singapore. In the current study, the statistical differences in yeast and mould within each vegetable are shown in figure 4.3. There was no significant difference ($p<0.05$) in yeast and mould between retail shops and the range of yeast and mould from

all retail shops were similar to previous studies. None of the vegetables sampled showed significant differences ($p<0.05$) in yeast and mould between retail shops vegetables (not shown).

4.5.2. Prevalence of *E. coli* O157:H7 and *Salmonella* spp.

According to Himathongkham *et al*. (1999), cattle are the main source of *E. coli* O157:H7 and therefore some types of soil enrichment (those that use cattle faeces) may have an increased risk of vegetable contamination. Sagoo *et al*. (2001) examined 3,200 whole organic vegetables collected from retail shops in the UK and no *E. coli* O157:H7 was detected. Similar results were observed by Johnston *et al*. (2005) in approximately 400 whole vegetables collected from retail shops in the USA. In contrast, Mohammad et al. (2012) reported that 6.5% of fresh cut vegetables and 11.4% of ready-to-eat herbs collected from retail shops in Iran contained *E. coli* O157:H7. In the current study, though many colonies were observed on specific plates (61 of 64 samples), the results of the confirmation test conducted using a novel molecular detection system (3M, USA) (22 samples) were all negative. One reason for this anomaly may be the limitation of MacConkey agar with sorbitol, cefixime and tellurite plates (HAEDY Diagnostics, 1996). It has been reported that sometimes sorbitol negative colonies are produced below sorbitol positive colonies (which are the majority of colonies) and therefore negative colonies are difficult to distinguish (HAEDY Diagnostics, 1996). Hence, it is recommended to test the sample using biochemical and serological tests.

FSANZ (2001) has suggested no *E. coli* O157:H7 should be present in ready-to-eat food. According to Lauri and Mariani (2009), the molecular detection system sometimes may give false positive results because of contamination from the environment and/or equipment, a PCR inhibitor in the sample, or the DNA of the vegetable and soil itself because of its size (larger than bacteria). As the results obtained were all negative, this limitation of the technique is not applicable to this study.

Salmonella spp. are the most common and important pathogens in food. According to Nguz *et al*. (2005), 0.75% of cut vegetable samples collected from retail shops in Zambia contained *Salmonella* spp.. Froder *et al*. (2007) reported 3% of leaf salad vegetables collected from retail shops in Brazil contained *Salmonella* spp., while Mohammad et al. (2012) reported contamination of 9.4% of ready-to-eat herbs and 5.6% of fresh cut vegetables collected from retail shops in Iran. In the current study, 6.3% to 12.5% of samples from each shop contained *Salmonella* spp. According to FSANZ (2001), any *Salmonella* spp. in ready-to-eat food is not acceptable. However, the range of contamination observed in the current study is similar to those reported above.

There have been many kinds of detection methods and confirmation methods invented and recently a commercial kit has come into the market which offers an easy way to analyse *Salmonella* spp. Reiter *et al*. (2010) analysed four different commercial kits and reported that the Reveal system (Oxoid, UK) was sensitive and could detect *Salmonella* spp. from twice as many samples than other methods. Maistro *et al*. (2012) also examined 18 samples and nine *Salmonella* spp. positive results were observed using the Reveal kit (Oxoid, UK). Among them, Maistro *et al*. (2012) was able to confirm only one *Salmonella* spp. positive sample by the conventional method and confirmation kit (Vidas®, France). According to Reiter *et al*. (2010), this may have been a false-positive. However, in the current study, all *Salmonella* spp. positive samples (five) when examined with the Reveal kit (Oxoid, UK) showed positive results in the latex agglutination test (Oxoid, UK). According to Joseph and Carlos (2012), the Reveal kit, based on lateral flow immunoassays, sometimes gives false positive results because antibodies that are used for the detection may connect with denatured, captured antibodies which may be produced during the reaction. Therefore, the result obtained in this study may have some positive results.

4.5.3. Antibiotic resistance

Antibiotic resistance of pathogens, especially Methicillin-resistant *Staph. aureus* (MRSA), are regarded as dangerous and cause human infections in the clinical environment (European Food Safety Authority, 2012). Therefore, antibiotic resistance of pathogens found in food is of concern to the food industry. Antibiotic resistance tests for foodborne pathogens have been conducted by many researchers previously. Yildirim *et al*. (2011) reported that *Salmonella* spp. from raw chicken carcasses showed a higher resistance to penicillin, oxacillin, clindamycin, vancomycin, erythromycin and ampicillin. In contrast, Dallal *et al*. (2010) reported that *Salmonella* spp. from retail chicken and beef showed higher resistance to nalidixic acid, tetracycline, trimethoprim and streptomycin. In the current study, all *Salmonella* spp. positive samples showed resistance to vancomycin and penicillin, and most were resistant to ampicillin also. These results are similar to the study of Yildirim *et al*. (2011). For *Staph. aureus*, according to Gündoğan *et al*. (2006), *Staph. aureus* appears to be resistant to antibiotics, especially β-lactams such as penicillin, vancomycin, ampicillin and others. In the current study, all *Staph. aureus* positive strain samples showed resistance to penicillin, which is in agreement with previous studies (Gündoğan *et al*., 2006).

