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Microbiological survey of ready-to-eat foods prepared and displayed in retail delicatessens

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CHAPTER 1

INTRODUCTION

INTRODUCTION TO FOODBORNE DISEASE

Foodborne disease results from the consumption of foods contaminated with microbiological pathogens or toxic chemicals (Mossel *et al*., 1995). A broad spectrum of microorganisms has been associated with foodborne diseases (Table 1.1). Various symptoms resulting from the ingestion of pathogenic foodborne microorganisms include vomiting, diarrhoea, abdominal pain, fever, meningitis, septicaemia, endocarditis and kidney failure, to name a few. The high incidence of foodborne disease has made food safety a global concern. Mead *et al*., (1999) reported that during the period 1992 – 1997, 76 million people in the USA alone suffered from a significant foodborne disease annually, resulting in 5,000 deaths, and that the etiological agents for 64% of these diseases were unknown.

In an attempt to understand the nature and sources of foodborne disease several countries have established surveillance systems aimed at monitoring outbreaks. In the USA two surveillance systems have been established, Food Net (Foodborne Diseases Active Surveillance Network - http://www.fsis.usda.gov) and Pulse Net (National Molecular Subtyping Network - http://www.cdc.gov/pulsenet/) which provide surveillance systems for the USA and certain territories within Canada. In Europe, the WHO surveillance system (http://www.euro.who.int/) aids in the monitoring of food-related outbreaks within 51 countries. A recent surveillance system has also been established in Australia called OzFoodNet (http://www.health.gov.au).

During production, processing, packaging, transportation, storage and consumption, food may become contaminated with potential bacterial foodborne pathogens (Table 1.2). Processing failures may lead to the survival of microorganisms, while temperature abuse may lead to the proliferation of pathogenic bacteria (ICMSF, 1996). If food products containing sufficient numbers of pathogenic bacteria are ingested, bacterial foodborne disease will result (ICMSF, 1996). Bacterial foodborne disease may result in either infections or intoxications. Foodborne infections are caused by ingesting sufficient amounts of potentially infectious bacteria, which then cause disease through the elaboration of toxins, or by invasion of the host through the small and large intestines (Pierson and Corlett, 1992; Mossel *et al*., 1995). Foodborne intoxications involve the ingestion of pre-formed toxins, produced and excreted by

bacteria whilst in foods (Pierson and Corlett, 1992). The predominant bacterial foodborne pathogens associated with various food commodities are summarised in Table 1.2.

Bacterial foodborne illness is a significant economic burden on society as it results in financial loss through decreased productivity, litigation, medical expenses, as well as the cost of product recall and disposing of contaminated food (Salin and Hooker, 2001; Whyte *et al.,* 2004; Normanno *et al*., 2005). For example, *ca* US$1.5 billion is spent annually in the USA due to *Staphylococcus* intoxications (Normanno *et al*., 2005). In another example, the total loss of productivity due to *Clostridium perfringens* infections was reportedly US$12.5 billion in the USA (Novak and Juneja, 2002).

READY-TO-EAT (RTE) FOODS

Consumer markets are showing an increased demand for a new class of processed foods, ready-to-eat (RTE) products (Kaneko *et al*., 1999). RTE foods are defined as food items, which are eaten as sold and they require no further cooking or processing by the consumer. There are several categories of RTE foods. These include airline foods served from flight kitchens (Hatakka and Asplund, 1993; Hatakka, 1998a; Hatakka, 1998b; Hatakka, 2000), restaurants (Swanger and Rutherford, 2004; Vazgecer *et al*., 2004), take-away premises (Nichols *et al*., 1999), school catering establishments (Martínez-Tomé *et al*., 2000; Tessi *et al.,* 2002; Rosset *et al*., 2004), hospitals (Ayçiçek *et al*., 2004) and college dining halls (Montville and Schaffner, 2004).

A. Bacterial foodborne pathogens associated with RTE foods

As indicated in Table 1.2 there are numerous potential bacterial pathogens, which may also be associated with RTE foods. The characteristics of selected bacterial foodborne pathogens will be discussed below.

i) *Bacillus* (*B.*) *cereus*

B. cereus is a spore forming rod-shaped bacterium (Jay *et al*., 2005) (Table 1.2). *B. cereus* is a major problem in convenience food, mass catering and starchy foods, such as rice, due to the heat and acid resistance of its spores and hence it is not eliminated by most pasteurisation or sanitation procedures (Ehling–Schulz *et al*., 2004). Most outbreaks are reportedly as a result of temperature abuse, which allows relatively low numbers of the bacterium to multiply within the food commodity (Ehling–Schulz *et al*., 2004). *B. cereus* food poisoning occurs after ingestion of food in which the microorganism has multiplied and produced heat-labile enterotoxins (ICMSF, 1996). Two types of toxins are produced, a heat-labile diarrhoeal toxin (protein) and a heat-stable emetic toxin (small peptide). *B. cereus* is generally present in foods at concentrations of 10^2/g – 10^3/g. These concentrations are considered innocuous since the minimum level to cause disease is estimated at $>10^5$/g (ICMSF, 1996). It has been reported that 10^7-10^8 cells/g have been isolated from foods which resulted in diarrhoeal syndrome, whilst a higher number of cells (*ca* 10^9 cells/g) are required to cause the emetic syndrome (Jay *et al*., 2005). The incidence of *B. cereus* poisoning is often underestimated, as it is not a notifiable disease (Ehling–Schulz *et al*., 2004), and symptoms often mimic that of *Staphylococcus* food poisoning (Jay *et al*., 2005). *B. cereus* was the most commonly isolated microorganism from foodborne disease outbreaks in Norway (14%) and Finland and Europe (42 %) between 1985 and 2000 (Ehling–Schulz *et al*., 2004).

ii) *Clostridium* (*C.*) *perfringens*

C. perfringens is a spore forming rod-shaped bacterium (Jay *et al*., 2005) (Table 1.2). The food commodities acting as vehicles for *C. perfringens* are cooked meat, poultry, fish and vegetable dishes stored at ambient temperatures with long cooling periods. *C. perfringens* food poisoning is frequently associated with temperature abuse as spores survive cooking and subsequently germinate whilst the food is held at ambient temperatures. (Novak and Juneja, 2002). Rapid cooling after cooking and subsequent refrigeration are the most effective means of limiting the growth of *C. perfringens* (Novak and Juneja, 2002). *C. perfringens* was found to be the third most common cause of foodborne disease in the USA in 1999 following *Campylobacter* and

Salmonella spp. Approximately 10^6 CFU/g (8 – 10 ng of toxin) are required to cause food poisoning (Pierson and Corlett, 1992; Novak and Juneja, 2002).

(iii) *Escherichia* (*E.*) *coli* O157:H7

E coli was first recognised as a foodborne pathogen in 1971 when imported cheeses to the USA were contaminated with an enteroinvasive strain causing illness in approximately 400 people (Jay *et al*., 2005). Pathogenic strains of *Escherichia* are serologically typed and *E. coli* has reportedly over 200 O serotypes. *E. coli* O157:H7 was first recognised as a pathogen in 1982 during an investigation of hemorrhagic colitis (Riley *et al*., 1983). *E.coli* O157:H7 infection may result in haemolytic uremic syndrome (HUS) from the production of Shiga-like toxins by the bacterium in the intestines of infected individuals (Park *et al*., 1999). It has been estimated that 73,480 illnesses are due to *E. coli* O157:H7 infection each year in the USA resulting in 2,168 hospitilzations and 61 deaths (Mead *et al*., 1999). Food originally associated with outbreaks was undercooked ground beef patties sold from a fast-food chain in 1993 (Bell *et al*., 1994). By 1994 *E. coli* O157:H7 was recognised as a significant emerging foodborne pathogen and became a notifiable infection in the USA (Park *et al*., 1999). Whilst ground beef still remains the most common vehicle for foodborne outbreaks, produce-associated outbreaks have been reported since 1991 and have accounted for 21% of 350 outbreaks in the USA between 1982-2002 (Rangel *et al*., 2005). Outbreaks have been associated with lettuce, apple cider, salads, coleslaw, melons, sprouts and grapes (Rangel *et al*., 2005).

iv) *Listeria* (*L.*) *monocytogenes*

L. monocytogenes is a foodborne microorganism of more recent concern (Pierson and Corlett, 1992) (Table 1.2). Foods normally associated with outbreaks include soft cheeses, pâtés, fermented sausages, coleslaw and other assorted salads. This bacterium is also harboured on wet surfaces in food processing plants and has the ability to multiply at refrigeration temperatures (4°C) (ICMSF, 1996). The incidence of *L. monocytogenes* infections in Japan is estimated at 1 per 1,000,000 people whilst in the USA it is *ca.* 4 cases per 1,000,000 people (Okutani *et al.,* 2004). The minimal infectious dose is estimated to be the ingestion of >100 viable cells/g for

immunocompromised individuals which show a greater susceptibility to listeriosis than otherwise healthy individuals (Jay *et al*., 2005). Consequently fatality rates are usually between 25 –30% but may be as high as 70% for severe forms if left untreated (Pierson and Corlett, 1992).

v) *Salmonella* (*Salm*) spp.

Salmonella spp. are non-spore forming, rod-shaped bacteria belonging to the family Enterobacteriaceae (Table 1.2). Previously, the 2,324 serovars of the genus *Salmonella* were treated as individual species, however, taxonomic changes within the genus has resulted in only two species being recognised, *Salm. enterica* and *Salm. bongori*. The serovars have subsequently been classified under either species. For example *Salm. typhimurium* has been reclassified as *Salm. enterica* serovar Typhimurium or *Salm*. Typhimurium (Jay *et al*., 2005). Different *Salm. enterica* serovars have different dose to infectivity ratios. It has been reported that generally 10^7-10^9 cells/g are necessary for salmonellosis whilst 10^5-10^{10} cells/g are necessary for gastroenteritis (ICMSF, 1996; Moon *et al*., 2004; Jay *et al*., 2005), however, certain serovars have been shown to exhibit low dose to infectivity ratios. For example 100 cells/100 g of *Salm*. Eastbourne in chocolate reportedly resulted in salmonellosis (Jay *et al*., 2005). In another example, it was estimated that 1-3 cells/g of *Salm*. Oranienburg present in German chocolate resulted in a salmonellosis outbreak (Werber *et al*., 2005). Many foods of animal origin have been identified as vehicles for transmitting these pathogens to human beings and spreading them to processing and kitchen environments due to faecal contamination of the environment and equipment (ICMSF, 1996).

vi) *Staphylococcus* (*S.*) *aureus*

S. aureus is a coccoid-shaped bacterium (Normanno *et al.,* 2005) (Table 1.2). *S. aureus* is ubiquitous and occurs within the mucous membranes of the nasal cavities as well as on the skin of most warm-blooded animals (ICMSF, 1996). It has been estimated that up to 50% of humans are carriers with nasal carriage being the most frequent. *S. aureus* is considered to be the third most important foodborne illness in the world (Normanno *et al.,* 2005). Contamination of food products with *S. aureus* is

frequently due to poor personal hygiene of food-handlers. Staphylococci are readily killed during cooking, however, the toxins they produce are heat stable. Thus staphylococcal food poisoning occurs most frequently in cooked foods, which are kept under warm conditions (20°C - 40°C) for several hours. The amount of enterotoxin causing disease depends on the weight of the patient and the individual's sensitivity, but it is generally estimated that *ca.* 1µl/kg will cause disease in humans (ICMSF, 1996). The incidence of staphylococcal intoxication is estimated at 1 million cases per year in the USA. Small outbreaks often go unreported as only outbreaks of food poisonings are reported when they result from large catering operations, such as banquets (Jay *et al*., 2005).

B. Incidence of bacterial foodborne pathogens in RTE products highlighted in media reports

Recent news reports have further highlighted the high incidence of bacterial foodborne pathogens in RTE foods. A 2 year (2005-2006) survey of newspapers, radio, television reports and government web sites worldwide, whilst not extensive, has yielded a wealth of information on the latest bacterial foodborne pathogens present in RTE foods.

i) Recalls

In the USA and UK recalls are the primary means by which authorities stem the consumption of foods contaminated with potentially pathogenic bacterial foodborne pathogens (Wong *et al.,* 2000). Numerous food products have been recalled as a result of *L. monocytogenes* contamination. These include sandwiches, such as E. A. Sween Co. sandwiches in Minnesota (Detroit Free Press Inc, March 18, 2005), Eastside Deli Supply Inc. sandwiches in Michigan (Seattle Post-Intelligencer, March 18, 2005), Prime Deli Corporation sandwiches in Texas (Foodconsumer, May 16, 2005), chicken wrap sandwiches in New York (Foodconsumer, April 28, 2005), Quik'n Tasty Foods sandwiches in Belton Missouri (Foodconsumer, May 9, 2005), Seven Eleven sliced processed meat sandwiches in Texas (FDA Enforcement Report, July 20, 2005), Mountain Fresh Deli's triple wedge sandwiches in Western Virginia (FDA Enforcement Report, January 18, 2006), Classic Delight Inc. egg sandwiches in Ohio

(FDA Enforcement Report, May 31, 2006) and Jumbo Foods, Inc. turkey sandwiches in West Virginia (U. S. Food and Drug Administration, October 4, 2006).

Salads recalled due to *L. monocytogenes* contamination include Golden Taste Inc. home style tuna salads (FDA Enforcement Report, January 4, 2006), Town and Country Meats salads in Maine (United States Department of Agriculture, April 5, 2006), Ballards Farm Sausage Inc. salads in West Virginia (U. S. Food and Drug Administration, October 25, 2006), Boston Salads and Provisions Co., Cole Slaw salads in Boston (U. S. Food and Drug Administration, November 1, 2006a) and Krisp-Pak Company Inc. fresh cut fruit in Norfolk (U. S. Food and Drug Administration, November 1, 2006b). Recalls due to other potential foodborne bacterial pathogens have included Timco Worldwide Inc. cantaloupe in California (U. S. Food and Drug Administration, October 6, 2006) and Classic Salad's spring mix in California (FDA Enforcement Report, October 11, 2006) due to *Salmonella* contamination. Similarly, Dole Fresh Vegetables Inc., pre-packaged salads in California (FDA Enforcement Report, November 2, 2005) and Pacific Coast Fruit Company recalled salads containing spinach in Oregon (U. S. Food and Drug Administration, September 22, 2006) due to *E. coli* O157:H7 contamination.

Similarly, RTE ham products in Kentucky (Perkins, May 3, 2005) and RTE sliced processed meat products in Michigan (wwmt.com, 2005) were also recalled due to *L. monocytogenes* contamination. In addition, Jilbert Dairy recalled Vanilla Supreme ice-cream (Foodconsumer, May 24, 2005) and the importers of Italian Mauri brand, Bon Taleggio cheese, recalled their products in Ottowa (Canadian Food Inspection Agency, May 26, 2005) due to *L. monocytogenes* contamination. Recalls due to other potential foodborne bacterial pathogens have included mushrooms in oil due to possible contamination with *C. botulinum* in Montreal (Canadian Food Inspection Agency, March 24, 2005) and Ghandour Halva (butter containing sesame seeds) owing to possible contamination with *Salmonella* spp. in Illinois (Georgia) (Foodconsumer, May 12, 2005).

ii) Outbreaks

Bacterial foodborne disease outbreaks, as a result of the consumption of RTE foods, have been reported. Many of the outbreaks resulted in closure of the eateries concerned. Some re-opened once they had complied with local food safety regulatory standards. For example, a typhoid outbreak in Kelantan (Malaysia) resulted in 203 hospitalisations and 2 deaths. The source of the outbreak was thought to be from RTE foods and 30 food stalls were closed for two weeks (ABC Radio Australia, April 21, 2005). Similarly, two catering companies, which were used to supply hot meals for Thanksgiving dinners in Waterloo (Iowa), resulted in *ca* 400 people displaying symptoms of *C. perfringens* foodborne disease. It was postulated that the outbreak was due to the turkeys that were served at both events (WCF Courier.com, May 13, 2005). In Benton (Arkansas), a *Salmonella* outbreak causing 9 cases of illness was traced to an eatery Café Santa Fé. (McGehee, May 2, 2005). In addition, a drive-in in Calgary was closed after 6 people became ill with *E. coli*. (CFCN. Ca. Calgary news from CFCN. CTV, May 4, 2005). A buffet style restaurant in the USA was the source of a recent *Salmonella* outbreak where 1 person died, 65 people became ill and 32 people were hospitalised (Jordan, May 25, 2005). A food poisoning outbreak in Wales, UK was reportedly due to *E. coli* O157:H7 contamination of cooked meals. John Tudor and Son Inc. supplied cooked meals to schools, council run institutions and some delicatessens in South Wales (Food Standards Agency, December 20, 2005). Similarly *E. coli* O157:H7 was the cause of a multistate outbreak in USA due to contaminated spinach containing products such as salads. The outbreak spanned 19 states in the USA, 109 cases of illness with 16 individuals suffering from haemolytic uremic syndromes and 1 death was reported (U. S. Food and Drug Administration, September 17, 2006). More recently, a multistate *Salm*. Typhimurium outbreak in the USA resulted in 183 cases of illness from the consumption of tomatoes in restaurants (U. S. Food and Drug Administration, November 3, 2006).

iii) Litigation

Foodborne outbreaks sometimes result in litigation. For example, a Mothers' Day brunch at the Royal Gardens in Burlington, Ontario resulted in 81 salmonellosis cases (The Globe and Mail - Canadian press, May 16, 2005). Subsequently, the caterers of

that particular Mother's Day event were sued for $1 million by the Royal Gardens. (The Globe and Mail - Canadian press, June 6, 2005). In another incident, a civil law suit was filed against Sea Specialities Inc., a supplier of RTE smoked salmon products to cruise ships, restaurants and retailers, due to operational inconsistencies at their Miami facility, as it was found that some of their products contained *L. monocytogenes.* The litigation resulted in their Miami facility being closed until it was properly sanitised and complied with the United States Food and Drug Administration regulations (Law Fuel, April 21, 2005). Suppliers of food for human consumption may also be held liable for class–action lawsuits as has been demonstrated in the recent Coronet Foods Inc. case. A judge allowed more than 80 plaintiffs to sue the supplier of Roma tomatoes, reservoirs of *Salmonella*, served at local convenience stores in the USA. As a result of these law suits, the company concerned has filed for bankruptcy (Pittsburgh Post Gazette, June 2, 2005).

C. Incidence of bacterial foodborne pathogens in RTE products highlighted in scientific journals

Many studies concerning the microbiological content of RTE foods have been conducted internationally. From these studies the predominant bacterial foodborne pathogens were *B. cereus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp. and *S. aureus.* Several studies conducted on RTE foods have highlighted disparity in the microbiological quality and safety of these products. The incidence and level of bacteria associated within the different RTE foods often varies between countries and even between different areas within a country. In a document prepared by the CIES – The Food Business Forum, it was suggested that these discrepancies may largely be due to local agricultural practices, endemic microbiological loads, water quality and cultural traditions (Bone and Parker, 2005).

i) Assorted salads

The aerobic bacterial counts obtained for assorted salads prepared in the UK, USA, Spain and Tokyo ranged from 2.4 – 8 Log colony forming units (CFU)/g (Christiansen and King, 1971; Fowler and Clark, 1976; Paradis and Stiles, 1978; Hagenmaier and Baker, 1998; Kaneko *et al*., 1999; Martinéz-Tomé, 2000; Montville

and Schaffner, 2004). In Tokyo the bacterial foodborne pathogens isolated from RTE assorted salads were *B. cereus*, *L. monocytogenes* and *S. aureus*. Two separate studies conducted in Ireland and Wales both confirmed the absence of viable *Campylobacter* spp. in RTE assorted salads in those areas (Meldrum and Ribeiro, 2003; Whyte, 2004). Cold tuna and corn salads contaminated with 10^6 CFU/g *L. monocytogenes* resulted in 1,473 scholars and teachers becoming ill and 292 hospitalisations in Italy (Aureli *et al*., 2000).

ii) Sandwiches

Sandwiches, like assorted salads, have shown variance between different areas. For example, aerobic plate counts of sandwiches prepared in Taiwan and the UK varied between 2 – 8 Log CFU/g (Christiansen and King, 1971; Fang *et al*., 2003). The bacterial foodborne pathogens isolated from sandwiches prepared in Taiwan contained *B. cereus, E. coli* and *S. aureus* (Fang *et al*., 2003).

iii) Sliced processed meats

Studies on sliced processed meats have revealed similar findings. For example, aerobic plate counts generally ranged between 3.2 - 3.7 Log CFU/g (Gillespi *et al*., 2000; Montville and Schaffner, 2004). *L. monocytogenes* (2.3% incidence) has been isolated from sliced processed meats in the USA and Nordic countries (Gombas *et al*., 2003; Gudbjörnsdóttir *et al*., 2004).

iv) Hot meals

Pathogens, such as *B. cereus* and *C. perfringens*, were shown to be present at counts of 2.3 – 5 Log CFU/g in the USA, Taiwan and Turkey (Christiansen and King, 1971; Fang *et al*., 2003; Ayçiçek *et al*., 2004).

v) Other RTE foods

L. monocytogenes was isolated from seafood (4.8%) products within the Nordic countries (Gudbjörnsdóttir *et al*., 2004). Chicken kebabs, retailed in Turkish restaurants, reportedly contained *B. cereus*, staphylococci and coliforms, while *Salmonella* spp. were not detected (Vazgecer *et al*., 2004). In two separate studies, cooked meat, cooked poultry, sauces, ice-creams, pizzas and cheeses did not yield *Campylobacter* (Meldrum and Ribeiro, 2003; Whyte *et al.,* 2004).

D. Sources of microbiological contamination in RTE foods

Contamination of RTE products can originate from many sources. Contamination of RTE foods may arise from (i) ingredients used to prepare RTE foods; (ii) poorly sanitised preparation surfaces; (iii) utensils used to prepare the food (Jay *et al*., 2005); (iv) the microbiota on the hands and outer garments of food handlers (Jay *et al*., 2005), as well as contamination arising from the mouths, nasal cavities and gastrointestinal tracts of food handlers due to poor personal hygiene (Jay *et al*., 2005) and (v) ice prepared in retail environments and subsequently used to cool RTE foods such as assorted salads.

i) Ice used to cool assorted salads

A preliminary study has implied that the ice used to cool RTE foods displayed in retail operations may represent a source of contamination. In a survey conducted in the UK samples of ice were taken from displays of RTE assorted salads. Of these samples 23% contained coliforms, 5% contained *E. coli* and 8% contained enterococci. The study suggested that the quality of ice may become a concern in terms of pathogen transmission to RTE food products during display (Nichols *et al.,* 2000).

ii) Food handlers

Food handlers have the potential to act as vectors for the transmission to or the contamination of food products with foodborne pathogens (Martínez-Tomé *et al*., 2000). Hygienic food preparation and education of food preparation personnel is crucial for the prevention of microbiological foodborne disease (Martínez-Tomé *et al*., 2000). It has been reported that normal hand washing can lower the numbers of transient microorganisms, but does not lower the numbers of *S. aureus* (Williams, 1963). South African legislation concerning the levels of microorganisms on the hands of food handlers is limited, with the exception of the total number of viable cells on aprons (1 x 10 CFU/cm^2) (South African National Standard 18593:2004, 2004). A recent study showed that the usage of disposable plastic gloves did not significantly reduce the risk of microbiological contamination from food handlers, but may in fact exacerbate the contamination levels. This may be due to gloved food handlers not adhering strictly to good glove practices, such as frequent changing of disposable plastic gloves and hand washing, as well as multiple usage of the same pair of disposable plastic gloves (Lynch *et al*., 2005).

iii) Food contact surfaces

As specified by Public Health Regulations a surface shall be cleaned and washed before food comes into contact with it so that contamination is prevented. Such surfaces will not contain more than 100 viable CFU/cm^2 (Republic of South Africa, Regulation R918, 1999). In a ten year study at Rutgers University (USA), plastic surfaces, such as pastry brushes and cutting boards, showed a higher mean microbial count compared to metal surfaces (food preparation surfaces) (Montville and Schaffner, 2004). In a separate study, *S. aureus* was isolated from swabs of food contact surfaces in Italy (Normanno *et al*., 2005). In a further study, when the degrees of transfer of *Campylobacter jejuni* and *Salm. enterica* from a stainless steel contact surface to an RTE food, such as lettuce, were evaluated, it was found that relatively high numbers of bacteria might be transferred from the food contact surface to the lettuce within 1-2 h after surface contamination (Moore *et al*., 2003).

iv) Utensils, sponges and cleaning cloths

Early studies in the USA have indicated that cross-contamination via cleaning cloths, sponges, cutting boards and utensils may contribute to the occurrence of food-borne salmonellosis (Bryan, 1998). In subsequent studies, kitchen sponges have been implicated as vehicles for pathogen transmission to food contact surfaces or food commodities (Hilton and Austin, 2000; Kusumaningrum *et al*., 2002; Kusumaningrum *et al*., 2003). Further studies have shown cleaning cloths and sponges to harbour *E. coli*, *Salmonella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. (Raloff, 1996). Structural surfaces and utensils have also been implicated in the harbourage of *Listeria* spp. at concentrations of $10^3 – 10^4$ CFU per object (Beumer *et al*., 1997).

1.3. RTE FOODS PREPARED AND DISPLAYED IN RETAIL DELICATESSENS

It has recently been estimated that 52% of meals are consumed outside of the home in the USA (Bone and Parker, 2005). For the consumer, this has resulted from longer working hours and less time for daily shopping and cooking (Novak and Juneja, 2002). This change in lifestyle has placed convenience store retailers in competition with restaurants and fast food outlets (Bone and Parker, 2005). Thus, retailers have incorporated kitchens in-store to prepare RTE foods and named them delicatessens (Bone and Parker, 2005). In the USA, restaurants, dining halls, and corporate canteens are also defined as retail delicatessens. For the purpose of this South African study, a retail delicatessen will be defined as a convenience store, which prepares and supplies RTE foods to consumers. Delicatessens provide a source of readily available and nutritious meals to consumers who want convenience foods that taste, smell and look like home-made foods (Novak and Juneja, 2002). RTE delicatessen foods are comprised of assorted salads, pies, sliced processed meats, sandwiches, hot meals, roasted chickens, prepared meals, fast foods and snacks. The preparation of these food types requires handling within the convenience store delicatessen kitchen, thus potentially exposing these foods to cross-contamination (Bone and Parker, 2005). As these RTE products are often consumed without further cooking, the presence of potential bacterial foodborne pathogens presents a major food safety concern.

A. Incidence of bacterial foodborne pathogens in retail delicatessen RTE products highlighted in scientific journals

i) Assorted salads

Surveys conducted in retail delicatessens outside of South Africa reported on the microbiological ecology of delicatessen assorted salads and sandwiches, Aerobic plate counts ranged between 2.1 - 6 Log CFU/g, coliform counts between 0.1 – 2.3 Log CFU/g, while *S. aureus* and *L. monocytogenes* were only detected in some delicatessen foods (Christiansen and King, 1971; Kaneko *et al*., 1999). In a study conducted in Ireland *Campylobacter* spp. could not be isolated from RTE retail sandwiches, assorted salads, vegetables or cheeses, while it was reportedly present in raw meat, poultry and unpasteurised milk (Kaneko *et al.,* 1999; Whyte *et al.,* 2004). Aerobic bacteria, *Salmonella* spp., *S. aureus*, *C. perfringens,* streptococci and Enterobacteriaceae were isolated from assorted salads in Spain (Martínez-Tomé *et al.,* 2000).

ii) Sandwiches

Coliforms and *E. coli* were reportedly detected in sandwiches from retail outlets in Taiwan (Fang *et al*., 2003). Interestingly, *S. aureus* and *B. cereus* were isolated more frequently from sliced processed meat sandwiches than sandwiches containing vegetables (Fang *et al*., 2003).

iii) Hot meals

In the UK it was found that cooked and stored rice had higher counts of *B. cereus* and *E. coli* compared to RTE rice cooked at point of sale (Nichols *et al*., 1999).

B. RTE food preparation in South African delicatessens

Retail delicatessens in South Africa prepare and display various RTE foods, such as roast chickens, pies, pizzas, baked goods, filled baguettes, fruit or vegetable salads, hot meals, and sliced processed meats in-house. All RTE foods are usually prepared within an allocated area of the general store. Cleaning and sanitation practices within the delicatessens include the use of general detergents with sanitizers, which are used to clean and disinfect preparation surfaces and utensils as well as general hand

washing procedures. Hairnets, aprons, uniforms and sometimes disposable plastic gloves are worn to maintain personal hygiene standards and prevent cross contamination between the foods and food handlers.

Depending on the food type, RTE delicatessen foods are generally cooled during display. For example, assorted salads are prepared on the day of sale and kept cool by either placing the display containers in ice or on a cooled display counter. Similarly, filled baguettes and sliced processed meats are also prepared on the day of sale and kept cool by placing on a cooled display counter. The temperature of the cooling counters ranges from 0 - 5 °C. By contrast hot meals are prepared on the day of sale and placed on hot display counters (Bain Marie) (65°C), which will inhibit the growth of pathogenic bacteria. All such food products have a one day shelf life.

C. Motivation for research

RTE foods have increased in popularity worldwide as they are convenient and nutritious. As RTE foods represent new potential sources of foodborne illness outbreaks worldwide, and as shown previously, foodborne disease outbreaks subsequently lead to economic losses and low worker productivity. These 'new foods' are a relatively recent phenomenon to the South African market and are also gaining popularity. Previous studies conducted internationally have shown that RTE foods may harbour bacterial foodborne pathogens, such as *B. cereus, C. perfringens, L. monocytogenes, Salmonella* and *S. aureus*, and consumption of these contaminated foods has led to foodborne disease outbreaks. Furthermore, several foodborne pathogens, such as *L. monocytogenes*, are opportunistic pathogens affecting immunocompromised individuals. A large portion of the South African population is immunocompromised due to age, pregnancy, chronic illnesses or HIV infection. Opportunistic foodborne pathogens infect these individuals more readily than immunocompetent people. Thus, as RTE delicatessen foods gain popularity within the South African context, their safety should be monitored.

Studies on the microbiology and safety of RTE retail delicatessen foods within South Africa are limited. A recent study evaluated the personal hygiene practices of the food handlers within the delicatessen, tested for the presence of airborne microorganisms

within the delicatessen and evaluated the efficacy of staff training pertaining to the safe handling of foods (van Tonder, 2004). There have, however, been no published studies concerning the presence of bacterial foodborne pathogens associated with these food types or the general microbiological content of RTE retail delicatessen foods in South Africa.

Table 1.1: Summary of potential foodborne pathogens associated with foodborne illness.

Foodborne pathogens	Examples	References
Bacteria	*Bacillus cereus, Campylobacter, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella, Shigella*	ICMSF, 1996; Tauxe, 2002; Mayrhofer *et al*., 2004.
Biotoxins in fish	Paralytic shellfish toxin	Tauxe, 2002; American Dietetic Association, 2003.
Marine dinoflagellates	Diarrhoeic shellfish poisoning	De Schrijver *et al.,* 2002; Tauxe, 2002.
Pathogenic fungi	Aflatoxins associated with corn, Patulin associated with apples	ICMSF, 1996; Cheraghali *et al*., 2005; Fandohan *et al*., 2005.
Parasites	*Cyclospora cayetanensis, Cryptosporidium parvum, Giardia lamblia*	Monge and Chinchilla 1996.
Self-inducing prions	Bovine spongiform encephalopathy (Mad cow disease)	Taylor and Woodgate, 1997.
Viruses	Norwalk-like viruses, Hepatitis A	Khan *et al*., 1994.

Table 1.2: Summary of predominant bacterial pathogens associated with various food commodities (Mossel *et al*., 1995; ICMSF, 1996; Jay *et al*., 2005).

Bacterial Pathogen	Gram type	Morphology	Oxygen requirements	Source in nature	Severity of illness	Associated foods
Bacillus cereus	Positive	Rod	Aerobic	Soils, water, cereals, dried foods, spices, dairy products, vegetables	Mild	Milk, meat, cereal, vegetables, cream pastries, soups, puddings
Campylobacter spp.	Negative	Rod	Microaerophilic	Soil, sewage, intestinal tracts of animals	Mild	Water, raw milk, meat
Clostridium botulinum	Positive	Rod	Anaerobic	Soil, intestinal tracts of fish, seafood	Severe	Low-acid canned foods, meats, fish, vegetables, seafood
Clostridium perfringens	Positive	Rod	Anaerobic	Soil, water, intestinal tracts of humans and animals	Mild	Meat, poultry, fish, vegetables, dehydrated food products such as gravies, sauces, soups and prepared foods stored at ambient temperatures
Escherichia coli O157:H7	Negative	Rod	Facultatively anaerobic	Intestinal tracts of animals and humans	Moderate	Undercooked ground beef, raw milk, unprocessed cheese and salads
Listeria monocytogenes	Positive	Rod	Facultatively anaerobic	Soil, water, environmental sources, birds, mammals, fish	Moderate	Raw chicken, milk, meat, assorted salads
Salmonella spp.	Negative	Rod	Facultatively anaerobic	Water, soil, birds, intestinal tracts of animals	Severe/ moderate	Meat, milk. eggs, poultry, vegetable products, meat salads, chocolate, dried coconut, baked goods
Shigella spp.	Negative	Rod	Facultatively anaerobic	Polluted water, intestinal tracts of humans	Severe/ moderate	Dairy products, raw vegetables, assorted salads
Staphylococcus aureus	Positive	Coccoid	Facultatively anaerobic	Hands, throats, nasal passages of humans	Mild	Cooked foods stored at ambient temperatures
Vibrio cholera	Negative	Rod	Facultatively anaerobic	Estuarine and marine waters	Moderate	Raw / improperly cooked fish, shellfish
Yersinia enterocolitica	Negative	Rod	Facultatively anaerobic	Soil, intestinal tracts of animals	Mild	Meat, fresh vegetables, milk

CHAPTER 2

MICROBIOLOGICAL SURVEY OF READY-TO-EAT FOODS, ASSOCIATED PREPARATION ENVIRONMENTS AND CLEANING TOOLS SAMPLED FROM RETAIL DELICATESSENS: A PILOT STUDY

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ABSTRACT

This pilot study evaluated the microbiological quality and safety of 77 ready-to-eat (RTE) food samples prepared and displayed by four retail delicatessens in Johannesburg, South Africa. Furthermore, the bacteriological status of associated food preparation surfaces, selected utensils, food handlers' hands, disposable plastic gloves and cleaning tools was determined. For all RTE food samples, aerobic bacteria, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* counts were quantified and *Salmonella* spp., *Listeria monocytogenes* and *Clostridium* spores were detected by standard methods. During three replicate surveys the aerobic plate counts APCs were consistently the highest bacterial counts for filled baguettes (n = 14), sliced processed meats (n = 34) and assorted salads (n = 15). Coliform counts (CC) were statistically significantly ($P < 0.05$) higher for filled baguettes and assorted salads compared to sliced processed meats. *E. coli* (ECC), *S. aureus* and *B. cereus* counts of *ca*. 1 Log CFU/g were found for the above-mentioned food categories. No *Clostridium* spores were detected but *Salmonella* spp. and *L. monocytogenes* were present in assorted salads, filled baguettes and sliced processed meats. By contrast, lower APCs and no foodborne pathogens were found in hot meal (n = 14) samples, indicating that adequate cooking temperatures were reached. Furthermore, preliminary incubation (PI) of samples at 15°C for 18 h showed that PI did not statistically significantly ($P > 0.05$) alter bacterial counts when results were compared to samples analysed on the day of collection. Similarly, the vacuum packaging of sliced processed meats prior to display did not statistically significantly ($P > 0.05$) alter bacterial counts compared to sliced processed meats displayed without further packaging. The highest APC counts for utensils (n = 57), food preparation surfaces (n = 35) and handlers' hands (n = 35) were obtained for the knives. Floor mops (n = 9), used within ready-to-eat food preparation environments were identified as potential sources of contamination by bacteria as APCs, CCs and ECCs were statistically significantly ($P < 0.05$) higher on floor mops compared to cleaning cloths (n = 13) and disposable plastic gloves (n = 8).

INTRODUCTION

Due to modern fast-paced lifestyles, consumer markets are showing an increased demand for a new class of processed foods generally referred to as ready-to-eat (RTE) products (Kaneko *et al*., 1999). RTE foods are defined as food items, which are eaten as sold, requiring no further cooking or processing by the consumer (Bone and Parker, 2005). This change in lifestyle has placed convenience store retailers in competition with restaurants and fast food outlets, prompting retailers to incorporate kitchens, called delicatessens, in-store to prepare RTE foods (Bone and Parker, 2005). Delicatessens provide sources of readily available and nutritious convenience meals that taste, smell and look like homemade foods to consumers (Novak and Juneja, 2002).

Foodborne pathogens, such as *Bacillus* (*B*.) *cereus*, *Clostridium* (*C*.) *perfringens*, *Escherichia* (*E*.) *coli*, *Listeria* (*L*.) *monocytogenes*, *Salmonella* (*Salm*.) spp. and *Staphylococcus* (*S*.) *aureus*, have reportedly been associated with foodborne illness from the consumption of sandwiches, assorted salads, sliced processed meats and cooked foods (Kaneko *et al*., 1999; Tessi *et al*., 2002; Novak and Juneja, 2002; Fang *et al*., 2003; Gombas, 2003; Montville and Schaffner, 2004). Several studies conducted on RTE foods have highlighted disparity in the microbiological quality and safety of these products (Nichols *et al*., 1999; Montville and Schaffner, 2004; Swanger and Rutherford, 2004; Vazgecer *et al*., 2004). The incidence and level of bacteria associated within different RTE foods often varies between and within countries.

For example, aerobic plate counts obtained for assorted salads prepared in the UK, USA, Spain and Japan ranged from 2.4 – 8 Log CFU/g (Christiansen and King, 1971; Fowler and Clark, 1976; Paradis and Stiles, 1978; Hagenmaier and Baker, 1998; Kaneko *et al*., 1999; Martinéz-Tomé, 2000; Montville and Schaffner, 2004). Bacterial foodborne pathogens isolated from RTE assorted salads included *B. cereus, L. monocytogenes, S. aureus* and *Campylobacter* spp. (Aureli, 2000; Meldrum and Ribeiro, 2003; Whyte, 2004). Sandwiches, like assorted salads, have shown variable results between different countries. For example, aerobic plate counts for sandwiches prepared in Taiwan and the UK varied from 2 to 8 Log CFU/g (Christiansen and

King, 1971; Fang *et al*., 2003). Foodborne pathogens isolated from sandwiches have included *B. cereus, E. coli* and *S. aureus* (Fang *et al.,* 2003). Studies on sliced processed meats have revealed similar findings. For example, aerobic plate counts generally ranged from 3.2 – 3.7 Log CFU/g (Gillespi *et al*., 2000; Montville and Schaffner, 2004) and foodborne pathogens such as *L. monocytogenes, B. cereus* and *C. perfringens* have been isolated from sliced processed meats (Christiansen and King, 1971; Fang *et al.,* 2003; Gombas *et al.,* 2003; Ayçiçek *et al*., 2004). Similarly, *E. coli* and *S. aureus* have been isolated from hot meals prepared in military hospitals (Ayçiçek *et al*., 2004).

The preparation of these food types requires handling within the delicatessen environment, potentially exposing these foods to contamination (Bone and Parker, 2005). As these RTE products are often consumed without further cooking, the presence of potential foodborne pathogens in them is a particular food safety concern. Contamination of RTE products can originate from many sources including (i) ingredients used to prepare RTE foods; (ii) poorly sanitised preparation surfaces (Montville and Schaffner, 2004; Normanno *et al.,* 2005); (iii) utensils used to prepare the food (Jay *et al*., 2005; Martínez-Tomé *et al*., 2000); (iv) the microorganisms on the hands and outer garments of food handlers (Jay *et al*., 2005); (v) contamination arising from the mouths, nasal cavities and gastrointestinal tracts of food handlers due to poor personal hygiene (Williams, 1963; Jay *et al*., 2005); (vi) disposable plastic gloves used by food handlers (Lynch *et al*., 2005); (vii) ice prepared in retail environments and subsequently used to cool RTE foods such as assorted salads (Nichols *et al*., 2000) and (viii) cleaning tools such as kitchen sponges and cleaning cloths (Bryan, 1998; Hilton and Austin, 2000; Kusumaningrum *et al.,* 2002; Kusumaningrum *et al*., 2003).

As a result of the ever increasing demand for RTE foods, retail delicatessens in South Africa prepare and display a wide variety of RTE foods, such as roast chickens, pies, pizzas, baked goods, filled baguettes, fruit or vegetable salads, hot meals, and sliced processed meats. RTE foods are usually prepared within a defined area of the general store. Strategies to prevent contamination of RTE foods in retail delicatessens include; cleaning and sanitation practices within the delicatessens, such as the use of general detergents in combination with sanitizers, which are used to clean and disinfect

preparation surfaces, utensils and hands. Hairnets, aprons, uniforms and sometimes disposable plastic gloves are worn to maintain personal hygiene standards and prevent cross-contamination between foods and food handlers.

A recent study in South Africa evaluated the personal hygiene practices of food handlers within delicatessens, tested for the presence of airborne microorganisms within delicatessens and evaluated the efficacy of staff training pertaining to the safe handling of foods (van Tonder, 2004). Although there is a growing demand for RTE foods, published data available in South Africa regarding the microbiological quality of these products and the bacterial load within the delicatessens preparation environment, on cleaning tools and disposable plastic gloves is limited. The present study was hence undertaken to determine the microbiological quality of a variety of RTE food products retailed in Johannesburg, South Africa. In addition, pre-incubation of RTE food samples under laboratory conditions (15°C for 18 h) was assessed as a potential method to enhance detection of foodborne pathogens. Furthermore preparation surfaces, utensils, handlers' hands, cleaning tools and disposable plastic gloves associated with the preparation of RTE foods were evaluated for their bacteriological status. Since this is the first investigation of its kind in South Africa these results provide basic information on the microbiological quality of these RTE foods and the bacteriological status of the preparation environment, cleaning tools and disposable plastic gloves.