4.6. Conclusion

In conclusion, the microbiological quality of fresh produce for APC ranged from 0.00-7.21 $\log_{10}$ CFU/g; coliforms from 0.00-7.07 $\log_{10}$ CFU/g; *E. coli* from 0.00-6.10 $\log_{10}$ CFU/g; *Staph. aureus* from 0.00-5.11 $\log_{10}$ CFU/g; and yeast and mould from 0.00-5.23 $\log_{10}$ CFU/g. No *E. coli* O157:H7 was detected from the samples tested using a novel molecular detection system (3M, USA), however, 7.8% of samples were positive *Salmonella* spp. presence. Based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods, the microbiological status of fresh produce from different retail shops in Christchurch, New Zealand obtained in this study was acceptable for *E. coli* O157:H7 but not for APC, coliform, *E. coli*, *Staph. aureus* or *Salmonella* spp., though these results and the yeast and

mould were similar to studies previously for samples purchased in retail shops in the USA, Zambia, Spain, Turkey and other countries, except for *E. coli*. Retail shop B had the highest unsatisfactory levels for APC, coliform, *E. coli* and *Staph. aureus* based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. In addition, *Staph. aureus* and *Salmonella* spp. showed resistance to β-lactam antibiotics. There were significant differences ($p<0.05$) between the retail shops for APC, coliform, *E. coli* and *Staph. aureus.* In addition*, E. coli* in carrots between the retail shops tested also showed significant difference ($p<0.05$). Furthermore, the levels (log_{10} CFU/g) of APC between the vegetables obtained from retail shop A, and the average (log_{10} CFU/g) of *E. coli* between retail shop A and other shops showed significant difference ($p<0.05$).

Chapter 5 General Discussion and Conclusion

5.1. General discussion

5.1.1. Important factors that affect food safety of fresh produce

As outlined in the literature review, there have been several foodborne disease outbreaks related to fresh produce in several countries. In the USA, the pathogens responsible for a high percentage of foodborne outbreaks between 1998 and 2012 were norovirus, *Salmonella* and *E. coli* and more recent studies have shown similar findings. For most countries listed in the literature review, such as USA, New Zealand and Japan, trends in the number of outbreaks did not change between 2002 and 2012. In order to manage the food safety, guidelines are useful for the supply chain to set goals against hazards. According to WHO (2008), the examples of hazards which can enter foods are during production systems, post-harvest practices, water, local environment, fertilizers, worker health and hygiene, and consumption patterns and practices. However, many countries have no guidelines, especially for fresh produce. It is therefore prudent to develop maximum permissible levels of pathogens to manage fresh produce and monitor these pathogens regularly as consumer demand for more natural products increases. Approaches such as the use of hurdle technology to reduce pathogens may be useful for improving the safety of the fresh produce.

5.1.2. Significance of microbiological risk assessment of fresh produce from three different soil enrichment systems in Canterbury, New Zealand

This study revealed that vegetables from three different farm systems (compost, crop-livestock rotation and conventional) all contained a relatively high microbial load (APC, coliform, *E. coli, Staph. aureus* and *Salmonella*) at levels above the safe limits set for ready-to-eat food by FSANZ (2001) or PHLS (2000). An even higher microbial load was evident in soil samples than the vegetables samples. Samples that tested *Salmonella* and *Staph. aureus* positive strains also showed higher resistance to β-lactam antibiotics. Yeast and mould were similar to those reported in previous studies in the USA, Zambia, Spain,

Turkey and other countries. No *E. coli* O157:H7 was detected; this was a satisfactory result based on the safe limits set for ready-to-eat food by FSANZ (2001). For the comparison between the different farm types, the bacterial contamination of silver beet was significantly higher from the crop-livestock rotation farm than silver beet from the conventional farm. In addition, there were significant differences ($p<0.05$) between the conventional farm vegetable samples and those of the organic farms for the average APC, coliform, yeast and mould and *Staph. aureus,* and between the compost farm vegetable samples and those of the other farms for the average *E. coli.* Soil seems to be one of the major sources of pathogen contamination and indeed soil from the crop-livestock rotation farm contained higher *E. coli*. Therefore, managing microbiological status in organic farms and checking soil to manage pathogens, especially *E.coli*, would enhance food safety in fresh produce.

5.1.3. Significance of microbiological risk assessment of fresh produce from four different retailers in Canterbury

This study revealed that vegetables purchased from four different retail shops did not satisfy the 'safe' levels of APC, coliform, *E. coli*, *Staph. aureus* or *Salmonella* suggested for ready-to-eat food in FSANZ (2001) or PHLS (2000). In particular *E. coli* were higher than reported in previous studies, though APC, coliform, *E. coli*, *Staph. aureus* or *Salmonella* results and yeast and mould in this study were comparable to the previous studies. Some results were identified in this study, in that cucumber had a higher microbial load (especially *E. coli* and *Staph. aureus*) than reported in previous studies. Also, there was a significant difference in *E. coli* between carrots collected from the four retail shops. *Salmonella* spp. and *Staph. aureus* positive isolates from vegetable samples were resistant to β-lactams. No *E. coli* O157:H7 was detected. From these results, microbiological status seems to depend on the type of vegetable sampled and the place of sale; therefore, setting regulations for distributors and retailers to manage food safety in fresh food is an

urgent need in New Zealand. In addition, conducting research to find natural agents to reduce microbial load is a priority.