MATERIALS AND METHODS

In a typical retail delicatessen RTE foods are prepared, displayed and sold to the public on a daily basis. Table 2.1 describes the in-house preparation procedures for RTE foods and cleaning practices in the retail delicatessen. Figure 2.1 illustrates the general floor plan of a typical retail delicatessen. Three independent replicate surveys of retail delicatessens at four branches (Fig 2.2) of a leading retail chain were conducted in Johannesburg, South Africa over a 6-month period, from May to October 2005. During each survey, RTE foods (Fig. 2.3 and 2.4) and swabs of the associated preparation environment and cleaning tools (Table 2.1 and Fig. 2.4) were aseptically collected at weekly intervals.

a) Sample collection

RTE food samples consisted of filled baguettes, assorted salads, hot meals and sliced processed meats (vacuum packaged or sliced upon consumer request), all of which were prepared by food handlers at each of four retail delicatessens (Fig. 2.2 and 2.4). Factory-processed assorted salads and sliced processed meats also sold by these delicatessens were excluded from this study. All food samples were purchased as sold and transported to the laboratory on ice. All samples were analysed on the day of collection and again after preliminary incubation (PI) at 15°C for 18h. A previous study on yeast samples taken along the processing line showed that PI enhanced the detection of low levels of *E. coli*, coliforms, *Enterococcus* and aerobic bacteria (O'Brien *et al*., 2004). This methodology was applied here to evaluate whether PI would enhance detection of *E. coli* and other potential bacterial pathogens in RTE food samples.

Evaluation of the associated preparation environment consisted of sampling preparation utensils, preparation surfaces and handlers' hands after cleaning. Surfaces integral to the preparation of each category of RTE foods were swabbed using sterile swabs (Merck, RSA). Each sterile swab was moistened in Neutralising Buffer (NB) (Difco, New Jersey, USA) and the tip moved over the surface in two directions at right angles to each other for *ca*. 15 seconds whilst rotating the swab between thumb and finger (Bell *et al*., 2005). The swabs were placed on ice during transport and analysed on the same day.

Cleaning tools including floor mops (*ca*. 15g) used to clean floors of the delicatessens in which RTE foods are prepared, cleaning cloths (*ca*. 15g) used to clean the preparation surfaces and disposable plastic gloves (1 pair) used to handle RTE foods, were aseptically collected using sterile scissors and forceps at each of the preparation areas and placed into sterile Whirl Pak bags (Nasco, USA) (Fig. 2.4). All samples were transported to the laboratory on ice and analysed on the same day.

b) Sample processing and analysis

Samples were prepared for analysis according to sample type (RTE food, swabs of associated preparation surfaces, preparation environment cleaning tools or disposable plastic gloves) (Fig. 2.4). For RTE foods, 20g samples of each category were homogenized for two minutes in 180 ml sterile peptone saline solution (0.1% peptone and 0.85% NaCl) using a Colworth 400 Stomacher (Seward Medical, London, UK). For assessment of the preparation utensils, food preparation surfaces and handlers' hands, each swab was placed in 10ml NB (Difco) and vortexed for 30 seconds. For cleaning tool samples, 1g was aseptically placed in 9ml NB (Difco) with 20g sterile glass beads and shaken vigorously by hand for 10 minutes to dislodge attached bacterial cells (Lindsay *et al*., 2002). The solution was allowed to stand for 20 minutes to allow cell recovery from injury (Lindsay *et al*., 2002).

c) Bacterial counts

Following sample homogenization, RTE foods were tested for aerobic bacteria (APC) (Bell *et al*., 2005), *E. coli* (ECC), coliform bacteria (CC) (International Standards Organisation 4832, 1991), *S. aureus* (SAC) (International Standards Organisation 6888-1, 1992) and *B. cereus* counts (BCC) (International Standards Organisation 7932, 1993) (Table 2.2) whilst cleaning tool samples and swabs were only tested for aerobic bacteria, *E. coli* and coliform bacteria (Table 2.2). Hand swabs and disposable plastic gloves were also tested for *S. aureus* counts. Serial ten fold dilutions in sterile peptone saline solution were prepared and plated in duplicate using standard pour- and spread-plate techniques, onto different media (Table 2.2 and Fig. 2.5). Plates were inverted and incubated aerobically. Plates containing between 30 and 300 colony forming units (CFU) (or the highest number if below 30) were counted.

For the presumptive *B. cereus* counts, pink-purple colonies with red centers on *Bacillus cereus* Agar (Scharlau, Barcelona, Spain) were streaked onto Nutrient Agar (Oxoid). Colonies which were Gram-positive, catalase positive rod-shaped bacteria were considered *B. cereus* (International Standards Organisation 7932, 1993). For the *E. coli* and coliform count, purple colonies were counted as *E. coli* and green colonies were counted as coliforms on Rapid' *E. coli* 2 agar (Bio-Rad, Marnes-La-Coquette)

(International Standards Organisation 4832, 1991). For the presumptive *S. aureus* count, only black or grey colonies on Baird Parker agar medium (Scharlau, Barcelona, Spain) surrounded by a clear zone were streaked onto Nutrient Agar (Oxoid). Colonies, which were Gram-positive, catalase positive, coccoid-shaped bacteria were considered presumptive *S. aureus*. The presumptive *S. aureus* isolates were further streaked onto DNAse Agar (Scharlau). The latter plates were incubated at 35°C for 24 h and flooded with 1 ml hydrochloric acid (1M) and colonies showing a clear zone were considered *S. aureus* (International Standards Organisation 6888-1, 1992).

d) *Clostridium* spores, *L. monocytogenes* and *Salmonella* spp. detections

For *Clostridium* spore detection, the food samples were prepared as described above and the 10^{-1} dilution was heated in a water bath to 82-85°C for 60 seconds. Thirteen ml of the heated homogenate was transferred to 12.5ml Reinforced Clostridial Medium (Scharlau) and incubated at 30°C for 5 days. The contents were examined daily for black discolouration of the medium (South African Bureau of Standards 761, 1975) (Table 2.2).

For *L. monocytogenes* detection, pre-enrichment was carried out by homogenizing 25g of the sample in 225 ml Fraser ½ Broth (Bio-Rad, Marnes-La-Coquette, France) for 2 minutes in a Colworth 400 Stomacher (Seward Medical) followed by incubation for 24 h at 30°C. For secondary enrichment 0.1ml of the incubated Fraser ½ Broth was inoculated into 10ml Fraser 1 Broth (Bio-Rad) and incubated at 37°C for 48 h. A loopful of blackened Fraser 1 broth was streaked onto one Rapid' *L mono* agar (Bio-Rad) plate and incubated at 37°C for 48h. Blue colonies without yellow halos were streaked onto Nutrient Agar (Oxoid). Isolates, which were Gram-positive, catalase positive, rod-shaped bacteria, were considered *L. monocytogenes* (Scotter *et al.*, 2001) (Table 2.2).

For *Salmonella* spp. pre-enrichment was carried out by homogenizing 25g of the sample in 225 ml Buffered Peptone Water (BPW) (Oxoid, Basingstoke, UK) for 2 minutes in a Colworth 400 stomacher and incubated for 18 hours at 37°C. Aliquots of the enriched culture in BPW were inoculated into two selective enrichment broths. Firstly 1 ml of BPW enriched broth was inoculated into 10ml Müller-Kauffmann

Medium (MKM) (Scharlau, Barcelona, Spain) plus Brilliant Green and Novobiocin Selective Supplement (1vial / 500ml) (Scharlau) and 200 µl Gram's Iodine and incubated for 24 h at 30°C. Secondly 0.1 ml of the enriched broth sample was inoculated into Rappaport-Vassiliadis Broth (RVB) (Scharlau) and incubated for 24 h at 41.5°C. A loopful of the MKM and RVB were each streaked onto Xylose-Lysine Deoxycholate (XLD) Agar (Scharlau) and Brilliant Green Modified Agar (BGAM) (Scharlau) (Fig. 2.5 and Table 2.2) (South African Bureau of Standards 6579:1993, 1993). Red colonies with black centers on XLD and pink colonies on BGAM were considered presumptive *Salmonella* spp. (Fig. 2.5 and Table 2.2) (South African Bureau of Standards 6579:1993, 1993). The presumptive *Salmonella* spp. were further streaked onto Nutrient Agar (Oxoid). Colonies which were Gram-negative, oxidase negative rods and showed a fermentative reaction for the Oxidative-Fermentative test were considered *Salmonella* spp.

e) Statistical analysis

Counts of RTE foods, preparation utensils, associated food preparation surfaces, handlers' hands, cleaning tools and disposable plastic gloves over the three replicate surveys were meaned and standard deviations between samples were calculated. In addition colony count data was statistically analysed using analysis of variance (ANOVA) at the 95% confidence level (STATGRAPHICS 7.0, Manugistics Inc. and Statistical Graphics Corporation, USA) programme.

RESULTS

Means, standard deviations and statistically significant differences ($P < 0.05$) of aerobic bacterial, coliform, *E. coli*, *S. aureus* and *B. cereus* counts are shown in Table 2.3. Detection of *L. monocytogenes* and *Salmonella* spp. are shown in Table 2.4. Results of before and after PI of delicatessen RTE food samples including filled baguettes (n=14), assorted salads (n=15), sliced processed meats (n=34) and hot meals (n=14) are shown in Fig. 2.8. Means, standard deviations and statistically significant differences for aerobic bacteria, coliform and *E. coli* counts for the preparation utensils (n=57), associated food preparation surfaces (n=35) and handlers' hands (n=35) (Fig. 2.6), cleaning tools (n=22) and disposable plastic gloves (n=8) are shown

in Fig. 2.7. In addition, counts obtained for all sample categories showed no statistically significant difference ($P < 0.05$) between the four retail delicatessens included in this study.

a) RTE food samples

The highest bacterial numbers on RTE foods were consistently the APCs (*ca*. 5.5 Log CFU/g), followed by CCs, ECCs, SACs and BCCs in decreasing order (Table 2.3). Similarly, the highest incidence of pathogens were *Salmonella* spp. followed by *L. monocytogenes,* whilst *Clostridium* spores were absent (Table 2.4).

There were no statistically significant differences ($P > 0.05$) in bacterial counts obtained between sliced processed meats, which were sliced and displayed in a fridge, or those which were vacuum packaged (data not shown).

Statistically significantly ($P<0.05$) lower APCs were obtained for hot meals (3.5 Log CFU/g) compared to filled baguettes, assorted salads and sliced processed meats (*ca*. 5.5 Log CFU/g) (Table 2.3). The CCs for filled baguettes and assorted salads (*ca*. 2.6 Log CFU/g) were statistically significantly ($P<0.05$) higher than sliced processed meats (1.4 Log CFU/g), whilst CCs were below the lower detection limit (1 Log CFU/g) for hot meals (Table 2.3). SACs were statistically significantly ($P<0.05$) higher for filled baguettes (1.4 Log CFU/g) compared to the other RTE food categories (*ca*. 1.1 Log CFU/g) (Table 2.3). In addition ECCs and BCCs were low (*ca*. 1.1 Log CFU/g) for filled baguettes, assorted salads and sliced processed meats; and below the lower detection limit (1 Log CFU/g) for hot meals (Table 2.3).

The incidence of *L. monocytogenes* was similar in filled baguettes (13%) and sliced processed meats (11%) but lower in assorted salads (5%) (Table 2.4). By contrast the incidence of *Salmonella* spp. in sliced processed meats (21%) displayed in a fridge after slicing was double that of filled baguettes (7%) and assorted salads (14%). Hot meals harboured neither *L. monocytogenes* nor *Salmonella* spp. (Table 2.4). *Clostridium* spores were absent from all RTE food categories.

b) Pre-incubation (PI) of RTE food samples

For each of the food categories tested, no statistically significant differences ($P > 0.05$) were obtained between bacterial counts from samples analysed on the sampling day and corresponding samples pre- incubated at 15°C for 18 h (Fig. 2.8). In addition PI decreased the detection of *L. monocytogenes* and *Salmonella* spp. below the lower detection limit (absent/25g) (Table 2.4).

c) Preparation environment

No statistically significant differences in counts ($P>0.05$) were obtained between food contact surfaces associated with filled baguette, salad, hot meal and sliced processed meats. However, preparation knives harboured statistically significantly ($P<0.05$) higher APCs (4.8 Log CFU/cm^2) compared to preparation spoons, associated food preparation surfaces and handlers' hands (2 Log CFU/cm^2) (Fig. 2.6). CCs were only associated with the preparation utensils (1.5 Log CFU/cm^2) whilst ECCs were below the lower detection limit (1 Log CFU/cm^2) on all food contact surfaces. In addition SACs were below the lower detection limit (1 Log CFU/cm^2) for handlers' hands.

d) Cleaning tools and disposable plastic gloves

In general, APCs for floor mops (6.4 Log CFU/g) were statistically significantly higher ($P < 0.05$) than cleaning cloths (4 Log CFU/g) and disposable plastic gloves (2.2 Log CFU/g) (Fig. 2.7). Similarly the floor mops statistically had significantly ($P<0.05$) the higher CCs (4.4 Log CFU/g) and ECCs (2.1 Log CFU/g). Disposable plastic gloves had the lowest corresponding CC and ECC (1 Log CFU/g) (Fig. 2.7) and low SACs (1.5 Log CFU/g) (Fig. 2.7).

DISCUSSION

a) RTE food samples

Previous studies have shown that the APCs for sandwiches may vary greatly between countries, for example APCs between sandwiches prepared in Taiwan and the UK varied between 2 – 8 Log CFU/g (Christiansen and King, 1971). Results from the present study showed that APCs obtained from filled baguettes were on the higher side of this range (5.7 Log CFU/g). *B. cereus*, *E. coli* and *S. aureus* have also previously been isolated from sandwiches (Fang *et al*., 2003) and these findings are similar to the present study.

B. cereus, *S. aureus* and *L. monocytogenes* have been reported elsewhere in assorted salads (Meldrum and Ribeiro, 2003, Whyte, 2004). The coliform counts for assorted salads were statistically significantly higher ($P < 0.05$) than those obtained for hot meals and sliced processed meats. This may be attributed to coliforms occurring naturally on fresh produce (De Roever, 1999).

In contrast to results obtained in the present study, Fang *et al*., (2003) reported *C. perfringens* from sliced processed meats in Japan and Meldrum *et al*., (2005) reported low incidences of *C. perfringens* in assorted salads from the UK. Vacuum packaging reduces the oxygen content of the atmosphere around the sliced processed meats by evacuating air from gas-impermeable pouches, followed by sealing. This reduces the air pressure from 1 bar to 0.3-0.4 bar, therefore removing some oxygen. Upon storage of the vacuum packaged sliced processed meat the carbon dioxide increases in part from microbial respiration (Jay *et al*., 2005). A previous study of the effect vacuum packaging has on the microbial populations of Greek taverna sausage revealed that *Lactobacillus* predominated after 30 days storage (Samelis and Georgiadou, 2000). By contrast, in the present study the vacuum packaged processed sliced meats were only stored for a short period of time. This may explain the absence of statistically significant different bacterial counts between non-vacuum and vacuum packaged sliced processed meats sampled.

Previous studies in the UK have reported the absence of potential foodborne pathogens in hot meals (Meldrum *et al*., 2005). These findings concurred with the results of the present study. The APCs for hot meals (3.5 Log CFU/g) were statistically significantly ($P<0.05$) lower than APCs for the other RTE food categories (*ca*. 5.5 Log CFU/g) included in this study and the potential foodborne pathogens were below the lower detection limits of 1 Log CFU/g. These results were expected, as any potential foodborne pathogens present in the hot meals would be inactivated during cooking (Jay *et al*., 2005). These results suggest that hygiene practices and holding temperatures were adequate to prevent cross contamination of the hot meals and to prevent the multiplication of potential foodborne pathogens.

Results from this pilot study showed that filled baguettes and assorted salads contained the highest bacterial counts and incidences of potential foodborne pathogens. Therefore further investigation should focus on these two RTE food types.

b) Pre-incubation (PI) of RTE food samples

PI appeared to decrease the incidence of *Salmonella* spp. and *L. monocytogenes* in the present study. The preliminary incubation (PI) technique has previously been shown to be a useful technique for increasing the detection of low-level bacterial contamination of commercial yeast products by aerobic, coliform, *Enterococcus* and *E. coli* bacteria (O'Brien *et al*., 2004). However, the implementation of the PI method in this study did not statistically significantly ($P>0.05$) influence bacterial counts and rather resulted in less detection of potential foodborne pathogens. In addition, these results confirm that the 1 day shelf life of these RTE foods is suitable.

c) Preparation environment

Previous studies have implicated food preparation surfaces in the transfer of foodborne pathogens to food commodities. For example, structural surfaces and utensils have been implicated in the harbourage of *Listeria* spp. at concentrations of $10^3 – 10^4$ CFU per object, contributing to the occurrence of salmonellosis (Beumer *et al*., 1997; Bryan, 1998; Normanno *et al*., 2005). When the transfer of *Campylobacter jejuni* and *Salmonella enterica* from stainless steel contact surfaces to RTE foods such

as lettuce was investigated, it was found that high numbers of bacteria might be transferred from the food contact surface to the lettuce within 1-2 h after surface contamination (Moore *et al.,* 2003).

As specified by Public Health Regulations, a surface shall be cleaned and washed before food comes into contact with it so that contamination is prevented. Such surfaces will not contain more than 2 Log viable CFU/cm^2 (Republic of South Africa, Regulation R918, 1999). Of the three food contact surface types (knives, spoons and preparation surfaces) associated with delicatessen RTE food preparation, knives and spoons did not comply with these Public Health Regulation standards. By contrast, the preparation surfaces harboured the lowest number of bacteria.

It has been reported elsewhere that plastic surfaces, such as cutting boards, have higher microbial counts compared to metal surfaces, such as food preparation surfaces (Montville and Schaffner, 2004). By contrast, results from the present study showed no statistically significant differences ($P>0.05$) between the bacterial counts associated with either plastic chopping boards (filled baguette and salad preparation) or stainless steel surfaces (hot meal and sliced processed meat preparation).

Food handlers have the potential to act as vectors for the transmission to or the contamination of food products with foodborne pathogens (Martínez-Tomé *et al*., 2000). Hygienic food preparation and education of food preparation personnel is crucial for the prevention of microbiological foodborne disease (Martínez-Tomé *et al*., 2000). Results from the current study indicated that the food handlers preparing RTE foods in retail delicatessens followed hygienic food preparation as low APCs (1.4 Log CFU/cm^2) were determined and CCs, ECCs or SACs counts were below the lower detection limit (1 Log CFU/cm^2).

d) Cleaning tools and disposable plastic gloves

Several studies have highlighted cleaning tools, such as kitchen sponges, as well as disposable plastic gloves, as sources of contamination, and implicated them in outbreaks of gastroenteritis (Mitakakis *et al*., 2004; Rayner *et al.,* 2004). Previous reports have shown that bacteria may survive for hours or days on sponges and

cleaning cloths after initial contact with bacteria (Scott and Bloomfield, 1990; Kusumaningrum *et al*., 2002). It has previously been reported that RTE foods may become contaminated with foodborne pathogens such as *E. coli, Staphylococcus* spp., *Salmonella* spp. and *Campylobacter* spp. through contact with cleaning tools, such as kitchen sponges and disposable plastic gloves used by food handlers during preparation (Kusumaningrum *et al*., 2002; Hilton and Austin, 2000). Thus the floor mops, cleaning cloths and disposable plastic gloves examined in the present study may act as potential vehicles for pathogen transmission in retail delicatessens during the preparation of RTE foods. However, few studies have concentrated on the role that cleaning tools and disposable plastic gloves may play in RTE food contamination in retail delicatessens.

In general, aerobic plate counts for floor mops were statistically significantly higher ($P < 0.05$) than cleaning cloths and disposable plastic gloves (Fig. 2.7). The floor mops consistently had the highest coliform and *E. coli* counts whilst disposable plastic gloves had the lowest corresponding counts (Fig. 2.7). Coliform bacteria and *E. coli* have long been used as indicators of food processing environment hygiene and food safety with respect to foodborne bacterial pathogens (Jay *et al*., 2005). Their presence on cleaning tools used during the preparation of RTE foods in retail delicatessens suggests that improved cleaning regimes may be required (Jay *et al*., 2005). Furthermore, their detection suggests that other foodborne pathogens may be present. It has previously been reported that RTE foods may become contaminated with foodborne pathogens such as *E. coli, Staphylococcus* spp., *Salmonella* spp. and *Campylobacter* spp. through contact with cleaning tools, such as kitchen sponges and disposable plastic gloves used by food handlers during preparation (Hilton and Austin, 2000; Kusumaningrum *et al*., 2002). Thus the floor mops, cleaning cloths and disposable plastic gloves should be further examined for the presence of potential foodborne pathogens. Furthermore, the low APC, CC, ECC, and SACs associated with the disposable plastic gloves suggests good glove practices were used by the food handlers.

CONCLUSION

This study confirmed that RTE foods prepared in selected South African retail delicatessens contained several potential bacterial foodborne pathogens, with the exception of *Clostridium* spores. Filled baguettes and assorted salads showed the highest incidence of potential foodborne pathogens, whilst hot meals contained no bacterial pathogens. In addition, the presence of coliforms on display areas, preparation surfaces and utensils may indicate inadequate hygiene practices and potential reservoirs for bacterial foodborne pathogen transmission to RTE foods. Bacterial counts confirmed the association of bacteria with cleaning tools and disposable plastic gloves associated with the preparation areas of RTE foods in retail delicatessens, suggesting that cleaning tools may provide additional reservoirs for the bacterial contamination of RTE foods.

This pilot study allowed the parameters for the remainder of the study to be set. Filled baguettes and assorted salads have been identified as risk products for consumers; therefore further replicate surveys should be performed on these two RTE foods. Furthermore, display areas, preparation surfaces and utensils should be surveyed in order to assess which food contact surface acts as the greatest reservoir for contamination of filled baguettes and assorted salads. Results from such a study are described in Chapter 3. In order to quantify the level of bacterial contamination of cleaning tools and disposable plastic gloves, replicate surveys focusing on the incidence of foodborne pathogen should be performed on cleaning tools and disposable plastic gloves. Results from such a study are described in Chapter 4.

Table 2.1. Description of RTE foods, environmental swabs and cleaning tools

SAMPLE CATEGORY	NAME	DESCRIPTION
Ready-to-eat foods	Filled Baguette	Baguette (made by in-store bakery) cut longitudinally, filled with mayonnaise, diced lettuce, sliced tomato and in-store sliced processed meat or in-store rotisserie chicken.
	Assorted salads	a) Fruit and vegetables sliced and diced by in-store food handler. b) Various in-store sliced processed meats mixed together with mayonnaise.
	Sliced processed meats	Sliced processed meats sliced in-store and either displayed in a fridge or vacuum packaged.
	Hot meals	Rice, pap* and lasagna prepared from the raw materials in-store and then displayed in a Bain Marie.
Environmental swabs	Knives	Different knives used for each food category (24 cm^2).
	Spoons	Different spoons used for each food category (58 cm^2).
	Preparation surfaces	Plastic cutting boards used for baguette and salad preparation (25 cm^2). Stainless steel surfaces used for hot meal and sliced processed meat preparation (25 cm^2).
	Handlers' hands	All five fingers and the palm of both hands were swabbed (92 cm^2).
Cleaning tools	Floor mops	Dedicated floor mops used to clean the floors of the retail delicatessen, stored in dilute sanitizer when not in use.
	Cleaning cloths	Cleaning cloths dedicated to each RTE food preparation area, stored in dilute sanitizer when not in use.
	Disposable plastic gloves	Used during the preparation of sliced meats and occasionally for the other food categories.

* Stiff maize porridge

Table 2.2: Culture media, times, temperatures and plating techniques used in the microbiological survey of RTE foods, associated preparation surfaces and cleaning tools sampled from four retail delicatessens.

Analysis	Incubation		Methodology	Sample type analysed	Growth Medium
	Time (h)	Temperature (°C)			
Aerobic Plate count (APC)	48	30	Pour plate	RTE foods, swabs, cleaning tools	Plate Count Agar (Scharlau, Barcelona, Spain)
Bacillus cereus count (BCC)	24	30	Spread plate	RTE foods	*Bacillus cereus* Agar plus Egg's Yolk Sterile Emulsion (10% v/v) plus Polimixin B Sulfate Selective Supplement (1 vial/500ml) (Scharlau, Barcelona, Spain). Pink-purple colonies selected and Gram stained.
Coliform count (CC) and *Escherichia coli* count (ECC)	24	37	Pour plate	RTE foods, swabs, cleaning tools	Rapid' *E. coli* 2 agar (Bio-Rad, Marnes-La-Coquette, France). Green colonies = Coliforms, Purple colonies =. *E coli*.
Staphylococcus aureus count (SAC)	48	37	Spread plate	RTE foods, handlers' hands, disposable plastic gloves	Baird-Parker agar medium plus Egg's Yolk Tellurite Sterile Emulsion (0,5% v/v) (Scharlau, Spain). Black colonies with a halo selected and Gram stained.
Clostridium spores detection	5 (days)	30	-	RTE foods	Reinforced Clostridial Medium (Scharlau, Barcelona, Spain)
Listeria monocytogenes detection	24	30	Pre-enrichment	RTE foods	Fraser ½ (Bio-Rad, France)
	48	37	Enrichment	RTE foods	Fraser 1 (Bio-Rad, France)
	24	37	Streak plate	RTE foods	Rapid' *L. mono* agar (Bio-Rad, France), Blue colonies selected and Gram stained.
Salmonella spp. detection	18	37	Pre-enrichment	RTE foods	Buffered Peptone Water (Scharlau, Spain)
	24	37	Enrichment	RTE foods	Müller Kauffmann Medium plus Brilliant Green and Novobiocin Selective Supplement (1 vial/ 500ml) plus 200 µl Grams Iodine/ 10ml (Scharlau, Spain)
	24	41.5	Enrichment	RTE foods	Rappaport-Vassiliadis Broth (Scharlau, Spain)
	24	37	Streak plate	RTE foods	Brilliant Green Modified Agar (Scharlau, Spain). Pink colonies selected and Gram-stained
	24	37	Streak plate	RTE foods	Xylose Lysine Deoxycholate Agar Scharlau, Spain). Red colonies with black centres were selected and Gram stained

Table 2.3: Mean bacterial counts obtained for RTE foods (n = 77) sampled from four retail delicatessens.

RTE food category	Bacterial count (Log CFU/g) RTE food sample				
	Aerobic Plate count	**Coliform count**	***E. coli* count**	***S. aureus* count**	***B. cereus* count**
Filled baguettes (n = 14)	5.7 ± 1.4[a] (4.2 – 6.8)[b]	2.7***** ± 0.2 (2.5 – 2.9)	1.1 ± 0.1 (1.0 – 1.2)	1.4***** ± 0.3 (1.1 – 1.7)	1.2 ± 0.2 (1.0 – 1.3)
Assorted salads (n = 15)	5.1 ± 1.5 (3.4 – 6.7)	2.5***** ± 1.1 (1.2 – 3.8)	1.1± 0.2 (1.0 – 1.4)	1.0 ± 0.1 (1.0 – 1.1)	1.1 ± 0.1 (1.0 –1.3)
Hot meals (n = 14)	3.5***** ± 3.4 (1.0 – 2.62)	<1.0 -	<1.0 -	<1.0 -	<1.0 -
Sliced processed meats (n=34)	5.5 ± 0.5 (4.1 – 6.4)	1.4 ± 0.3 (1.0 – 1.6)	1.0 ± 0.1 (1.0 – 1.1)	1.1 ± 0.2 (1.0 – 1.3)	1.1 ± 0.1 (1.0 – 1.2)

[a] Mean ± standard deviation

[b] Range

* Denotes a statistically significant difference (P<0.05)

Table 2.4: Incidences of *Listeria monocytogenes* and *Salmonella* spp. in RTE foods (n=77) sampled from four retail delicatessens before and after preliminary incubation (PI).

RTE food category	Detection (present or absent / 25g of RTE food sample) % positive samples			
	Listeria monocytogenes		*Salmonella* spp.	
	Non-PI[a]	PI[b]	Non-PI	PI
Filled baguettes (n = 14)	13[c]	7	7	0
Assorted salads (n = 15)	5	0	14	0
Hot meals (n = 14)	0	0	0	0
Sliced processed meat (open) (n = 17)	11	5	21	0

a Samples analysed on the day of collection

b Corresponding samples analysed after preliminary incubation at 15°C for 18 h

c Percentage of positives detected / 25g of RTE food samples

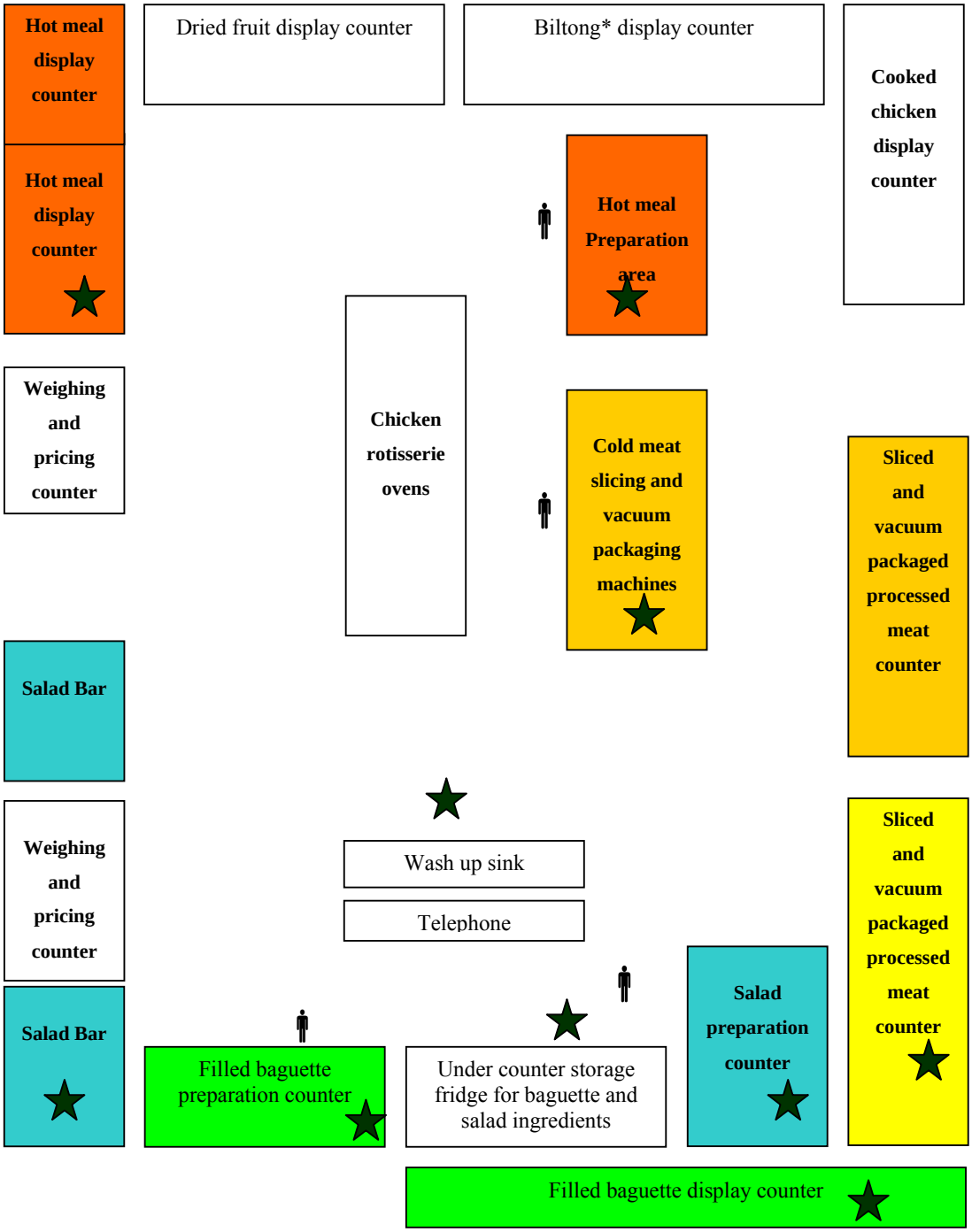


Figure 2.1: Schematic diagram representing the layout of a typical retail delicatessen. Indicates the location of the food handlers, ★ depicts the preparation surfaces and display areas sampled.

*Dried and spiced meat product

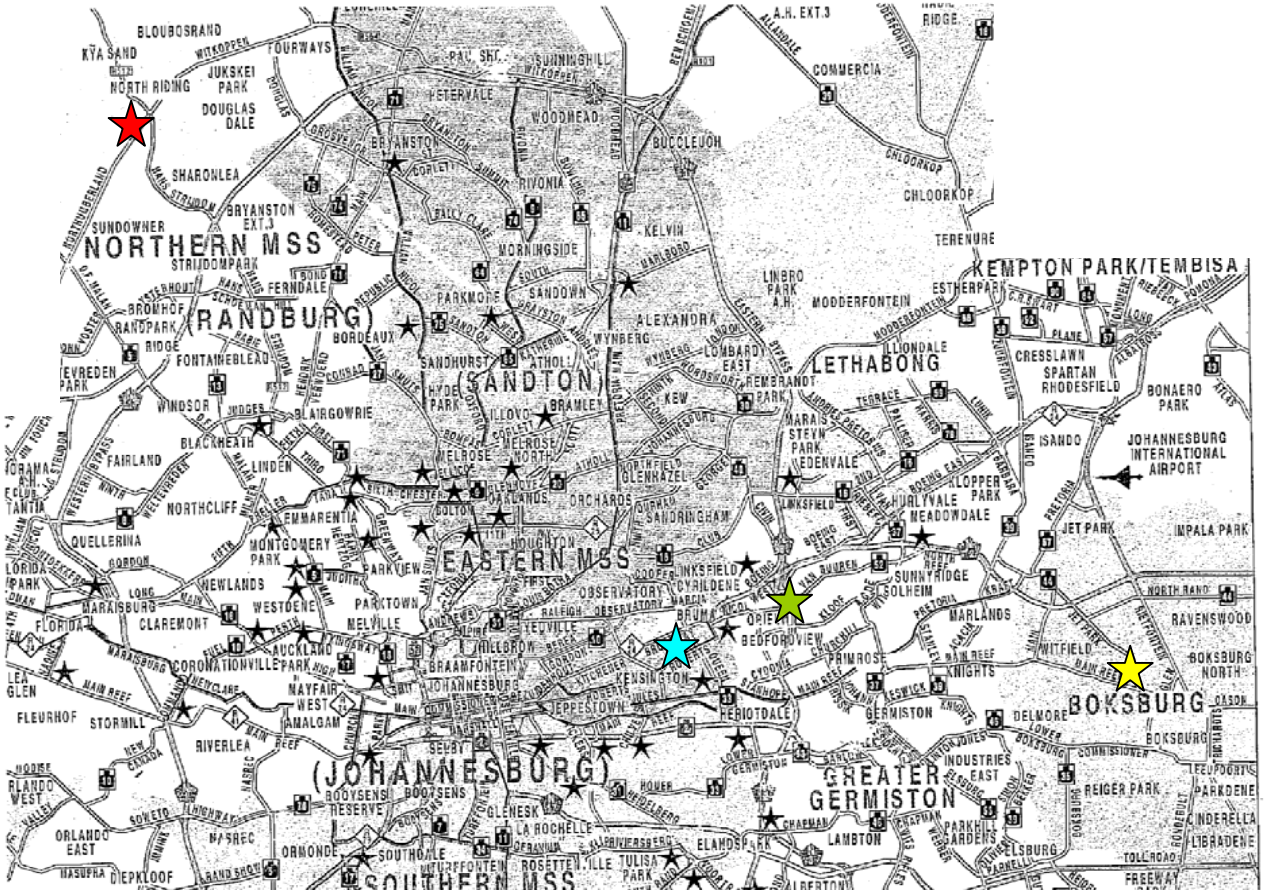


Figure 2.2: Map of Johannesburg indicating the location of four branches of a South African retail chain at which RTE foods are prepared on site and which have delicatessens where samples were taken. Boksburg ★ Bedfordview ★ Kensington ★ and Northgate ★. (Adapted from Map studios).

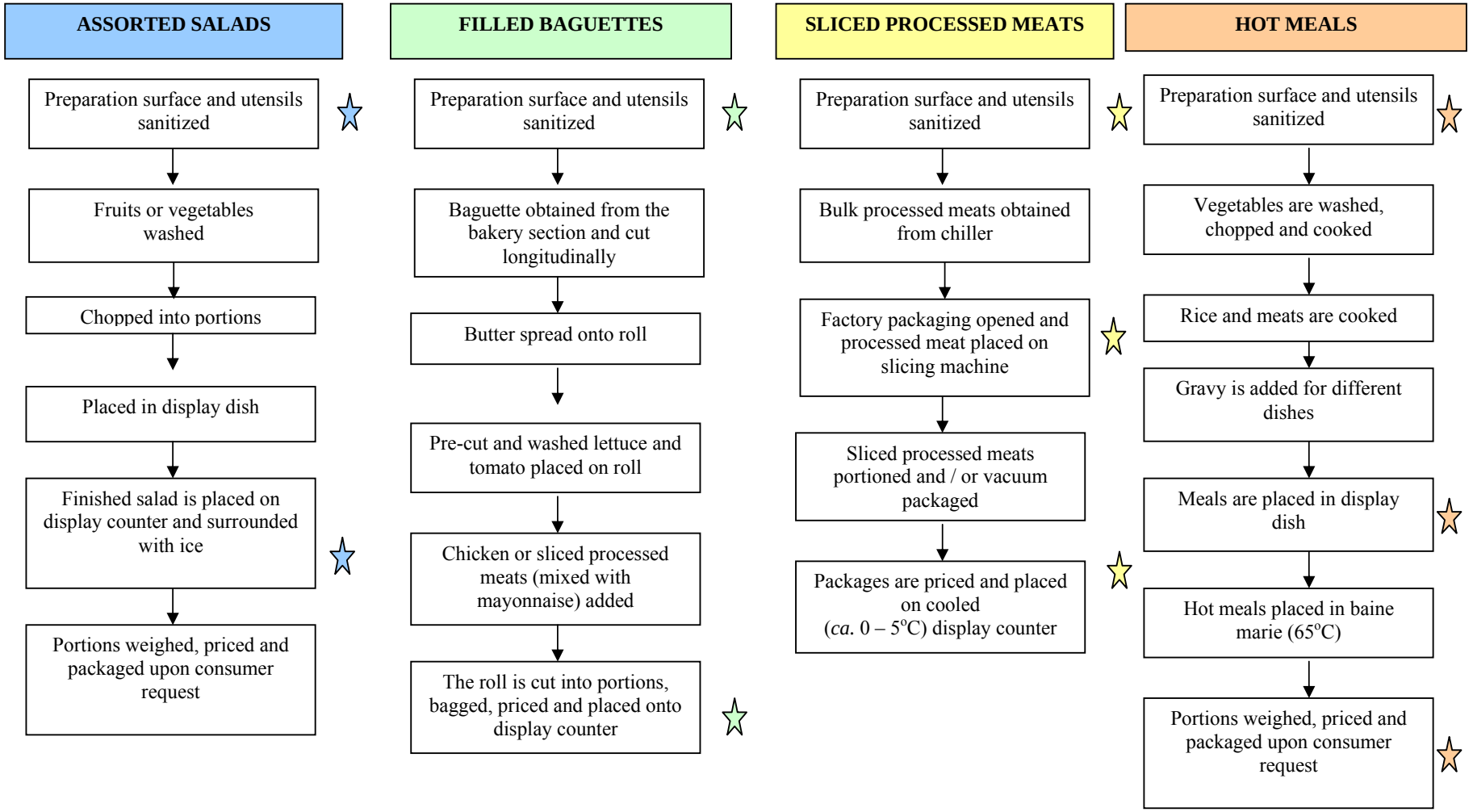


Figure 2.3: Flow diagrams illustrating the preparation process for selected retail delicatessen assorted salads, filled baguettes, sliced processed meats and hot meals. Stars represent points within the preparation process where samples were taken.

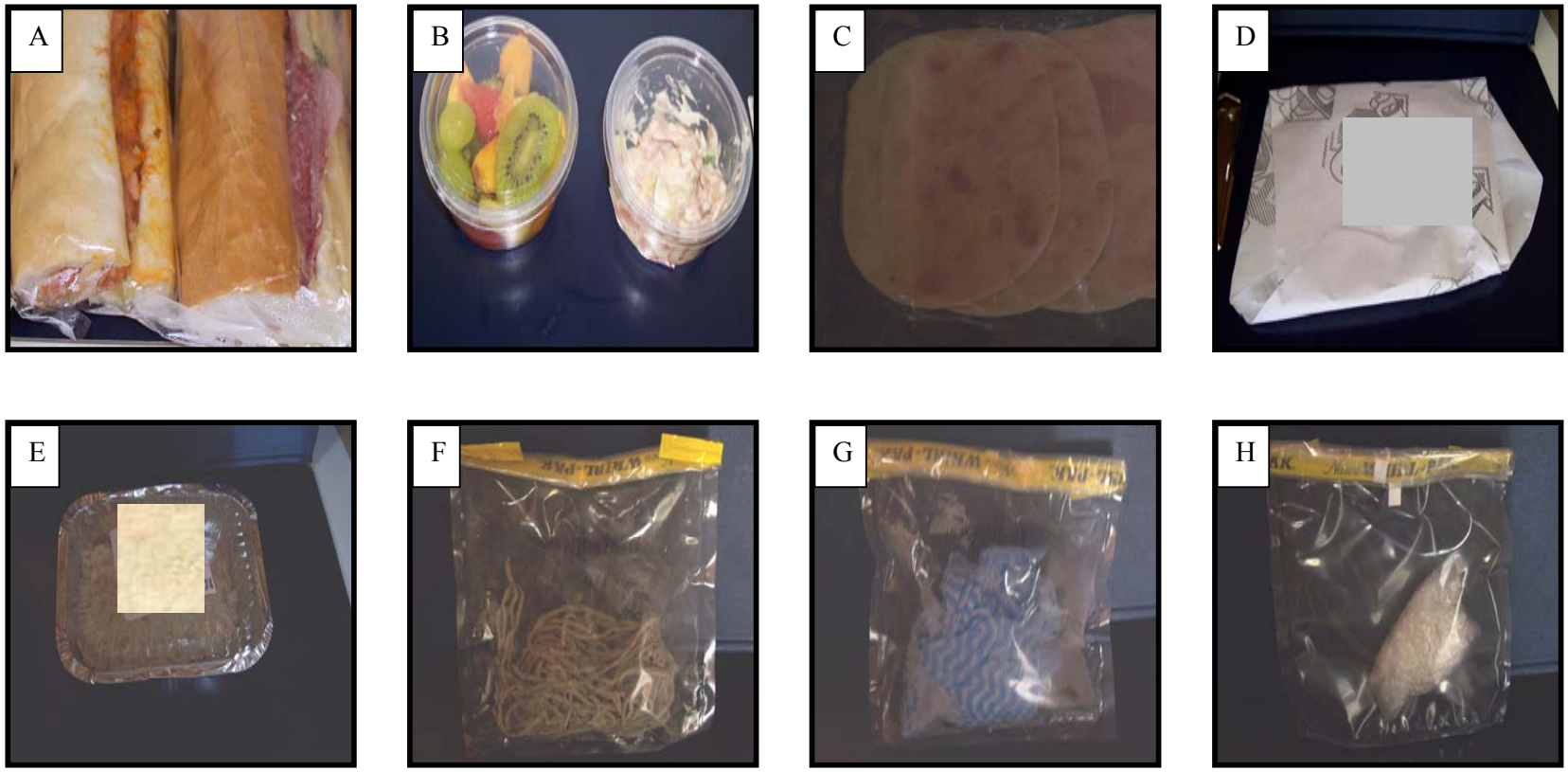


Figure 2.4. Examples of RTE food products such as filled baguettes (A); assorted salads (B); vacuum packaged sliced processed meats (C); hand wrapped sliced processed meats (D) and hot meals (E). In addition examples of cleaning tools such as floor mops (F), cleaning cloths (G) and disposable plastic gloves (H) sampled from four retail delicatessens.