5.1.4. Significance and findings of the research

From an overall perspective, there is a risk of food poisoning irrespective of whether consumers purchase fresh produce from retailers or directly from farms, based on the 'safe levels' recommended by the FSANZ (2001) or PHLS (2000). To gain an overall food safety perspective for fresh produce in New Zealand, further studies should be conducted in samples collected from many cities and rural areas. Setting regulations to manage fresh produce from farming systems through to retailers is required.

5.2. Conclusions

The aim of this thesis was to analyse microbiological risks associated with fresh produce from farms and retail shops in Canterbury. In the process of this study, the following information was gathered or generated:

A - Literature review

1. The current situation of microbiological risks in fresh produce in different countries was identified.

2. The potential risks of organic farming, such as the effects of contamination of fresh produce from faeces of animals, were identified.

3. The risks of organic farming, such as pathogen contamination from soils or internalization which means that pathogens can pass from soil and water to the inside of plants through their root systems, were identified.

From the literature research, it was found that norovirus was the most common pathogen causing foodborne disease in the USA, followed by *Salmonella* and enteropathogenic *E. coli*. Most studies showed similar results for APC from fresh produce, which ranged between 10^0 and 10^{10} $\log_{10}$CFU/g. Most studies found no *E. coli* O157:H7 from fresh

produce and 0-28.3% of samples were *Salmonella* spp. positive. Frequency of foodborne illness related to fresh produce was different between the USA, Japan and New Zealand, and no particular trend was observed among them. As there are many factors affecting the microbial status of fresh produce, further studies should be conducted to understand the status of microbiological risks of fresh produce in many more countries and about how to manage pathogens in fresh produce.

The number of pathogens in the faeces of animals is approx. one thousand cells per mil. In addition, pathogens can survive in faeces for more than two hundred days, depending on conditions. However, high temperatures generated by composting or aging can reduce pathogens. Inappropriate use of faeces without such prior treatment can lead to a high microbial load in fresh produce and be a potential risk to the consumers of produce from organic farms.

Though internalization of *Salmonella* spp. has been observed from the root system, this may not significantly affect the microbial load in fresh produce for two reasons. Firstly, the diversity of soil microorganisms in the organic farming leads to supress the large amount of internalization of *Salmonella* to fresh produce and secondly, the number of pathogens which can travel through the root systems to leaves are quite low and it is not enough to harm human health. These may be why relatively few pathogens are detected in fresh produce. However, the risks of organic farming are a concern and ideally the microbial load should be considered and checked regularly, although it may not be possible to implement such measures easily because it is not practical to do so.

B - The research work

1. The risks of soil enrichment were illustrated by comparing different types of soil enrichment. Differences in microbial contamination were observed between fresh produce from the organic and conventional farms and there appeared to be a tendency

for the organic farm produce to be contaminated with more pathogens than produce from the conventional farm.

2. The risks associated with the microbial load in different retail shops were identified, such as the microbial load in most of the vegetables showed unsatisfactory results compared to the guideline FSANZ (2001) or PHLS (2000).

Finding 1: In this research, microbiological testing data analysis showed differences in the level of microbial load between organic farms and the conventional farm. When soil samples and individual vegetable samples were compared, *E. coli* for silver beet and soil samples were significantly higher ($p<0.05$) in the fresh produce from organic farms than from conventional farming systems. The fresh produce samples collected from different farms in Canterbury, New Zealand was acceptable for *E. coli* O157:H7 (not detected) but not for APC, coliform, *E. coli, Staph. aureus* or *Salmonella* spp. based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. In addition, there were significant differences ($p<0.05$) between the conventional farm vegetable samples and those of the organic farms for APC, coliform, yeast and mould, and *Staph. aureus.* In addition, there were significant differences ($p<0.05$) between *E. coli* in the compost farm vegetable samples and those from farms. Therefore, further study should be conducted to compare fresh produce grown under different soil enrichment farming systems to obtain a better understanding of the situation.

Finding 2: In this research, similar to the previous results from farms, microbiological testing data analysis showed differences in the level of microbial load between different retail shops. Percentage of unsatisfactory to the guideline by FSANZ (2001) or PHLS (2000) varied within a retail shop, such as 75-87.5% samples had unsatisfactory for APC, 75-91.7% samples had unsatisfactory for coliform, 80-100% samples had unsatisfactory for *E. coli*, and 17.4-21.7% samples had unsatisfactory for *Staph. aureus*. When data for total samples from each retail shop was combined, significant differences ($p<0.05$) between

the retail shops were observed for of APC, coliform, *E. coli* and *Staph. aureus*. In addition, of *E. coli* tended to be lower in supermarket produce (retail shop A) than produce from other retail shops and retail shop B had the highest unsatisfactory levels for APC (87.5%), coliform (91.7%), *E. coli* (100%) and *Staph. aureus* (21.7%) based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. There is possibility that each retail shop system has different specifications (i.e., pre-packing treatment and type of package) for suppliers. Therefore treatments applied in the supply chain may have affected the microbiological status of the fresh produce, though no such information was collected in this study.

5.3. Future research

In this research, there were some limitations. Firstly, there was a lack of data, such as temperature and weather conditions on the day of harvest, fertilizer applications, treatment of different types of fertilizer including animal faeces, processing of the vegetables in each retail shop, and the number of days after harvesting the vegetables had been in each retail shop. Seasonal influences may have been a significant factor: samples were collected in winter when humidity in the farms was very high. This may have affected the growth and diversity of microbes. Comparing microbiological status between seasons may be a future study.