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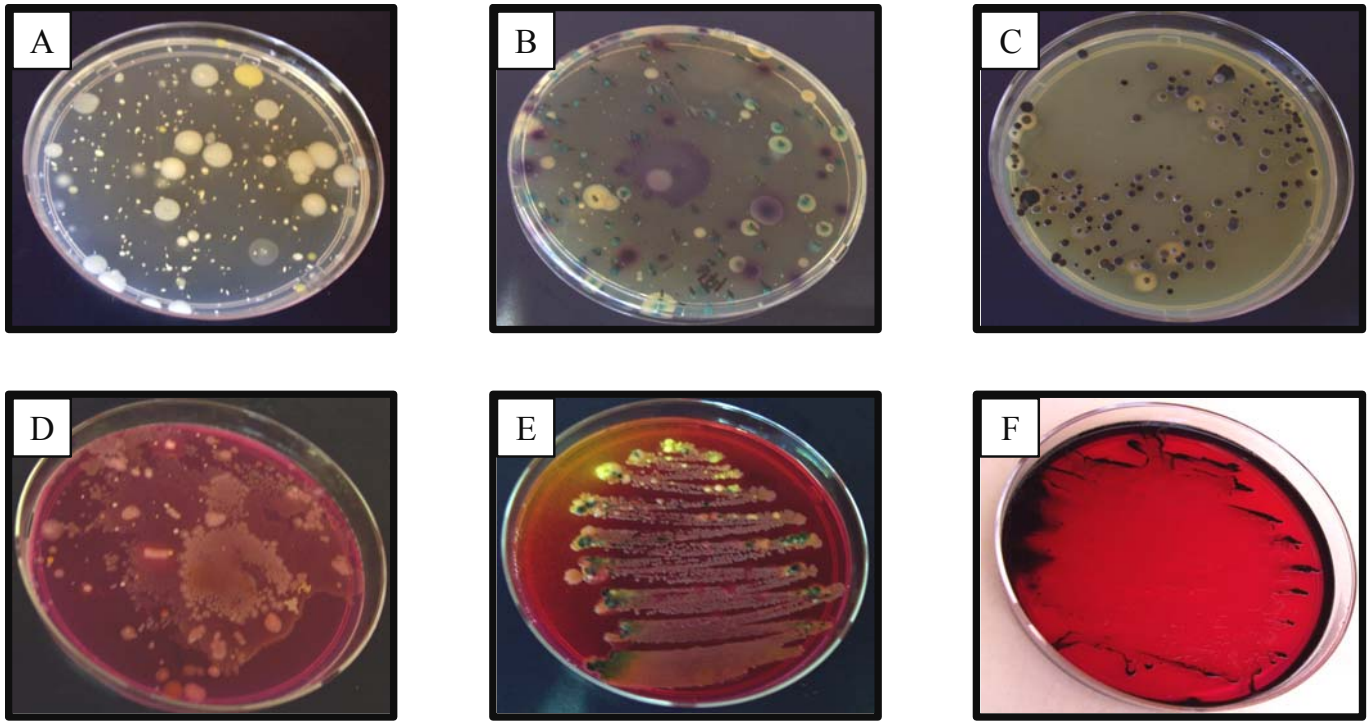


Figure 2.5. Microbiological tests conducted on RTE foods and associated preparation surfaces, cleaning tools and disposable plastic gloves sampled from four retail delicatessens. Plate Count Agar used to evaluate the aerobic bacterial population (A); Rapid' *E. coli* 2 agar used to determine *E. coli* (purple colonies) and coliform bacteria (green colonies) (B); Baird Parker agar medium plus 0.05% Egg's Yolk Tellurite Sterile Emulsion for *S. aureus* (C); *Bacillus cereus* Agar plus 10% Egg's Yolk Sterile Emulsion plus 1 vial of Polimixin B Sulfate Selective Supplement for *B. cereus* (D); Xylose-Lysine Deoxycholate Agar for *Salmonella* spp. (E); Rapid' *L mono* agar used for *L. monocytogenes* (F).

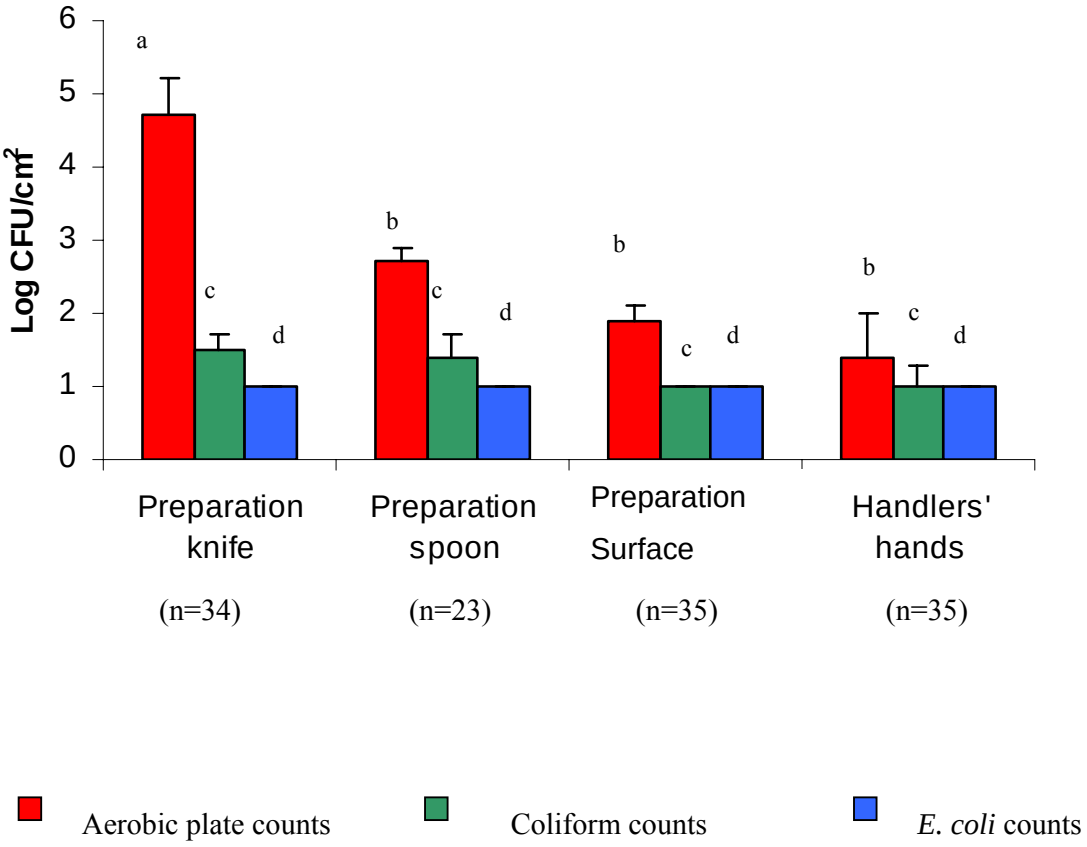


Figure 2.6: Mean aerobic bacterial, coliform and *E. coli* counts obtained for preparation knives (n=34), preparation spoons (n=23), preparation surface (n=35) and handlers' hands (n=35) sampled from four retail delicatessens (Lower detection limit: 1 Log CFU/cm^2). Means with different superscripts represent statistically significant differences (P<0.5) between counts on food contact surfaces.

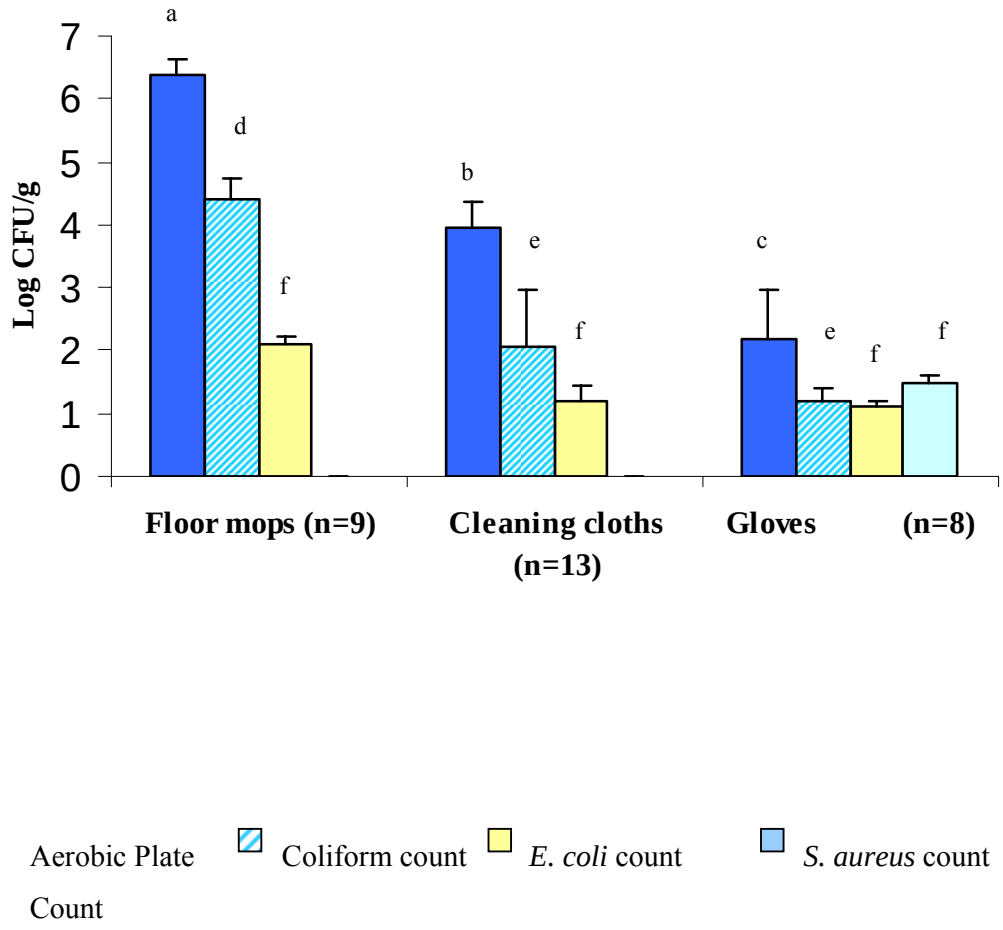


Figure 2.7: Counts of bacterial cells attached to floor mops and cleaning cloths used as cleaning tools and disposable plastic gloves from food handlers' hands associated with the preparation of RTE foods in retail delicatessens (Lower detection limit: 1 Log CFU/g). Means with different superscripts represent statistically significant differences ($P < 0.05$) between counts on cleaning tools.

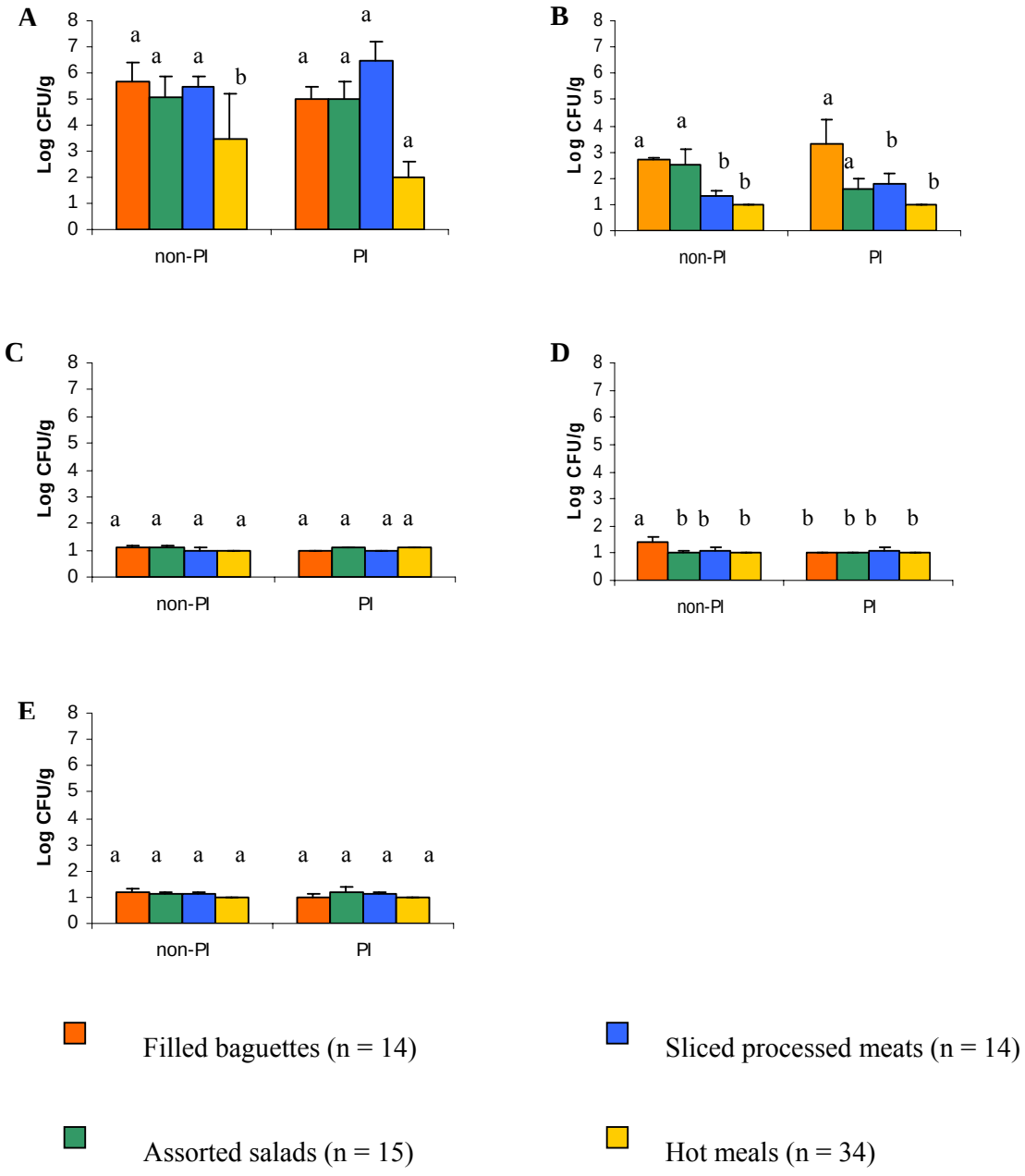


Figure 2.8: Mean aerobic bacterial (A), coliform (B), *E. coli* (C), *S. aureus* (D) and *B. cereus* (E) counts of RTE foods sampled from four retail delicatessens before and after preliminary incubation (PI). Means with different superscripts represent statistically significant differences ($P<0.05$) between non-PI and PI (Lower detection limit: 1 Log CFU/g).

CHAPTER 3

MICROBIOLOGICAL SURVEY OF READY-TO-EAT FILLED BAGUETTES, ASSORTED SALADS AND ASSOCIATED PREPARATION SURFACES SAMPLED FROM RETAIL DELICATESSENS

This work has been presented as:

Christison, C. A., D. Lindsay and A. von Holy, 2006. Cleaning tools as potential sources of cross-contamination in ready-to-eat foods prepared and displayed in retail delicatessens. **Food Micro 2006 20th International Symposium of the International Committee on Food Microbiology and Hygiene (ICFMH) (Bologna, Italy)**, pp 213.

ABSTRACT

A microbiological survey aimed at establishing the bacterial load of RTE filled baguettes (n = 35) and assorted salads (n = 35) prepared and displayed by 4 retail delicatessens in Johannesburg, South Africa was conducted. In addition the bacteriological status of associated plastic chopping boards (n = 23), selected associated preparation utensils (n = 46), storage refrigerators (n = 8) hands of food handlers' (n = 24) and display ice (n = 8) were also assessed. All samples were analysed using standard plate counting techniques and predominant populations (712) isolated and identified. Similar numbers of aerobic (*ca*. 7 Log CFU/g) and coliform (*ca*. 5.5 Log CFU/g) bacteria were isolated from filled baguettes and assorted salads. In addition *Escherichia* (*E*.) *coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp. and *Listeria monocytogenes* were associated with both RTE food types. ***E. coli*** O157:H7 were not detected. Gram positive (73.6%) populations dominated amongst filled baguettes while, Gram-positive (53.5%) and Gram-negative rods (42.2%) were present in similar proportions in assorted salads. Counts for ice used to cool salads during display were negligible and bacterial populations were dominated by Gram-negative rods (70%). Aerobic (4.6 CFU/cm^2), coliform (3.3 CFU/cm^2) and ***E. coli*** (4.6 Log CFU/cm^2) counts were obtained for assorted salad plastic chopping boards. Handlers' hands, plastic chopping boards, salad spoons and filled baguette knives were dominated by Gram-positive bacteria (52-75%). Results from this study suggested that plastic chopping boards, preparation knives and spoons were probable reservoirs for contamination of filled baguettes and assorted salads. Thus the potential exists for the transfer of potential foodborne pathogens to RTE foods in retail delicatessens. Furthermore, the prevalence of pathogens in RTE delicatessen foods studied here may pose foodborne illness potential.

INTRODUCTION

The production of minimally processed pre-cut fruit and vegetable salads as well as filled baguettes has increased over the last decade due to increasing consumer demands (Francis *et al*., 1999). As the demand for these types of foods increases a greater variety is becoming available, each with their own problems and constraints with regards to food safety, as they do not receive any heat treatment before consumption (Angelidis *et al*., 2006). The contamination of ready-to-eat (RTE) foods during factory production (Hagenmaier and Baker, 1998; Baş *et al*., 2006), in the domestic kitchens (Knabel, 1995; Scuderi, *et al*., 1996; Cogan *et al*., 1999; Beumer and Kusumaningrum, 2003), canteens (Abe *et al*., 2004; Ayçiçek *et al*., 2004) and street vended foods (von Holy and Makhoane, 2006) has been investigated. However, the potential for contamination of filled baguettes and assorted salads that occurs in retail delicatessens has received relatively less attention (Kaneko *et al.,* 1999; Angelidis *et al*., 2006; Balague *et al*., 2006).

Foodborne bacterial pathogens are generally of concern if the food which they contaminate can support their growth, multiplication or toxin production. The nature of fresh produce often allows for the aforementioned criteria, for example fruit and vegetables are on average 85-88% water (Jay *et al*., 2005). Vegetables contain 8.6% carbohydrates whilst fruits contain a much higher percentage of carbohydrates (13%). As a result of the carbohydrate content being low for both produce types, there is much free water available for use by microorganisms (Jay *et al*., 2005). In addition the pH of vegetables is well within the optimal range for bacterial growth whilst fruits are generally colonized by yeasts as a result of the fruit pH being in the acidic range (Jay *et al*., 2005). Thus fruits and vegetables provide an ideal environment in which contaminating microorganisms may proliferate. Previous reports have shown that certain foodborne pathogens may enter and multiply in fruits and vegetables before harvest and have been isolated from these types of produce after storage (Beuchat and Ryu, 1997; Burnett and Beuchat, 2000).

In retail delicatessens fruit and vegetables are washed, sliced, packaged or displayed and stored at chill temperatures, so that they are ready-to-eat upon purchase (Jay *et al*., 2005). Whilst the washing process reduces the microbial numbers on the fruit or

vegetables, cutting may potentially lead to recontamination (Francis *et al*., 1999). In addition fresh cut fruit and vegetables provide a higher level of moisture, nutrient availability and a higher surface area for microbial contaminants than the original whole produce (Francis *et al*., 1999), thus making minimally processed fruit and vegetable salads susceptible to microbial growth. In addition, ingredients such as cheese and sliced processed meats are added to the vegetable based salads. These ingredients bring a whole new spectrum of contaminating microorganisms to the salad microbiota. Overall, RTE assorted salads are in general not microbe free. As a result, there have been numerous studies investigating the safety of RTE assorted salads (Christiansen and King, 1971; Fowler and Clark, 1976; Burnett and Beuchat, 2000).