Secondly, this research concentrated only on analysing the status of microbiological risks and did not investigate how to reduce or manage those risks. There have been some studies conducted to investigate how to reduce microbial status from fresh produce. For example, Sirsat and Neal (2013) reported that diluted vinegar was useful to reduce *Salmonella*, *E. coli*, coliforms and spoilage microorganisms. Moore *et al*. (2011) reported that olive, apple and hibiscus extracts were capable of significantly reducing *S. enterica*. Since a consumer who prefers organic produce does not want chemical applied to

produce, it is important in future studies to investigate effective ways of reducing pathogens with natural agents.

Thirdly, it is interesting to conduct further study to the current study which analyses the effects of antimicrobial agent such as bacteriocin from bacteria to the pathogens. As mentioned in Chapter 1, Gálvez *et al*. (2007) reported that bacteriocin produced from lactic acid bacteria through lactic fermentationcan be an antimicrobial agent. As a fact, Vidhyasagar and Jeevaratnam (2013) reported that the bacteriocin produced from one of the lactic acid bacterial species (*Pediococcus pentosaceus* VJ13) has antimicrobial effects on *Mycobacterium smegmatis*, *Klebsiella pneumonia*, *C. perfringens* and *Staph. epidermidis*. Since bacteriocins are effective and produce few side effects, investigations into applying it to fresh produce would be helpful.

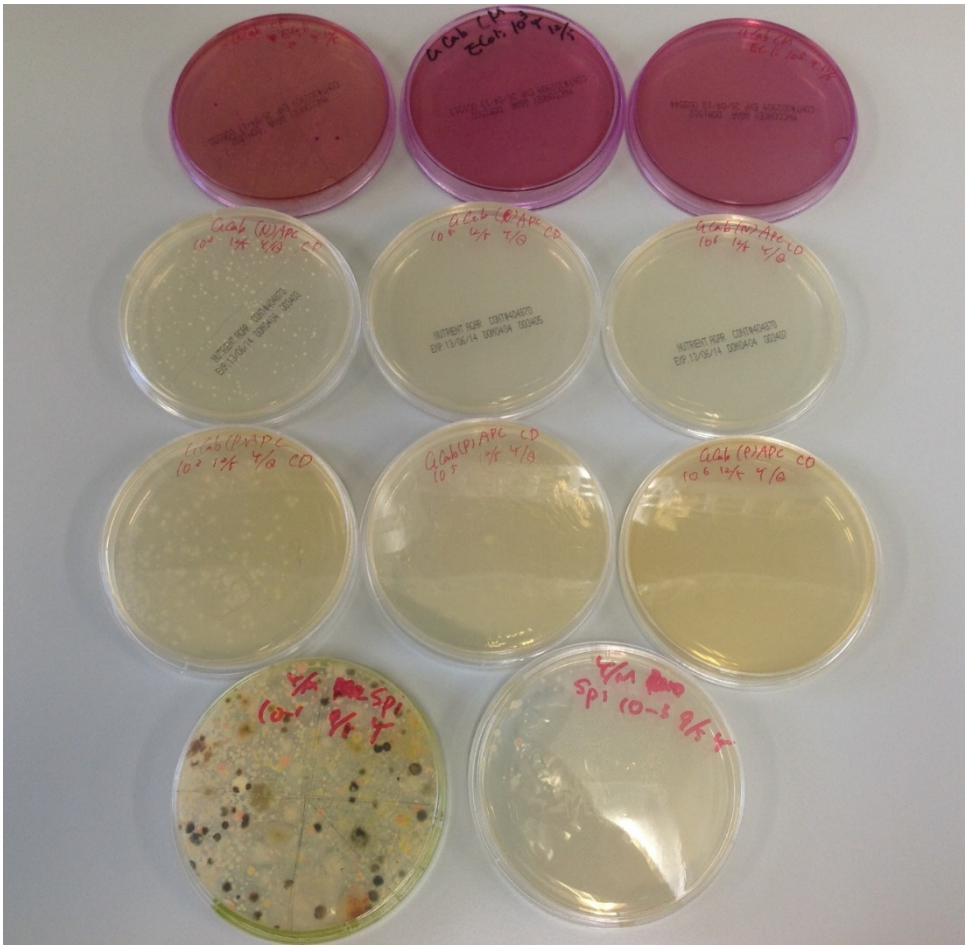
Finally, using computational models by quantitative assessment from data to determine the microbial risk is another option. For example, Franz *et al*. (2010) created a model for pathogen growth and conducted risk assessments of salad bars in the Netherlands. Similarly, Danyluk and Schaffner (2011) analysed the relationship of temperature and *E. coli* O157:H7 on cut leafy vegetables using a computational model. Likewise, Puerta-Gomez *et al*. (2013) assessed the risk of *Salmonella* spp. contamination in the processing line of baby spinach by using a computational model. Ding *et al*. (2011b) reported the prediction of the growth of *Staph. aureus* on fresh-cut spinach under different conditions such as temperature and relative humidity using computer models. These predictions can be an effective way of managing microbiological safety of fresh produce, though there have been very few studies on this in New Zealand. As modelling requires large sets of data, this analysis would be a good future study after more studies assessing the current microbial situation of fresh produce in New Zealand have been completed.

5.4. Final Summary

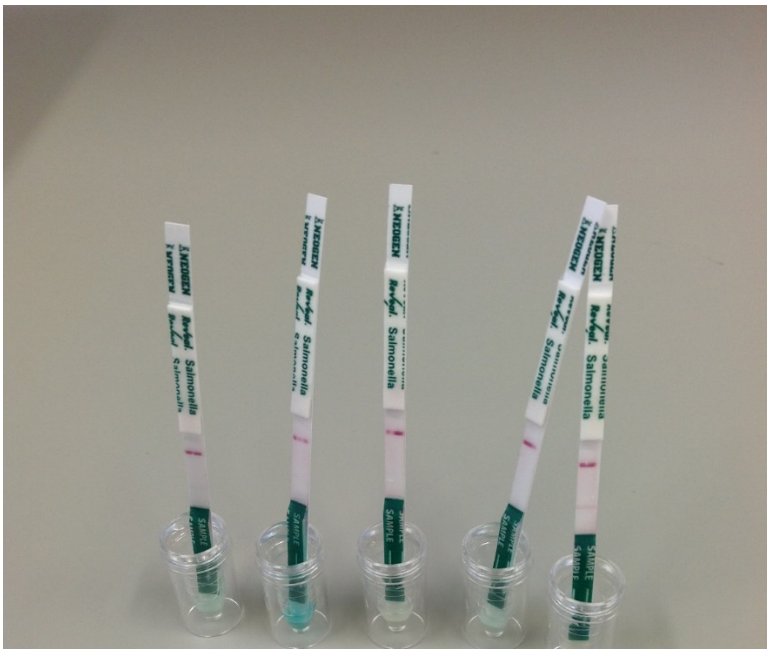
The type of soil enrichment affected total microbial load on fresh produce collected in this research. Most of the analysis showed significant differences ($p<0.05$) in microbial load on fresh produce or from soils between the organic farms and the conventional farm. Similarly, microbial load of fresh produce was significantly different ($p<0.05$) between different types of retail shops (supermarkets and other retail shops) and this was most noticeable in *E. coli*. In addition, Asian shop had the highest unsatisfactory levels for APC, coliform, *E. coli* and *Staph. aureus* based on the FSANZ (2001) or PHLS (2000) guidelines for ready-to-eat foods. This research has provided proof of concept of the potential risks of fresh produce with the *E. coli* in some vegetables in the crop-livestock rotation farm being significantly higher than in the conventional farm, and with some of the other pathogens in the organic farms being significantly higher than in the conventional farm. In addition, the potential risks of fresh produce in some retail shops were also identified. Further research is required to fully understand the current situation of microbiological risks of fresh produce in different parts of New Zealand.

Appendix

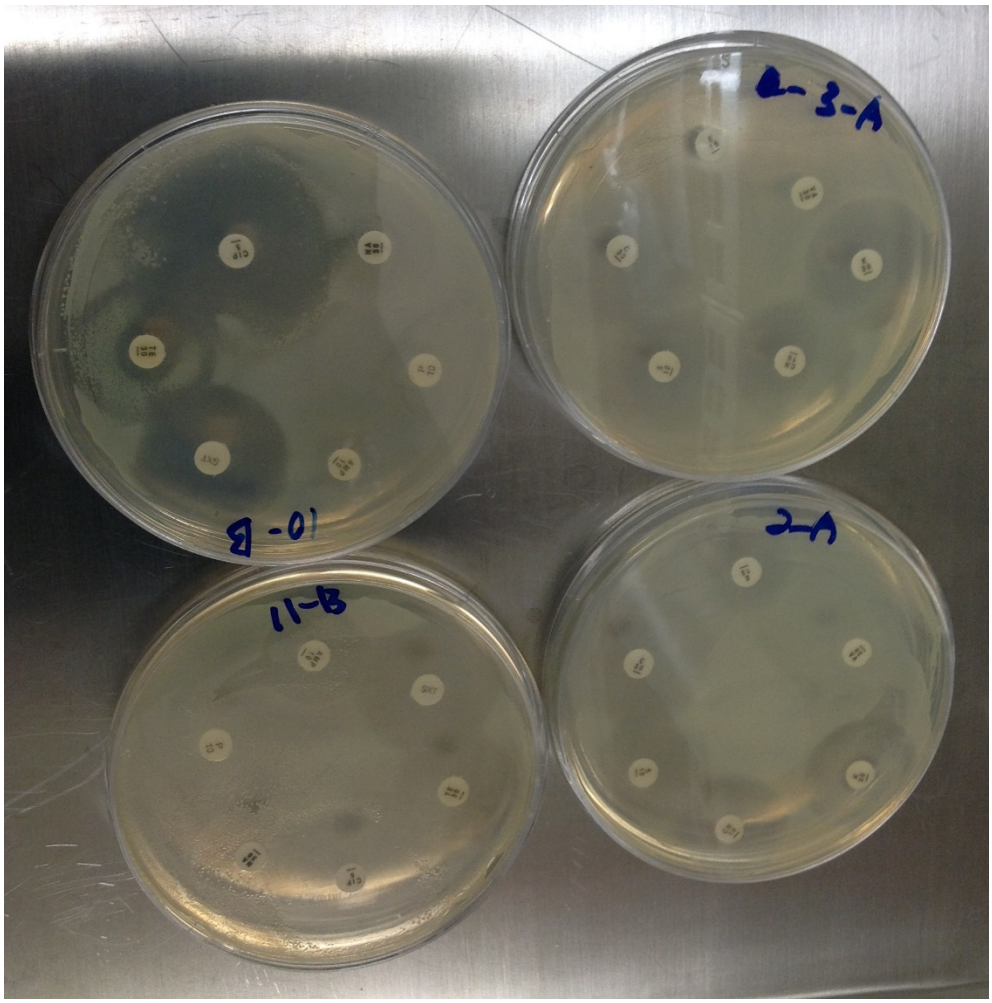
Appendix 1: Pictorial reference of the culture colony counting plates for APC, coliform, *E. coli*, *Staph. aureus* and yeast and mould