Foodborne disease outbreaks linked to filled baguettes and assorted salads have been associated with *Escherichia* (*E*.) *coli*, *Staphylococcus* (*S*.) *aureus*, *Bacillus* (*B*.) *cereus*, *Salmonella* (*Salm*.) spp. and *Listeria* (*L*.) *monocytogenes* (Goulet *et al.,* 1998*;* Bula *et al*., 1995; Aureli *et al*., 2000; Abe *et al*., 2004). Coliforms are defined on a biochemical basis and are classified as Gram-negative, facultative aerobic, non-spore forming rods that ferment lactose. This group of organisms is generally used as indicators of hygiene during food processing (Jay *et al.,* 2005). *E. coli* naturally forms part of the natural microflora of human intestines and other mammals. However several *E. coli* strains possess the enhanced ability to cause diseases, specifically diarrhea (Williams *et al*., 1999). *E. coli* has been recognized as a foodborne pathogen since 1971 when imported cheeses caused illness in 400 individuals in the USA (Jay *et al*., 2005). More importantly there are several serogroups, which have been associated with severe human illness (Griffin and Tauxe, 1991). For example *E. coli* O157:H7 (Enterohemorrhagic – EHEC) is associated with shiga-toxin production resulting in severe diarrhea in adults, hemolytic uremic syndrome (HUS) in children and possible death (Park *et al*., 1999). EHEC is predominantly associated with contaminated undercooked meat products (Doyle and Schoeni, 1987; Samadpour *et al*., 1994) but recent reports have identified this serogroup in outbreaks caused by fast-food restaurant sandwiches, garden salads, white radish sprouts, Alfalfa sprouts and fruit salads (Abdul-Raouf *et al*, 1993; Diaz and Hotchkiss, 1996; Jay *et al*., 2005).

The initial microbiological load on RTE food ingredients is important (Angelidis *et al*., 2006), factors such as handling (Jumaa, 2005), processing (Beuchat and Ryu,

1997), storage (Angelidis *et al*., 2006) and display (Nichols *et al*., 2000), influence the microbiological load of RTE foods at the point of sale. Delicatessen foods such as assorted salads and filled baguettes are often prepared by hand leading to an increased incidence of foodborne bacterial contamination with *Staphylococcus* (Jay *et al*., 2005). *Staphylococcus* may proliferate in these foods due to low numbers of competing spoilage populations as a result of cooking before production (Jay *et al*., 2005).

Contamination of the processing environment is frequently responsible for food product contamination (Kornacki, 2000). Pathogens may be spread onto preparation utensils and plastic chopping boards when preparing contaminated food. Bacteria that attach and if allowed to form biofilms, may become protected from cleaners and sanitizers (Notermans *et al*., 1991; De Beer *et al*., 1994; Dhir and Dodd, 1995). The potential for the recontamination of RTE foods exists from hands of food workers, associated preparation utensils and food contact surfaces (Ali and Spencer, 1996). Inadequate hand washing by food handlers is an important contributing factor to potential foodborne pathogen contamination of RTE foods in a retail concern (Montville and Schaffner, 2002). During the handling and preparation of RTE foods, bacteria on raw foods may be transferred to the hands of a food handler and subsequently to other food contact surfaces (Montville and Schaffner, 2002). In addition infected workers may transmit potential pathogens with contaminated hands (Jamaa, 2005). For example, a laboratory-controlled study showed that during the preparation of a chicken contaminated with *Salmonella* spp. the bacterium was transferred to handlers' hands, cleaning cloths and food contact surfaces. In addition, if adequate cleaning and sanitation procedures were not carried out, plastic chopping boards and handlers' hands, were still potential reservoirs for subsequent cross-contamination (Cogan *et al*., 1999).

The purpose of this study was to determine the microbiology of filled baguettes and assorted salads at the point of sale in four retail delicatessens in Johannesburg, South Africa, as well as the bacterial load on associated preparation and food contact surfaces. Therefore the microbiological load of associated preparation utensils, plastic chopping boards, handlers' hands, display ice and storage refrigerators during filled baguette and assorted salad preparation was also evaluated.

MATERIALS AND METHODS

Bacterial populations associated with filled baguettes, assorted salads and associated preparation utensils (knives and spoons), plastic chopping boards, handlers' hands, display ice and storage refrigerators in retail delicatessens were isolated during four replicate surveys of four retail delicatessens in Johannesburg, South Africa. All preparation-associated samples were taken during or immediately after filled baguette and assorted salad preparation.

a) Sample collection

Generally baguette and assorted salad ingredients were stored in a refrigerator within the delicatessen before preparation and fruit and vegetables were taken from the retailers' in-store supply. Bread was baked in-store and bulk rolls of processed meats were sliced if required on in-store slicing machines. Filled baguettes ready for sale were placed in a refrigerated display cabinet (8˚C), whilst bowls of assorted salads were placed on beds of ice.

Samples were collected at weekly intervals from October 2005 – May 2006. A total of 35 filled baguettes and 35 assorted salads (Fig. 3.1) were sampled and the core temperatures taken at the point of sale using a thermometer (Testo 826 T-4; 2 in 1 with laser sighting). All filled baguette and assorted salad samples were transported to the laboratory in a cooler box. In addition swabs of 46 cleaned preparation utensils, 23 plastic chopping boards, 24 washed handlers' hands, 8 storage refrigerators and 8 samples of the ice used to cool assorted salads during display were taken during or directly after filled baguette and assorted salad preparation. The swabs were transported to the laboratory in a cooler box.

b) Sample processing and analysis

RTE food samples and swabs of the food contact surfaces were prepared for analysis using the methodology described in Chapter 2.

c) Bacterial counts

RTE food samples were tested for aerobic bacteria, coliforms, *E. coli*, *S. aureus* and *B. cereus* counts using the methodology described in Chapter 2. In addition, swabs of the food contact surfaces were tested for aerobic bacteria, coliforms and *E. coli*. Hand swabs were tested for *S. aureus* using methodology described in Chapter 2.

d) *L. monocytogenes* and *Salmonella* spp. detections

RTE food samples were tested for *L. monocytogenes* and *Salmonella* spp. using the methodology described in Chapter 2.

e) Statistical analysis

Counts of RTE foods, preparation utensils, associated food preparation surfaces, handlers' hands were meaned over the four replicate surveys and standard deviations were calculated. In addition colony count data was statistically analysed using analysis of variance (ANOVA) at the 95% confidence level (STATGRAPHICS 7.0, Manugistics Inc. and Statistical Graphics Corporation, USA).

f) Characterisation of predominant bacterial populations from aerobic plate counts (APC)

A total of 712 predominant bacterial isolates were obtained from the APC plates of each sample category (Fig. 3.2A). Four predominant colonies (von Holy and Holzapfel, 1988) for each of the filled baguettes (140 isolates), assorted salads (140 isolates), preparation knives (92 isolates), preparation spoons (92 isolates), plastic chopping boards (92 isolates), handlers' hands (92 isolates), assorted salads display ice (32 isolates) and ingredient storage refrigerator (32 isolates) were isolated from

APC plates (Fig. 3.2) of the highest dilution showing growth (von Holy and Holzapfel, 1988). The bacterial isolates were purified by standard methods (von Holy and Holzapfel, 1988) and characterized according to the dichotomous key of Fischer *et al*. (1986) (Fig. 3.3 and Fig. 3.4). In addition *Micrococcaceae* were further divided into *Micrococcus* and *Staphylococcus* using a modified oxidase test (Faller and Schleifer, 1981) (Fig. 3.3). Gram-negative bacterial isolates were not further characterized. An incubation temperature of 30˚C was used for all biochemical tests.

g) Screening of random *Escherichia coli* and coliform populations for *Escherichia coli* 0157:H7

A total of 606 coliform colonies (green colonies) (Fig. 3.2B) and 324 *E. coli* colonies (purple colonies) (Fig. 3.2B) were randomly (Harrigan and McCance 1966) selected from Rapid' *E. coli* 2 agar plates containing 30 to 300 colonies of filled baguettes, assorted salads, preparation knives, preparation spoons, plastic chopping boards, handlers' hands, display ice and storage refrigerators. The bacterial isolates were purified by standard methods (von Holy and Holzapfel, 1988) and 200 coliform and 300 *E. coli* were selected and screened on BD BBL^{TM}CHROMagar^{TM}O157 (BD Biosciences, Becton, Dickinson and Company, USA) (Fig. 3.2C) for the presence of *E. coli* O157:H7. An incubation temperature of 37˚C was used for all tests.

RESULTS

a) Bacterial counts

i) Baguettes and assorted salads

The aerobic (APC), coliform (CC), *E. coli* (ECC), *S. aureus*(SAC) and *B. cereus* counts (BCC) and *L. monocytogenes*- and *Salmonella* spp. detection are shown in Table 3.1 and Table 3.2 and Fig. 3.5.

The highest bacterial counts from filled baguettes and assorted salads were consistently the APCs (7.2 Log CFU/g), followed by CCs, ECCs, SACs and BCCs in decreasing order (Table 3.1). Overall, APCs, CCs, ECCs and BCCs were not

statistically significantly (P>0.05) different between filled baguettes and assorted salads (Table 3.1). *S. aureus* counts were statistically significantly (P<0.05) higher for filled baguettes compared to assorted salads (2.1 Log CFU/g) (Table 3.1.).

Figure 3.5 shows a breakdown of counts for filled baguette and assorted salad samples by product type. APCs and BCCs from chicken filled baguettes were statistically significantly (P<0.05) 1 Log CFU/g lower than cheese-based and sliced processed meat filled baguettes (Fig. 3.5). In addition, CC and SAC for chicken filled baguettes were statistically significantly (P<0.05) (0.5 Log CFU/g) higher than the corresponding counts for cheese-based and sliced processed meat filled baguettes (Fig. 3.5). Furthermore, *E. coli* were recovered 4 Log CFU/g (P<0.05) less than from sliced processed meat baguettes than the other two filled baguette types (Fig. 3.5).

Fruit salads contained statistically significantly (P<0.05) (2 Log CFU/g) lower APCs and CCs than the corresponding counts for vegetable and other salads (Fig. 3.5). By contrast, *E. coli* counts were statistically significantly (P<0.05) higher on assorted salads containing cheese or sliced processed meat compared to assorted salads containing only fruit or vegetables (4 Log CFU/g) (Fig. 3.5). Furthermore, *S. aureus* and *B. cereus* counts were consistently similar between all assorted salad types (*ca*. 1.5 and 1.7 Log CFU/g, respectively) (Fig. 3.5).

L. monocytogenes was only isolated from cheese based (7%) and sliced processed meat filled baguettes (7%) (Table 3.2). Double the proportion of chicken filled baguettes (28.5%) were positive for *Salmonella* spp. compared to cheese based baguettes (14.3%) (Table 3.2). *L. monocytogenes* was only isolated from assorted salads (8.3%) containing cheese or sliced processed meats (Table 3.2). Similarly double the number of assorted salads (20%) containing cheese or sliced processed meats were positive for *Salmonella* spp. compared to only fruit (7.7%) or only vegetable containing (10%) salads (Table 3.2). Overall the incidence of *L. monocytogenes* (5.7 and 2.9%) and *Salmonella* spp. (20.0 and 11.4%) in filled baguettes was double that of assorted salads, respectively (Table 3.2). In addition, the incidence of *Salmonella* spp. was approximately four times that of *L. monocytogenes* in filled baguettes and assorted salads (Table 3.2).

Table 3.3 shows the microbiological guidelines suggested for filled baguettes and assorted salads by the retailer participating in this study and those recommended by the South African Department of Health (Anonymous, 1998). On comparison, 97.1% of the filled baguette and assorted salads sampled conformed with the aerobic plate count specifications of <8 Log CFU/g recommended by the South African Department of Health (Anonymous, 1998). However, on average only a third of the 70 RTE foods sampled from the retail delicatessen complied with *coliform*, *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* spp. guidelines recommended by the retailer (Table 3.3).

ii) Associated preparation utensils, plastic chopping boards and handlers' hands

In the present study cleaned preparation knives, spoons, plastic chopping boards and handlers' hands were sampled during filled baguette and assorted salad preparation. The aerobic bacterial, coliform and *E. coli* counts, plus the *S. aureus* counts for the handlers' hands are shown in Table 3.1 and Figure 3.6.

The highest bacterial counts on the associated preparation surfaces were consistently the APCs (4.6 Log CFU/cm^2), followed by the ECCs (4.6 Log CFU/cm^2) and CCs (3.3 Log CFU/cm^2). In addition, ECCs were statistically significantly ($P<0.05$) (3 Log CFU/cm^2) higher on the assorted salads food contact surfaces compared to the filled baguette food contact surfaces (Fig. 3.1). Aerobic plate counts obtained for filled baguette preparation spoons (2.2 Log CFU/cm^2) and salad preparation knives (1.8 Log CFU/cm^2) were statistically significantly ($P<0.05$) lower than the rest of the food contact surfaces sampled (Fig. 3.6). For both filled baguettes and assorted salads, coliform counts on plastic chopping boards and handlers' hands were statistically significantly ($P<0.05$) higher (2-4 Log CFU/cm^2) than counts obtained from the associated preparation utensils (Fig. 3.6). *E. coli* counts of both filled baguette and salad associated preparation utensils and handlers' hands were low and did not statistically significantly ($P>0.05$) differ from each other. By contrast, *E. coli* counts on salad plastic chopping boards were statistically significantly higher (4 Log CFU/cm^2) than those on plastic chopping boards used in filled baguette preparation areas. *S. aureus* counts were below the lower detection limit (1 Log CFU/cm^2) for handlers' hands (Fig. 3.6).

iii) Ice used for salad display

Ice used to cool the assorted salads during display was sampled from retail delicatessens. The aerobic bacterial, coliform and *E. coli* counts are shown in Fig. 3.5. The highest bacterial counts for the ice were the APCs (3.3 Log CFU/ml), followed by CCs (2.3 Log CFU/ml) and ECCs (1.4 Log CFU/ml) counts. In addition the counts for the ice were statistically significantly ($P<0.05$) lower than counts obtained for assorted salads (Fig. 3.5).

iv) Storage refrigerator

The walls of the storage refrigerator used to store ingredients such as sliced tomato, lettuce, cheese, mayonnaise, sliced processed meat, fruit and vegetables for making the filled baguettes and assorted salads was sampled from the retail delicatessens. The aerobic bacterial, coliform and *E. coli* counts are shown in Fig. 3.6. The highest bacterial counts for the storage refrigerator were the APCs (2.2 Log CFU/cm^2) followed by the CCs (1.1 Log CFU/cm^2). ECCs were below the lower detection limit (1 Log CFU/cm^2) (Fig. 3.6). The counts for the storage refrigerator were statistically significantly ($P<0.05$) lower than counts obtained for the associated preparation surfaces (Fig. 3.6).

b) Core temperature of filled baguettes and assorted salads at the point of sale

The core temperatures of the filled baguettes and assorted salads were taken at the point of sale. The core temperatures of the filled baguettes (16°C) were 6°C higher than those obtained for the assorted salads (9.7°C).

c) Predominant populations

Predominant bacteria were isolated from the APC plates and characterized by following the key suggested by Fischer *et al*., (1986) and Faller and Schleifer, (1981) (Fig. 3.3). In addition random colonies of *E. coli* and coliform isolates were isolated from Rapid' *E. coli* 2 agar for filled baguettes, assorted salads, associated preparation utensils, plastic chopping boards, handlers' hands, assorted salads display ice and filled baguette ingredient storage refrigerators and isolates screened for the *E. coli* O157:H7.

The percentage distributions of bacterial populations isolated from the APC plates from the storage refrigerator are shown in Fig. 3.8. Bacterial populations isolated from the storage refrigerator were dominated by Gram-negative rods (86%) (Fig. 3.8). The remaining isolates were represented by *Micrococcus* (14%) (Fig. 3.8).

i) Filled baguette and assorted salads

The percentage distribution of predominant bacterial populations isolated from the APC plates of filled baguette and assorted salads is shown in Table 3.4 and 3.5 and Fig. 3.7. Bacterial populations isolated from filled baguettes were dominated by Gram-positive bacteria (73.6%) (Table 3.4). *Bacillus*, *Lactobacillus*, *Micrococcus*, *Staphylococcus* and *Enterococcus* (140 isolates) collectively made up 73.6% of the total isolates (Table 3.5). The remaining isolates were represented by the Gram-negative rods (20.7%) and yeast (5.7%) populations (Table 3.4). *Bacillus* was isolated in approximately equal proportions from all filled baguette types (Table 3.5). *Lactobacillus* was isolated from cheese based filled baguettes in the lowest proportion (5.4%), whilst *Micrococcus* (25%) was only isolated from chicken filled baguettes (Table 3.5). By contrast *Staphylococcus* was absent from chicken filled baguettes and *Enterococcus* was isolated in higher proportions from cheese based filled baguettes (28.6%) (Table 3.5).

Gram-positive (53.5%) and Gram-negative rod populations (42.2%) were isolated in similar proportions for assorted salads (140 isolates) (Fig. 3.4). The remaining isolates were represented by yeast (4.3%) populations (Fig. 3.4). *Bacillus* was isolated in

approximately equal proportions from all assorted salad types (Table 3.5). *Lactobacillus* (14.6%) was only isolated from assorted salads containing cheese or sliced processed meat (Table 3.5). By contrast *Micrococcus* (11.5%) was only isolated from fruit salads (Table 3.5). Double the proportion of *Staphylococcus* was isolated from vegetable salads (31.4%) compared to fruit (15.4%) and other salads (16.6%). Similarly double the proportion of *Enterococcus* was isolated from other salads (8.3%) compared to fruit (3.8%) and vegetable (2.8%) salads (Table 3.5).

ii) Associated preparation utensils, plastic chopping boards and handlers' hands

The percentage distribution of isolates obtained from the APC plates of associated preparation utensils (184 isolates), plastic chopping boards (92 isolates) and handlers' hands (92 isolates) are shown in Table 3.4 and Fig. 3.7. In general, of the 368 isolates, Gram-positive (*Bacillus*, *Lactobacillus*, *Micrococcus*, *Staphylococcus* and *Enterococcus*) groups (57.6%) generally predominated on the preparation food contact surfaces followed by Gram-negative rods (39.5%) (Table 3.4). The remainder of the isolates comprised of yeast populations totaling 2.9% (Table 3.4). Interestingly, of the Gram-positive populations, *Staphylococcus* (21%) generally predominated on all food contact surfaces, whilst *Lactobacillus* was generally present in the smallest percentage (4.5%). With the exception of the filled baguette plastic chopping boards (14%) (Fig. 3.7). In addition *Micrococcus* was isolated from neither filled baguette nor assorted salad plastic chopping boards (Fig. 3.7).

Gram-positive groups such as *Bacillus* (18%), *Lactobacillus* (5%), *Micrococcus* (12%), *Staphylococcus* (23%) and *Enterococcus* (12%) collectively comprised 70% of the total bacterial populations from filled baguette preparation knives (Fig. 3.7A). By contrast, Gram-negative rods (56%) predominated on assorted salad preparation knives (Fig. 3.7B1). Similarly *Bacillus* (37%), *Micrococcus* (6%) and *Staphylococcus* (19%) collectively comprised 62% of assorted salad preparation spoon isolates (Fig. 3.7B2), whilst Gram-negative rods (60%) predominated among filled baguette spoons (Fig 3.7A2). No yeast groups were isolated from either preparation spoon types (Fig. 3.7).

Bacillus (27%) predominated amongst Gram-positive isolates of salad plastic chopping boards (numbering 92), followed by *Staphylococcus* (19%), *Enterococcus* (8%) and *Lactobacillus* (4%) (Fig 3.7B3). By contrast, *Staphylococcus* (28%), predominated amongst Gram-positive isolates (numbering 92) from filled baguette plastic chopping boards, followed by *Enterococcus* (19%) and *Bacillus* and *Lactobacillus* each contributing 14%. Gram-negative rod populations were isolated in smaller percentages from plastic chopping boards used for filled baguette preparation (21%) compared to assorted salad plastic chopping boards (42%). Yeast groups (4%) were only isolated from filled baguette plastic chopping boards (Fig. 3.7).

Of the Gram-positive populations associated with the handlers' hands, *Staphylococcus* (27%) predominated followed by *Micrococcus* (10%), *Enterococcus* (10%), *Bacillus* (8.5%) and *Lactobacillus* (2.5%) (Fig 3.7A4 and B4). Gram-negative rod populations were isolated in double the proportion from filled baguette handlers' hands (44%) compared to assorted salad handlers' hands (27%). Low percentages of yeast populations were isolated from both filled baguette (4%) and assorted salad (9%) handlers' hands.

iii) Ice used for salad display

The percentage distribution of bacterial populations isolated from the APC plates of assorted salad display ice are shown in Fig. 3.8. Bacterial populations isolated from the ice were dominated by Gram-negative rods (70%) (Fig. 3.8). The remaining isolates were represented by *Micrococcus* (30%) (Fig. 3.8).

c) Screening of *E. coli* and coliform populations for *E. coli* O157:H7

None of the 200 coliform and 300 *E. coli* isolates screened on BD BBL^{TM}CHROMagar^{TM}O157 (BD Biosciences, Becton, Dickinson and Company, USA) were positive (pink) for *E. coli* O157:H7 (Fig. 3.2).

DISCUSSION

a) Filled baguette and assorted salads

Bacterial counts and incidence of potential foodborne pathogens

APCs of 6.21 Log CFU/g have previously been reported from RTE baguettes containing similar fillings to those in the present study (Angelidis *et al*., 2006). By contrast APCs for cheese, sliced processed meat and chicken baguettes in the present study were collectively 1 Log higher than those obtained for sandwiches in the Greek study. (Angelidis *et al*., 2006). The importance of aerobic bacteria enumerated from filled baguettes containing cheese and sliced processed meat is limited as these commodities reportedly contain high numbers of lactic acid bacteria (Jay *et al*., 2005).

An average aerobic count of 7 Log CFU/g for assorted salads in the present study, agreed with results reported by Christiansen and King (1971); Jay, (1996) but differed from results of Arumugaswamy *et al*., (1994); Albrecht *et al*., (1995); Kaneko, (1999) and Angelidis *et al*., (2006). The latter reported a range of counts from 2 to 6 Log CFU/g. The discrepancy in mean aerobic counts between different countries may be attributed to local agricultural practices resulting in endemic microbial loads (Bone and Parker, 2005; Jay *et al*., 2005). Overall fruit salads contained the lowest APCs (5.4 Log CFU/g). This may be due to the low pH of the fruits such as pineapples which is inhibitory to the growth of many bacteria (Jay *et al*., 2005). This was shown to be true for the present study as fruit salads contained the lowest counts of aerobic bacteria (5.4 Log CFU/g).

Historically coliforms in RTE foods were used as indicators of cross-contamination by food handlers (Mossel and van Netten, 1991). However recently their use has been shown to be limited as coliforms may occur naturally on fresh produce (De Roever, 1999). In this study, the coliform counts were highest on the vegetable containing salads (6.2 Log CFU/g) i.e. fresh produce naturally carrying coliform loads. However the presence and numbers of *E. coli* may be more valuable as indicators of cross-contamination (De Roever, 1999; Jay *et al*., 2005). Results from the present study showed that *E. coli* counts were highest on products containing sliced processed

meats. In addition the presence of coliforms and *E. coli* in both filled baguettes and assorted salads may be as a result of contaminated ingredients, poor handling practices of food handlers, cross contamination from food contact surfaces such as the in-store slicing machine within the delicatessen or high storage temperatures (Fang *et al*., 2003). Both *E. coli* and coliforms were enumerated in filled baguettes and assorted salads in the present study. Thus the source may be from any of the above-mentioned practices. The core temperatures of both filled baguettes (16°C) and assorted salads (9.7°C) were double the temperatures recommended by the retailer concerned for filled baguettes (8°C) and assorted salads (4°C) at the point of sale. Ambient temperatures provide a niche for bacterial growth and multiplication (Jay *et al*., 2005). Thus sanitation and personal hygiene practices aimed at reducing the incidence of *E. coli* and coliforms below the lower detection limit (1 Log CFU/g) in filled baguettes and assorted salads are recommended. In addition, reducing the temperatures of the display refrigerators may maintain *E. coli* and coliform counts below unacceptable levels.

S. aureus is frequently the cause of food intoxication in both the USA (Bean *et al*., 1990) and Taiwan (Pan *et al*, 1997). Human nasal passages and skin are the main reservoirs for *S. aureus* (Jay *et al*., 2005), which may contribute to RTE food contamination during preparation if conditions allow for bacterial growth and toxin production. Other studies have suggested that the presence of *S. aureus* in RTE foods may be as a result of improper handling, cross contamination and poor temperature control (Garcia *et al*., 1986; Snyder, 1998). Interestingly, this potential pathogen was isolated in low numbers from both filled baguettes and assorted salads. However, statistically significantly more *S. aureus* were enumerated from filled baguettes (2.1 Log CFU/g) compared to assorted salads (1.5 Log CFU/g). This may be attributed to the method of preparation. The food handler physically handles every component of the filled baguettes whilst the salad ingredients are chopped on a plastic chopping board and scraped into a serving bowl without further handling.

B. cereus has been considered an emerging foodborne pathogen in recent years due to its cosmopolitan distribution (Granum and Lund, 1997). Microbiological data has shown that *B. cereus* can grow in refrigerated RTE foods, however, correct refrigeration practices provide sufficient control of this potential foodborne pathogen

(Jay, *et al*., 2005). Foodborne outbreaks have primarily been linked to time/temperature abuse, which allowed initial low levels of cells to increase and for toxin production (International Commission on Microbiological Specifications for Foods, 1996.). Low *B. cereus* counts of *ca*. 1.8 Log CFU were determined for both filled baguettes and assorted salads. However, the presence of low numbers of *B. cereus,* the higher core temperatures of the RTE foods at the point of sale and potential time/temperature abuse of the RTE foods after the point of sale by consumers may be of concern. Maintenance of correct refrigeration within the delicatessens is fundamental for the safety these RTE foods at the point of sale.

L. monocytogenes is a psychrotrophic pathogen commonly found in refrigerated RTE foods (Hwang, 2005). The incidence of *L. monocytogenes* found here in filled baguettes and assorted salads are comparable to those described in similar products elsewhere. Results from the surveillance and monitoring activities of the FDA, USDA, Belgium, France and Portugal have shown that an estimated 5% of delicatessen style assorted salads and 9% sliced processed meats salads contain *L. monocytogenes* (Hitchins, 1996; Uyttendale *et al*., 1999; Goulet *et al*., 2001; Guerra *et al*., 2001; Levine *et al*., 2001). By contrast, *L. monocytogenes* was not detected in sliced processed meat salads and sliced processed meat sold as RTE foods in Belgium (van Coillie *et al*., 2004). Therefore, the similar incidence of *L. monocytogenes* here and in other countries indicates *L. monocytogenes* is a general problem with respect to delicatessen filled baguettes and assorted salads.

There is however much contention in literature as to whether the simple presence of *L. monocytogenes* (zero tolerance) in RTE foods is sufficient to classify the foods as adulterated (Tappero, *et al*., 1995; Gombas *et al*., 2003). Some countries such as Germany, The Netherlands, France, Canada and Denmark, accept the presence of low numbers (below 100 CFU/g) of *L. monocytogenes* (Nørrung, 2000). Therefore the incidence of *L. monocytogenes* present on the RTE foods in the present study may need to be evaluated in future studies so as to determine the full food safety risk posed by *L. monocytogenes* to the South African population.

Microbial interference may play a role in food safety (Jay, 1997). The concept is loosely based on the premise that a food commodity containing a high number of

harmless bacterial flora for example 6 Log CFU/g, is less likely to allow low numbers of potential foodborne pathogens to proliferate than one which contains for example 4 Log CFU/g (Holzapfel, 1995; Jay, 1996). This is as a result of natural competition. A previous study showed that when a large inoculum of *S. aureus* was added to creamed chicken, very little multiplication of the cells occurred as they were out competed by background bacterial flora (Straka and Combes, 1952). Similarly when *Lactobacillus* and pediococci were inoculated into RTE vegetables there was a reduction in the recovery of mesophiles, especially coliforms and enterococci (Vescova *et al*., 1995). The aerobic plate counts (7 Log CFU/g) for filled baguettes and assorted salads sampled in this study were relatively high when compared to some other countries (Albrecht *et al*., 1995; Kaneko *et al*., 1999; Johannessen, 2002; Angelidis *et al*., 2006;), whilst the incidence of potential foodborne pathogens such as *S. aureus* (2 Log CFU/g), *B. cereus* (1.8 Log CFU/g) and *L. monocytogenes* (5%) were relatively low. Results suggest aerobic bacteria compete with potential foodborne pathogens through natural selection, hence indirectly ensuring the safety of the foods.

The microbiological guidelines recommended by the retailer concerned in this study are fairly stringent compared to the South African Department of Health as a high proportion of the South African population is immunocompromised. This coupled with the fact that RTE foods are not further processed before consumption has led the retailer to recommend microbiologically stringent guidelines. However 60% of the RTE samples complied with coliform, *E. coli*, *S. aureus*, *B. cereus*, *Salmonella* spp. and *L. monocytogenes* recommendations (Table 3.3).

Predominant populations

Predominantly Gram-positive populations were isolated from filled baguettes. This may be attributed to the ingredients used to prepare the filled baguettes. *Bacillus* may be associated with bread and bread products and is primarily a spoilage microorganism (Leuschner *et al*., 1998). *Lactobacillus* and *Enterococcus* are lactic acid bacteria reportedly associated with cheeses and sliced processed meats. They are primarily responsible for spoilage of foods (Jay *et al*., 2005). *Micrococcus* and *Staphylococcus* are reportedly associated with handlers' hands. Filled baguettes are prepared by hand and this direct contact may lead to an increased number of *Micrococcus* and *Staphylococcus* associated with these foods (Jay *et al*., 2005).

By contrast, the assorted salads were associated with roughly equal Gram-positive and Gram-negative populations. The higher proportion of Gram-negative populations associated with the assorted salads may be due to fresh produce naturally containing coliform bacteria (De Roever, 1999).

b) Associated preparation utensils, plastic chopping boards and handlers' hands

Bacterial counts

The attachment of bacteria to food contact surfaces increases the risk of cross-contamination to foods, which come into contact with these surfaces. Several studies have concentrated on the transfer of bacteria between handlers' hands, food and preparation surfaces in domestic kitchens (Zhao *et al*., 1998; Chen *et al*., 2001; Montville *et al*., 2002). The preparation knives, spoons, plastic chopping boards and handlers' hands sampled in this study were generally encountered wet, thus providing a niche for bacterial growth and providing the potential for higher bacterial transfer (Marples and Towers, 1979; Buckalew *et al*., 1996). The number of fresh produce components in assorted salads is greater than in filled baguettes. The fresh produce components of the assorted salads are chopped directly on the salad chopping board whilst only the baguette comes into contact with the filled baguette plastic chopping

board. Therefore if the plastic chopping boards are inadequately washed there is the potential for a greater concentration of *E. coli* on the salad chopping boards.

Plastic chopping boards had the highest coliform and *E. coli* counts and may act as possible reservoirs for contamination of filled baguettes and assorted salads, and correct sanitation of food contact surfaces are crucial in preventing contamination of RTE foods.

The average aerobic count of *ca*. 4 Log CFU/cm^2 for plastic chopping boards, agreed with results reported by Tessi *et al*., (2002) but differed from results of Montville and Schaffner, (2004). The latter reported much higher counts of 9.6 Log CFU/cm^2. By contrast the *ca*. 5 Log CFU/cm^2 for preparation spoons were comparable to the results of Montville and Schaffner (2004). Higher numbers of aerobic bacteria were enumerated from assorted salads' preparation spoons (5.1 Log CFU/cm^2) compared to filled baguette preparation spoons (2.2. Log CFU/cm^2). This may arise from the assorted salads' spoon being used to serve the assorted salads and may become contaminated by the food handlers. Interestingly the preparation knives in the present study harbored 2.8 Log CFU/cm^2 whilst Tessi *et al*. (2002) reported a mere 1 Log CFU/cm^2.

A separate study showed that correct hand washing procedures using antibacterial soap, alcohol free sanitizer and paper towels decreased hand contamination by 3 Log CFU (Montville and Schaffner, 2002). Therefore re-evaluating hand washing regimes used by food handlers within the delicatessens may aid in increasing the safety of the RTE foods displayed. In the present study aerobic bacteria, coliform, *E. coli* and *S. aureus* counts on handlers' hands for both filled baguette and assorted salads either equaled or exceeded 2 Log CFU/cm^2. A previous laboratory study using *Enterobacter aerogenes,* showed that *ca*. 2 Log CFU may be transferred from handlers' hands to lettuce during preparation (Chen *et al*., 2001). Thus the potential exists for the transfer of potential foodborne pathogens to RTE foods in the retail delicatessens.

Predominant populations

Predominant populations isolated from the plastic chopping boards correlated with the predominant populations isolated from the filled baguettes. This is possibly due to the filled baguettes being prepared directly on the plastic chopping boards. Similarly, the predominant populations on the preparation knives used to prepare the assorted salads correlated with the assorted salad populations. Of the filled baguettes, *Micrococcus* was isolated exclusively from the chicken baguettes. This was expected as *Micrococcus* has been found associated with chickens (Georgnaras *et al*., 1994).

c) Display ice

Bacterial counts

Ice is frequently used to cool assorted salads during display in retail delicatessens and catering establishments (Nichols *et al*., 2000). In accordance with British standards, ice should be made from potable water and should therefore be free from coliforms, *E. coli* and enterococci (Anonymous, 1989). A previous study reported mean aerobic bacterial counts of 3 Log CFU/ml plus coliform and *E. coli* counts of 2 Log CFU/ml (Nichols *et al*., 2000). These results were comparable to ice sampled from the retail delicatessen salad display counters in the present study (Fig. 3.5). Mean aerobic counts for ice have been deemed of little importance in the context of microbiological safety (Nichols *et al*., 2000), however an upper limit of 3 Log CFU/ml is recommended (Anonymous, 1989). Given this, the ice used to cool assorted salads during display was of satisfactory microbiological quality. The coliform count may be used as an indicator of the hygienic practices used during preparation, storage and display of ice. The presence of *E. coli* in the ice may suggest incorrect handling of ice or contaminated serving utensils (Nichols *et al.*, 2000). The latter may be true as an *E. coli* count of 2 Log CFU/cm^2 was enumerated from the assorted salads preparation spoons which may be a potential source of contamination.

Predominant populations

The predominant populations isolated from the ice used to cool the assorted salads were predominantly Gram-negative rods and *Micrococcus*. The *Micrococcus* may result from the handling of the ice whilst it is being prepared for display.

d) Storage refrigerator

Bacterial counts

Previous reports have shown that coliforms, *S. aureus*, *B. cereus* and *Enterococcus* and *L. monocytogenes* may be associated with refrigerators (Evans *et al*., 2004). These findings are in contrast to the results of this study as low coliform and *E. coli* counts were determined. It has been noted that the majority of bacteria associated with refrigerators are spoilage bacteria (Evans *et al*., 2004), thus the low numbers of aerobic bacteria found in the delicatessen storage refrigerators may represent spoilage bacteria.

Predominant populations

The predominant populations were comprised of Gram-negative rods and *Micrococcus*. Therefore, correct sanitation of the refrigerators should be maintained so as to prevent cross-contamination of potential pathogens to RTE foods

CONCLUSION

This study confirms existing knowledge that RTE filled baguettes and assorted salads harbour bacterial contaminants which may reduce the quality and safety of these foods. The numbers of *B. cereus* and *S. aureus* in both RTE food types were low. However, the presence of these potential foodborne pathogens in RTE foods is undesirable. Due to the production of toxins by these bacteria and the higher (Ca.15ºC) core temperatures of these foods at the point of sale *B. cereus* and *S. aureus* may pose foodborne illness risk potential. Therefore adequate refrigeration of these

foods is recommended. The prevalence of *L. monocytogenes* in filled baguettes and assorted salads is comparable to studies conducted in other countries. However, *L. monocytogenes* is known to be prevalent in refrigerated foods. Therefore, elimination of *L. monocytogenes* through improved cleaning and sanitation regimes of food contact surfaces may reduce the safety implications of this foodborne pathogen in retail delicatessen foods. Improper handling and preparation of foods in food establishments are often the primary source of *Salmonella* spp. contamination. Therefore correct handling and preparation practices should be emphasized during delicatessen staff training programs. The highest number of bacteria were associated with the plastic chopping boards and therefore these plastic chopping boards may act as the greatest reservoir for RTE food contamination. Similarly the hands of the food handlers preparing the filled baguettes and the serving spoons for the assorted salads may be likely sources of bacterial contamination of these RTE foods in the retail delicatessen.

Table 3.1: Mean aerobic bacterial, coliforms, *E. coli*, *S. aureus* and *B. cereus* counts from RTE filled baguettes (n = 35) and assorted salads (n = 35) sampled from four retail delicatessens.

Bacterial group	RTE food		Food contact surfaces	
	Filled baguettes (n=35)	Assorted Salads (n=35)	Filled baguette (n=83)	Assorted salads (n=80)
	(Log CFU/g)		(Log CFU/cm^2)	
Aerobic plate counts	7.4 ±1.35[A] (5.0-7.9)[B]	6.9 ±1.41 (3.3-7.6)	4.8 ± 0.89 (2.7-5.9)	3.9± 1.0 (1.4-5.1)
Coliform counts	4.7 ± 1.40 (3.5-6.1)	5.8 ± 1.65 (2.7-7.1)	2.6 ± 0.9 (1.0-3.6)	3.3 ± 1.4 (1.8-5.6)
***E. coli* counts**	4.5 ± 1.29 (2.7-4.9)	5.4 ± 1.21 (2.2-6.0)	2.0[a] ± 0.6 (1.0-3.4)	5.0[b] ± 0.9 (1.5-6.9)
***S. aureus* counts**	2.1[a] ± 0.60 (1.0-2.8)	1.5[b] ± 0.42 (1.0-2.3)	1.5* ± 0.2 (1.0-2.2)	1.5* ± 0.2 (1.0-2.2)
***B. cereus* counts**	1.9 ± 0.54 (1.0 – 2.8)	1.7 ± 0.47 (1.0-2.4)	N/A	N/A

* *S. aureus* test conducted on swabs taken from handlers' hands only

Different lowercase superscripts denote a statistically significant ($P<0.05$) difference

N/A = Not applicable

A = Mean ± standard deviations

B = range

Table 3.2: Incidences of *L. monocytogenes* and *Salmonella* spp. in RTE filled baguettes (n = 35) and assorted salads (n = 35) sampled from four retail delicatessens.

		% positive samples	
		Listeria monocytogenes	***Salmonella* spp.**
Filled baguettes	**35 samples**		
Cheese based filling[a]	14 samples	7.0	14.3
Sliced processed meat[b]	14 samples	7.0	21.0
Chicken[c]	7 samples	0	28.5
	Average incidence	**5.7**	**20.0**
Assorted salads	**35 samples**		
Fruit[d]	13 samples	0	7.7
Vegetable[e]	10 samples	0	10.0
Other[f]	12 samples	8.3	20.0
	Average incidence	**2.9**	**11.0**

a Gouda cheese, white cheese, soft cheese plain or with sliced ham, lettuce, tomato, mayonnaise
b Salami, ham, cheese, lettuce, tomato, mayonnaise or processed polony meats, achar (spicy mango and fruit relish) sauce, lettuce, tomato, mayonnaise
c Rotisserie chicken, mayonnaise, lettuce, tomato
d Bananas, green melon, kiwi fruit, paw-paw, pineapple, spanspek (cantaloupe), strawberries, watermelon
e Baby marrows, mushrooms, butternut, tomato and onion
f Lettuce, olives, Feta cheese, cucumber, onion rings, green peppers or sliced processed meats, mayonnaise, green peppers

Table 3.3: Microbiological guidelines suggested for RTE filled baguettes and assorted salads from the retailer participating in this study and the South African Department of Health. In addition the percentage of RTE samples (n = 70) not complying with these recommended guidelines.

	Recommended microbiological guidelines							
	Counts (Log CFU/g)					**Detection (present/absent/25g)**		
Country	**Aerobic plate counts**	**Coliforms**	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus cereus***	***Salmonella* spp.**	***Listeria monocytogenes***	**Reference**
South African retailer	NG	<2	ND	ND	ND	ND	ND	
South African Department of Health	<8	<2.3	ND	<2	<2	ND	ND	Anonymous, 1998
	% of samples <u>not</u> complying with recommended microbiological guidelines							
South African retailer	NG	76	31	41	37	44	13	Table 3.1 and 3.2 Fig. 3.5
South African Department of Health	2.9	83	31	16	13	44	13	

NG= no guideline given
ND= not detected

Table 3.4: Percentage distribution of aerobic bacteria associated with RTE filled baguettes and assorted salads (280 isolates) and associated preparation surfaces (368 isolates) sampled from four retail delicatessens.

	Number of Isolates	% Gram-positive bacteria	% Gram-negative bacteria	% Yeasts
Ready-to-eat food				
Filled baguettes	140	73.6	20.7	5.7
Assorted salads	140	53.5	42.2	4.3
Total	**280**	**63.6**	**31.4**	**5.0**
Preparation food contact surfaces				
Filled baguettes	192	59,8	38.0	2.2
Assorted salads	176	55.2	40.9	3.9
Total	**368**	**57.6**	**39.5**	**2.9**

Table 3.5: Percentage distribution of 280 predominant isolates from aerobic plate counts (APC) of RTE filled baguettes and assorted salads sampled from four retail delicatessens.