Appendix 2**:** Pictorial reference of the *Salmonella* spp. detection kit



Appendix 3: Pictorial reference of the antibiotic resistance test



Appendix 4: Sampling plan (farms)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Organic farm (Compost)	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil
Organic farm (C-L integration)	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil
Conventional farm	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil	5 vegetables	5 vegetables and soil

Appendix 5: Sampling plan (shops)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Retail shop A	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables
Retail shop B	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables
Retail shop C	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables
Retail shop D	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables

Appendix 6: Number of samples (farms)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Organic farm (Compost)	5 vegetables	5 vegetables	5 vegetables	5 vegetables	4 vegetables, 2 soil and 2 water	4 vegetable, 2 soil and 2 water	28 vegetable, 4 soil and 4 water
Organic farm (C-L rotation)	4 vegetables	4 vegetables	4 vegetables	5 vegetables	5 vegetables, 2 soil and 2 water	5 vegetables, 2 soil and 2 water	27 vegetable, 4 soil and 4 water
Conventional farm	5 vegetables	5 vegetables	5 vegetables	4 vegetables	4 vegetables, 2 soil and 2 water	4 vegetables, 2 soil and 2 water	27 vegetable, 4 soil and 4 water
Total	14 vegetables	14 vegetables	14 vegetables	14 vegetables	13 vegetables, 6 soil and 6 water	13 vegetables, 6 soil and 6 water	82 vegetables, 12 soil and 12 water

Appendix 7: Number of samples (shops)

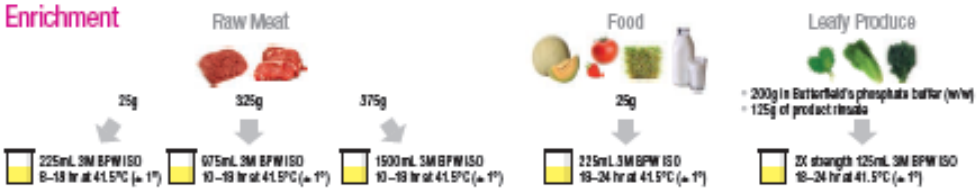
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
Retail shop A	5 vegetables	5 vegetables	5 vegetables	4 vegetables	4 vegetables	4 vegetables	27 vegetables
Retail shop B	4 vegetables	5 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	25 vegetables
Retail shop C	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	24 vegetables
Retail shop D	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	4 vegetables	24 vegetables
Total	17 vegetables	18 vegetables	17 vegetables	16 vegetables	16 vegetables	16 vegetables	100 vegetables

Appendix 8: Protocol for 3M Molecular Detection System

3M™ Molecular Detection System

E. coli O157 (including H7) Protocol Reference Guide

Enrichment

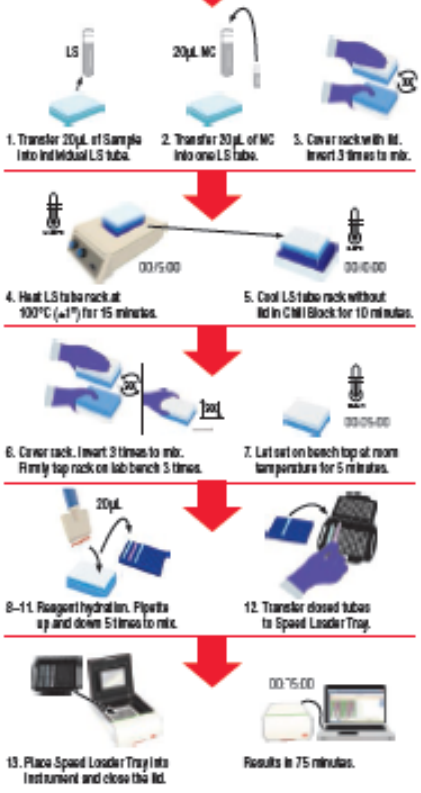


Lysis

1. Transfer 20µL of Sample into individual Lysis (LS) tube.
2. Transfer 20µL of Negative Control (NC) into one LS tube.
3. Cover rack of LS tubes with lid and invert 3 times to mix.
4. Place rack with LS tubes into a heating block (100°C ± 1°C) and heat for 15 minutes.
5. Remove the rack of LS tubes from heating block and remove lid. Place rack on Chill Block for 10 minutes.
6. Remove the rack from Chill Block and mix 3 times. Tap rack on bench 3 times.
7. Let set on bench top at room temperature for 5 minutes.

Amplification

8. Transfer 20µL of each sample lysate into Reagent tubes. Mix by gently pipetting up and down 5 times. Seal tubes with caps.
9. If needed, transfer 20µL of sample lysate into MC tubes. Mix by gently pipetting up and down 5 times. Seal tubes with caps.
10. Transfer 20µL NC lysate into one Reagent tube. Mix by gently pipetting up and down 5 times. Seal tube with cap.
11. Transfer 20µL NC lysate into one Reagent Control (RC) tube. Mix by gently pipetting up and down 5 times. Seal tube with cap.
12. Transfer closed tubes to Speed Loader Tray.
13. Place Speed Loader Tray into instrument and close the lid to start the assay.



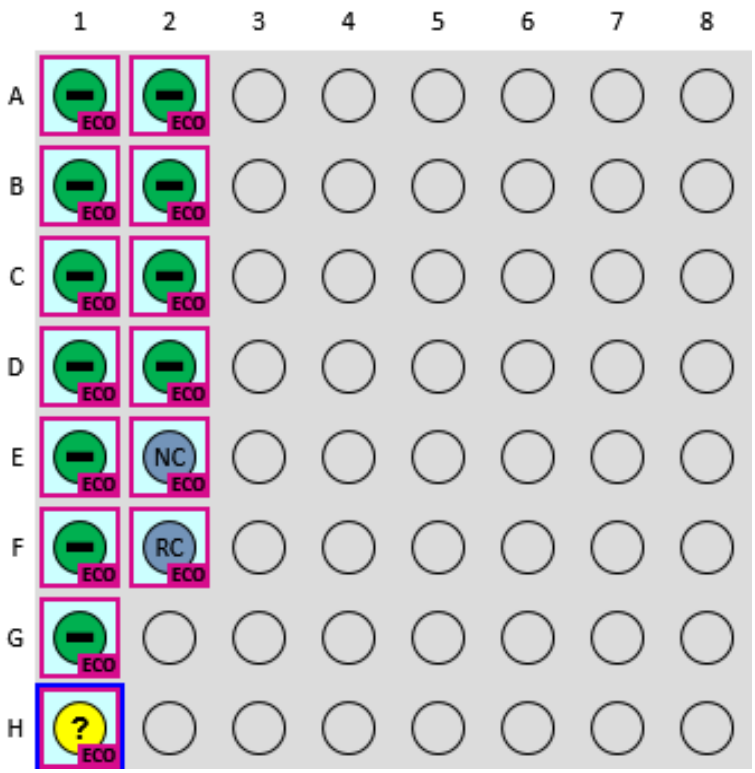
3M

Food Safety
3M Center
Building 275-5W-05
St. Paul, MN 55144-1000
USA
1-800-328-6553
www.3M.com/3MMolecularDetectionSystem

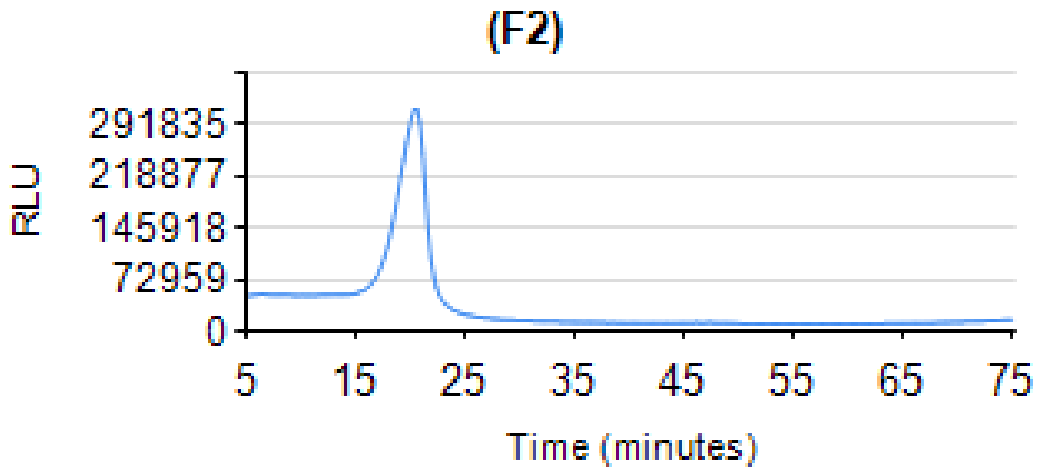
3M Canada
Post Office Box 5757
London, Ontario N6A 4T1
Canada
1-800-364-3577

70-2009-9511-9 Rev-0812

Appendix 9: Sample result for 3M Molecular Detection System (*E. coli O157:H7*)



Appendix 10: Reagent control (positive)



Appendix 11: Results of 3M Molecular Detection System (farms)

	Positive	Negative	Total	%
Conventional farm	0	8	8	0
Compost farm	0	9	9	0
Crop-Livestock Rotation farm	0	8	8	0

Appendix 12: Results of 3M Molecular Detection System (shops)

	Positive	Negative	Total	%
Retail shop A	0	5	5	0
Retail shop B	0	4	4	0
Retail shop C	0	7	7	0
Retail shop D	0	6	6	0
Total	0	22	22	0

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IJFST2015 Conference poster

Microbiological risk assessment of fresh produce grown in organic and traditional farms in New Zealand

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Introduction

Risk of food poisoning from organic fresh produce is probably more than from traditional fresh produce because of the use of manure and other types of soil enrichments. To our knowledge, there have not been many reports of microbiological assessment of fresh produce in the organic farming system and no reports of those of crop-livestock rotation farming system, which is a sustainable effective traditional organic farming method with rotational cropping and livestock production on the same land and offers many benefits (Vilrla et al., 2003) [1]. In this research, microbiological hazard status of fresh produce grown in three different types of farms (compost farm, crop-livestock rotation farm, traditional farm) in the Canterbury region of New Zealand was assessed by using a combination of detection methods (microbiological plating techniques, using rapid detection kits, and a molecular detection system).

Method

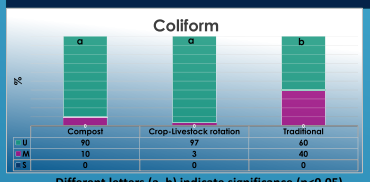
Picked vegetables from 3 farm types. → Washed, cut into pieces, & homogenized with broth. → Incubated in media (APC, coliform, *E.coli*, Yeast & mould, & *Staph.aureus*).

Detected & confirmed *S.typhimurium* with commercial kits.

Susceptibility to antibiotic test (*S.typhimurium* & *Staph.aureus*).

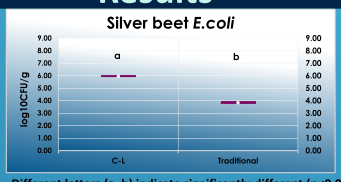
Confirmed *E.coli* O157:H7 with 3M molecular detection

Results



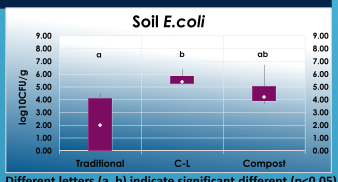
Different letters (a, b) indicate significance (p<0.05).

Fig 1: % unsatisfactory (U), marginal (M) & satisfactory (S) coliforms (based on FSANZ (2001) guidelines)



Different letters (a, b) indicate significantly different (p<0.05).

Fig 2: *E. coli* (log_{10}CFU/g) in silver beet from the 3 farms.



Different letters (a, b) indicate significant different (p<0.05).

Fig. 3: *E. coli* (log_{10}CFU/g) in soil from the 3 farms.

Food Standards Australia New Zealand (2001)	Satisfactory	Intermediate	Unsatisfactory
APC	<4	<5	>5
Coliforms	<2	2 – 4	>4
E.coli	<0.48		>2
Staphylococcus aureus	<2	2 – 3	>3

Table 1: FSANZ (2001) guidelines

Salmonella	Positive	Negative	Total	%
Traditional	2	25	27	7.4
Compost	3	23	26	11.5
Crop-Livestock rotation	4	22	26	15.4
Total	9	70	79	11.4

Table 2: *Salmonella* spp in vegetables

		VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Traditional	Leek	I	S	S	S	R	R	R	S	R	R	R	S
	Pac choi	S	S	S	I	S	S	S	S	S	S	S	S
	Radish	S	S	S	I	S	S	S	S	I	S	S	S
	Spring onion	I	S	S	R	R	R	I	S	R	R	I	S
Compost	Silverbeet	I	R	R	I	S	R	S	S	I	R	I	S
	Spinach	R	S	S	I	S	R	S	S	S	R	S	S
	Spring onion	R	S	S	S	S	R	S	S	S	R	R	S
	Carrot	S	S	S	S	R	R	R	S	R	R	I	R
	Coriander	S	S	S	S	S	S	S	S	S	S	S	S
C-L	Spinach	S	S	S	R	S	S	S	S	S	R	S	S
	Parsley	R	S	S	I	S	R	S	S	S	R	S	S
	Spinach	I	S	S	I	R	R	R	S	I	R	I	S
	Lettuce	S	S	S	S	S	S	S	S	S	R	S	S
	Parsley	I	S	S	I	S	R	S	S	I	R	S	S
	Cabbage	I	S	S	I	R	R	R	S	I	R	I	S
	Pac choi	I	S	S	I	S	I	S	S	I	R	S	S

Table 3: Susceptibility of *Staph.aureus* in vegetables to antibiotics

		VA	K	GN	S	C	E	TE	CIP	NA	P	AMP	SXT
Compost	Parsley	R	S	S	S	S	I	S	S	S	R	R	S
Compost	Parsley	R	S	S	I	S	S	S	S	S	R	R	S
C-L	Lettuce	R	S	S	R	S	R	S	S	S	R	R	S
C-L	Parsley	R	R	S	R	S	R	S	S	S	R	R	S

Table 4: Susceptibility of *Salmonella* in vegetables to antibiotics

*Vancomycin30 (VA), Kanamycin30 (K), Gentamicyn30 (GN), Streptomycin30 (S), Chloramphenicol30 (C), Eryhromycin15 (E), Tetracycline30 (TE), Coprofloxacin5 (CIP), Nalidixic acid30 (NA), Penicillin10 (P), Ampicillin10 (AMP), and Sulfamethoxazole23.75 & Trimethoprim1.25 (SXT). ** Zone Diameter (mm) S; Susceptible ≥20, I=Intermediate 15–19 and R=Resistant ≤14

Conclusions

E.coli in silver beet and soil were significantly higher (P<0.05) in the crop-livestock rotation farm than in the traditional farm. *E. coli* O157:H7 were within the acceptable level but APC, coliforms, *Staph. aureus* & *Salmonella* counts were higher in all farms and greater than the microbiological criteria for the ready-to-eat foods. Most *Staph. aureus* and *Salmonella spp.* positive isolates showed higher resistance to some β-lactams than other antibiotics . Fresh produce from the crop-livestock rotation farm had a higher microbial contaminant load than those from the traditional farm.

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[1]. Crop-Livestock Integration Benefits , translation available at:ww.fao.org/ag/AGP/AGPC/doc/integration/papers/integration_benefits.htm

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