	(%) *Bacillus*	(%) *Lactobacillus*	(%) *Micrococcus*	(%) *Staphylococcus*	(%) *Enterococcus*	(%) **Total Gram-positive**	(%) **Gram-negative rod**	(%) **Yeast**	(%) **TOTAL**
Filled baguettes (140 isolates)									
Cheese based filling[a] (56 isolates)	16.1	5.4	0	8.9	28.6	**59.0**	32.1	8.9	**100**
Sliced processed meat (56 isolates)	19.6	19.6	0	26.8	21.4	**87.4**	7.2	5.4	**100**
Chicken (28 isolates)	25	12.5	25	0	12.5	**75.0**	25	0	**100**
	19.3	**12.1**	**5.0**	**14.3**	**22.9**	**73.6**	**20.7**	**5.7**	**100**
Assorted salads (140 isolates)									
Fruit (52 isolates)	19.2	0	11.5	15.4	3.8	**49.9**	46.3	3.8	**100**
Vegetable (40 isolates)	14.3	0	0	31.4	2.8	**48.5**	48.6	2.9	**100**
Other (48 isolates)	22.9	14.6	0	16.6	8.3	**62.4**	33.3	4.3	**100**
	19.2	**5.0**	**4.3**	**20**	**5.0**	**53.5**	**42.2**	**4.3**	**100**

a Refer to Table 3.2 for filling ingredients

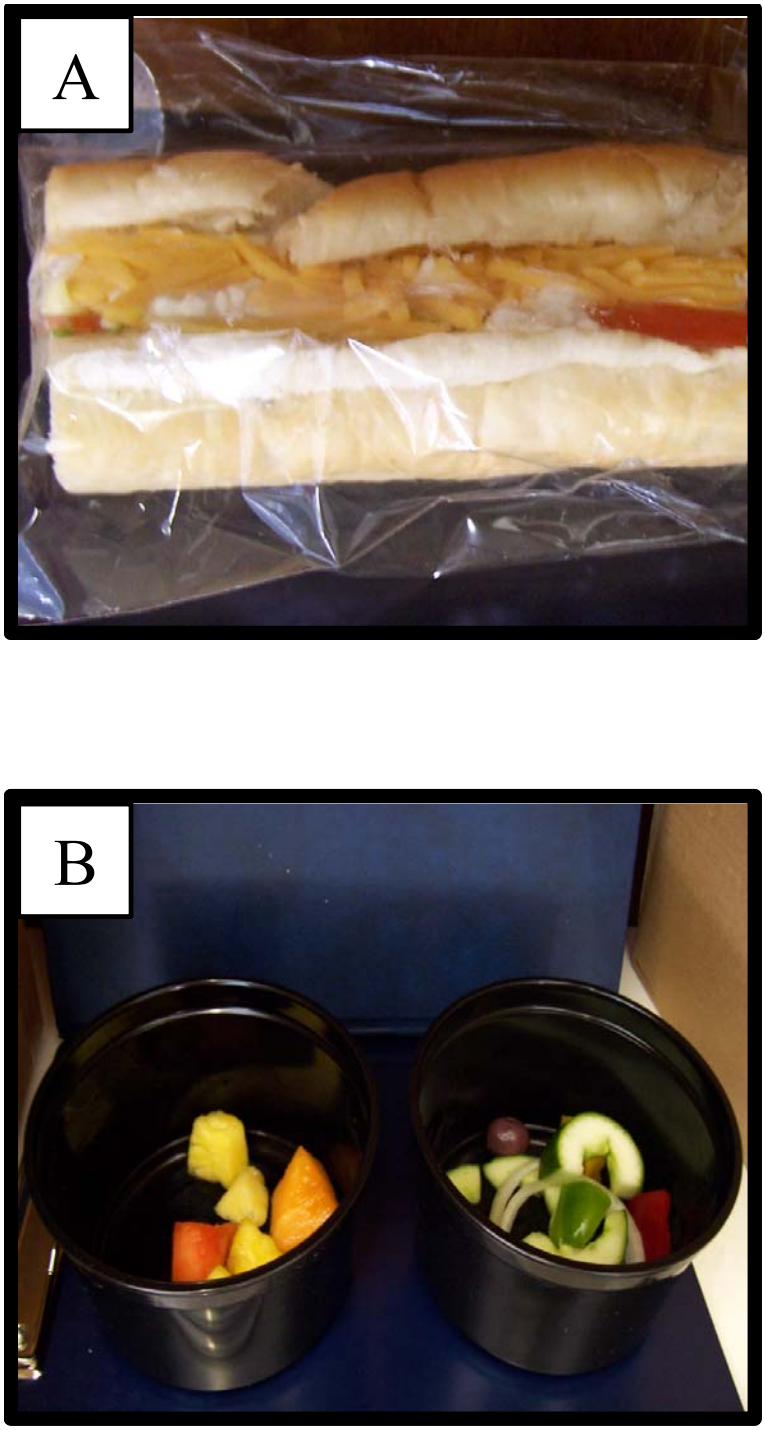


Figure 3.1: Examples of ready-to-eat filled baguettes (A) and assorted salads (B) sampled from four retail delicatessens in Johannesburg.

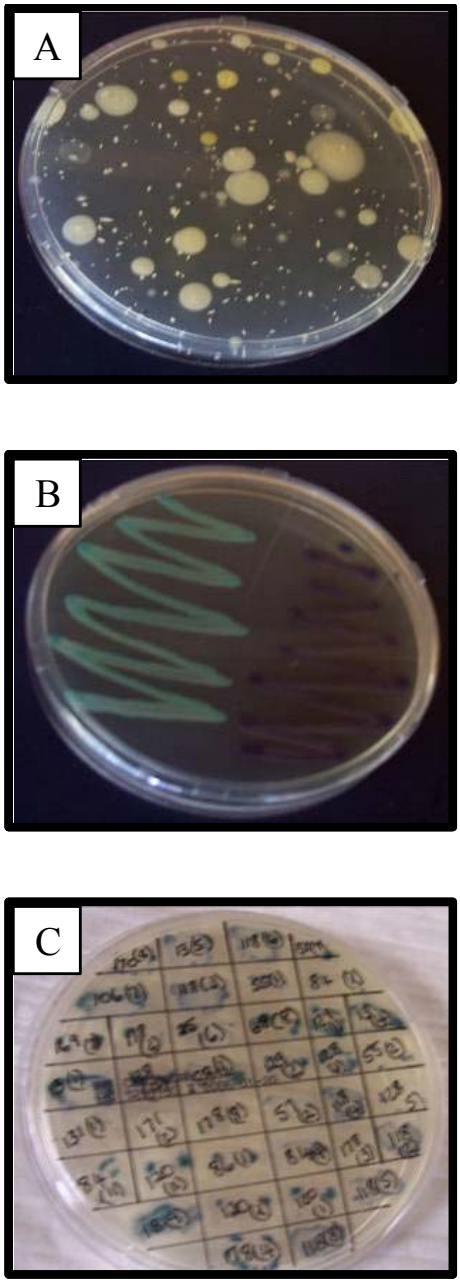


Figure 3.2: Plate Count Agar was used to determine total aerobic plate counts (A), Rapid' *E. coli* 2 agar for coliform (green colonies) and *E. coli* (purple colonies) counts (B) and BD BBL^{TM}CHROMagar^{TM}O157 (BD Biosciences, Becton, Dickinson and Company, USA) used to screen coliform and *E. coli* isolates for *E. coli* O157:H7 (C) in ready-to-eat foods, associated preparation utensils, plastic chopping boards, handlers' hands, display ice and storage refrigerators sampled from retail delicatessens.

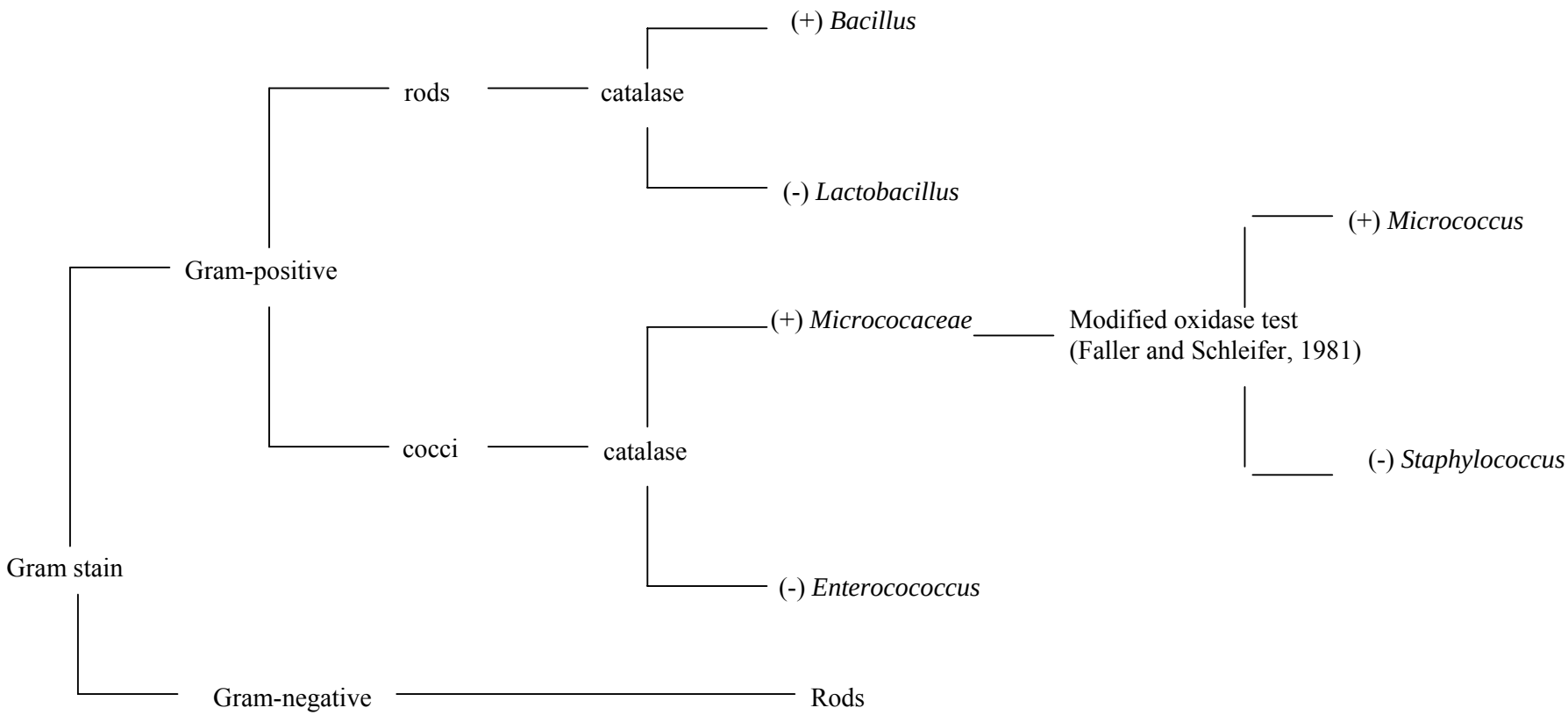


Figure 3.3: Modified characterisation key (after Faller and Schleifer, 1981; Fischer *et al*., 1986) used to characterise bacteria isolated from aerobic plate counts (APC) of filled baguettes, assorted salads, associated preparation utensils, plastic chopping boards, storage refrigerators, handlers' hands and ice used to cool salads during display sampled from four retail delicatessens.

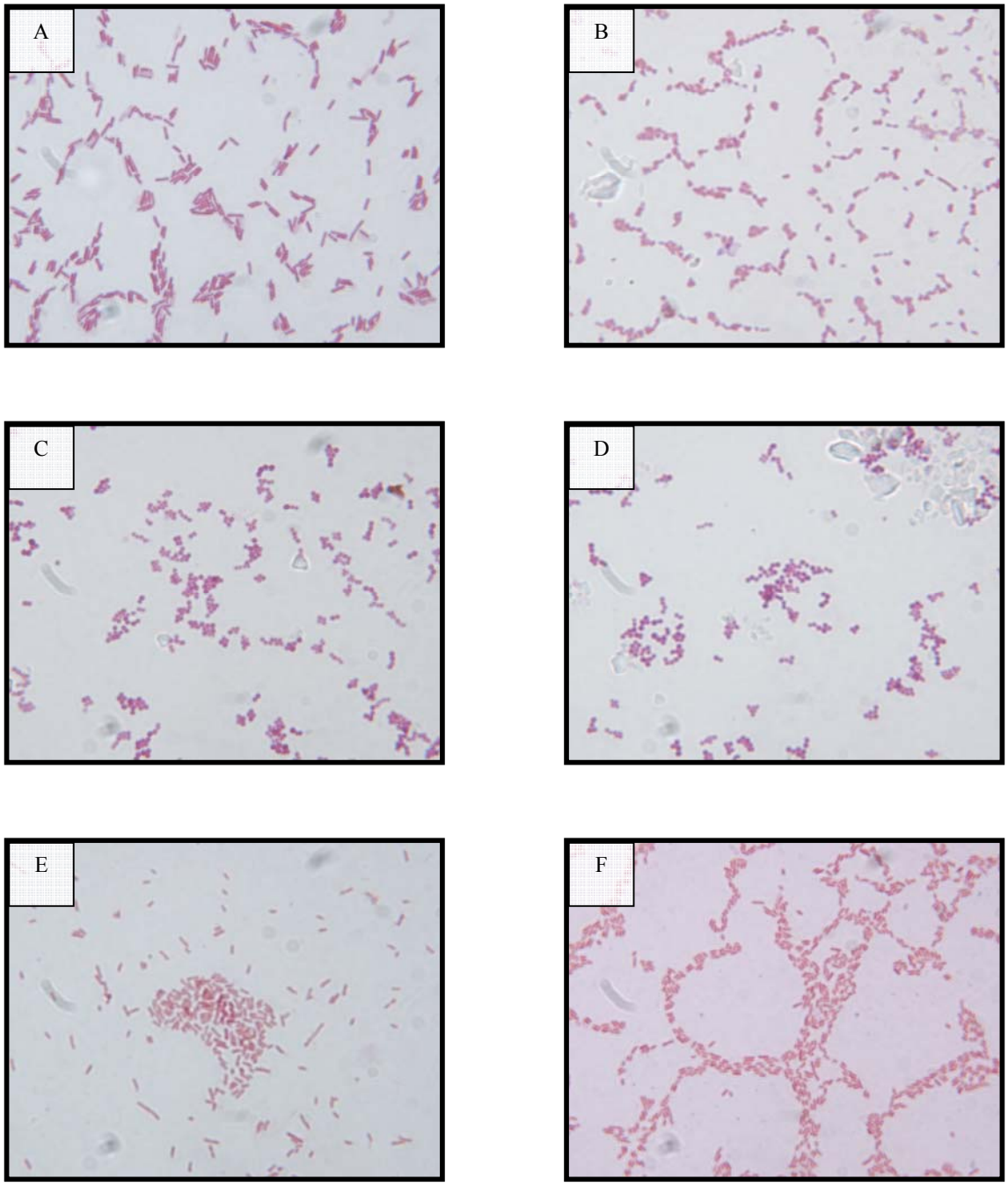


Figure 3.4: Light micrographs (x 1000 oil immersion) depicting morphologies of typical Gram-positive (purple) rod-shaped *Bacillus* (A) and *Lactobacillus* (B), Gram-positive (purple) cocci-shaped *Micrococcus* (C) and *Staphylococcus* (D) and Gram-negative (red) rods (E and F).

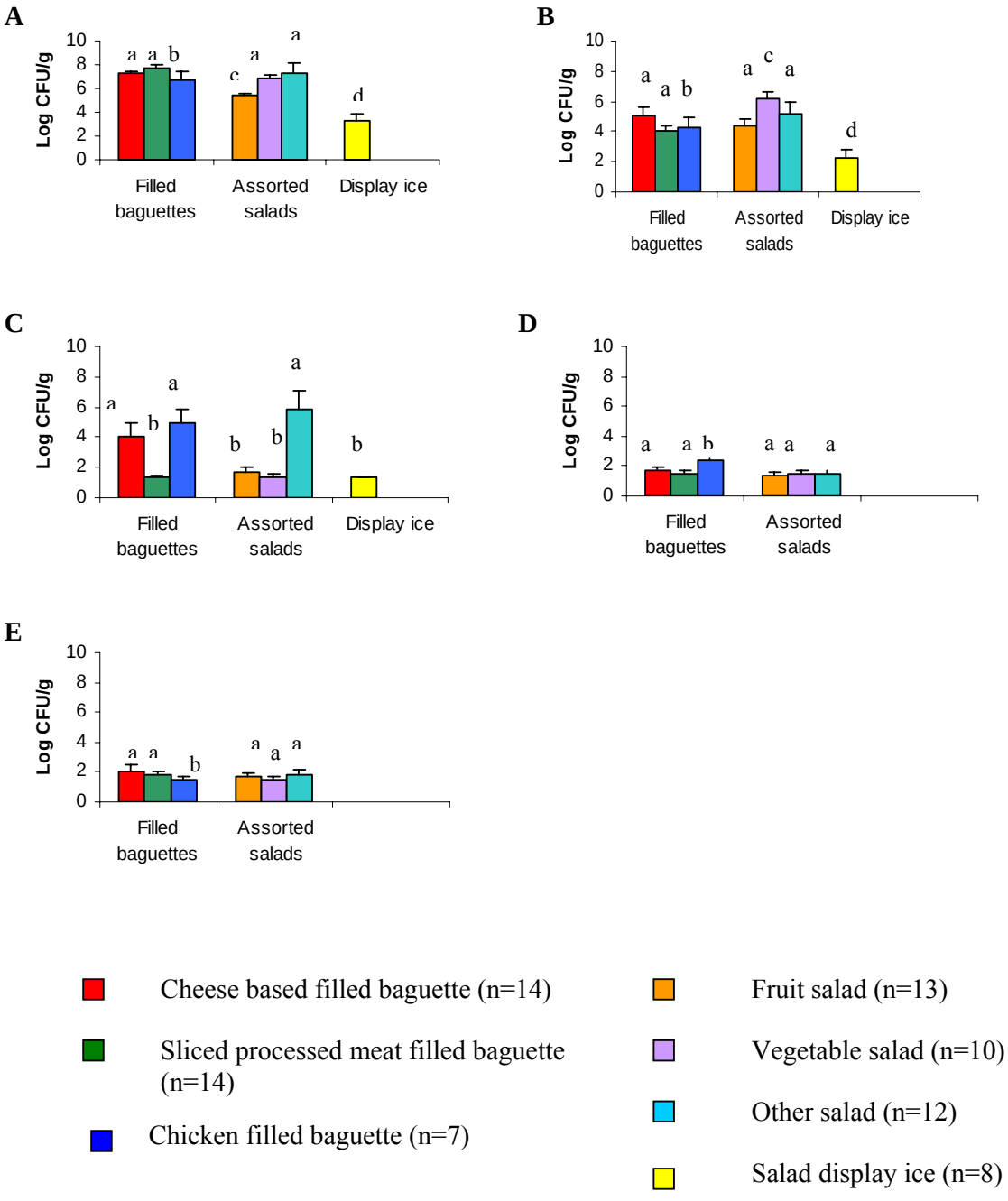


Figure 3.5: Aerobic bacterial (A), coliform (B), *E. coli* (C), *B. cereus* (D) and *S. aureus* (E) counts from filled baguettes, assorted salads and display ice sampled from four retail delicatessens. Means with different superscripts indicate statistically significant differences ($P<0.05$). (Lower detection limit: 1 Log CFU/g).

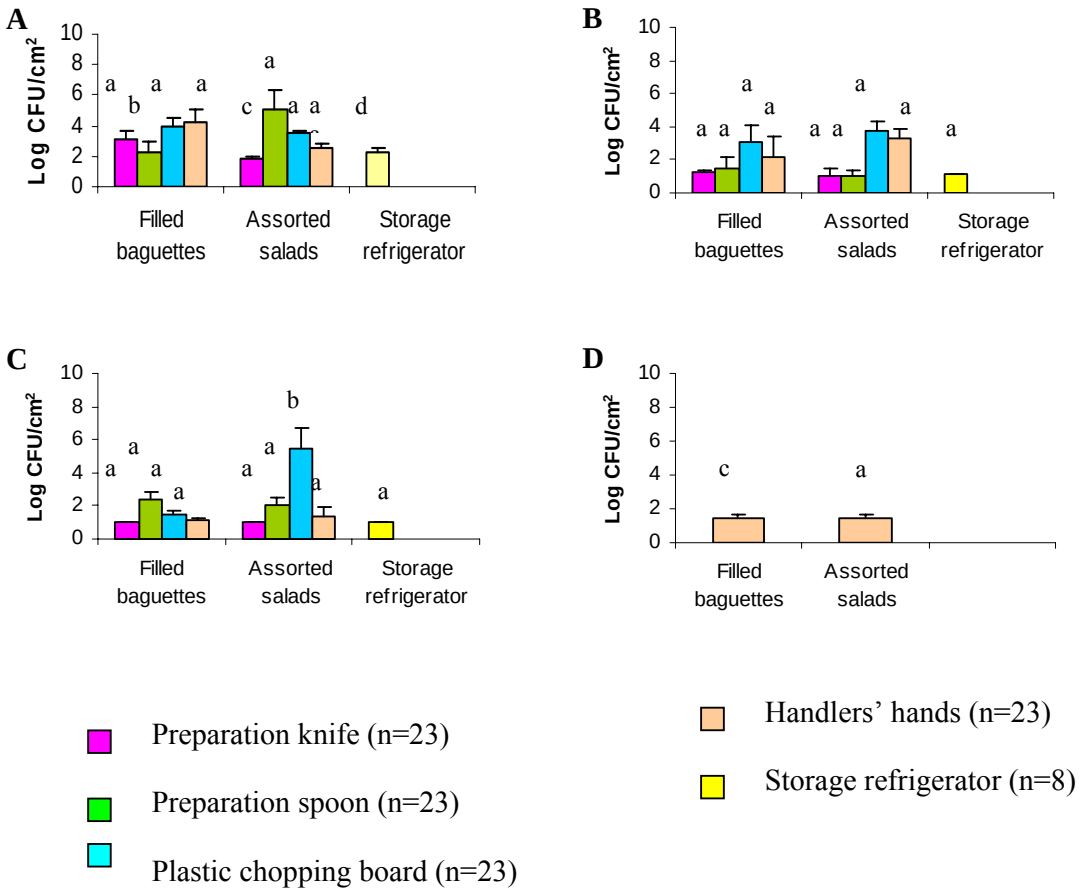


Figure 3.6: Aerobic plate (A), coliform (B) and *E. coli* counts (C) from filled baguettes and assorted salad preparation utensils, plastic chopping boards, handlers' hands and storage refrigerators. In addition *S. aureus* counts (D) for handlers' hands sampled from four retail delicatessens. Means with different superscripts indicate statistically significant differences ($P<0.05$). (Lower detection limit: 1 Log CFU/cm^2).

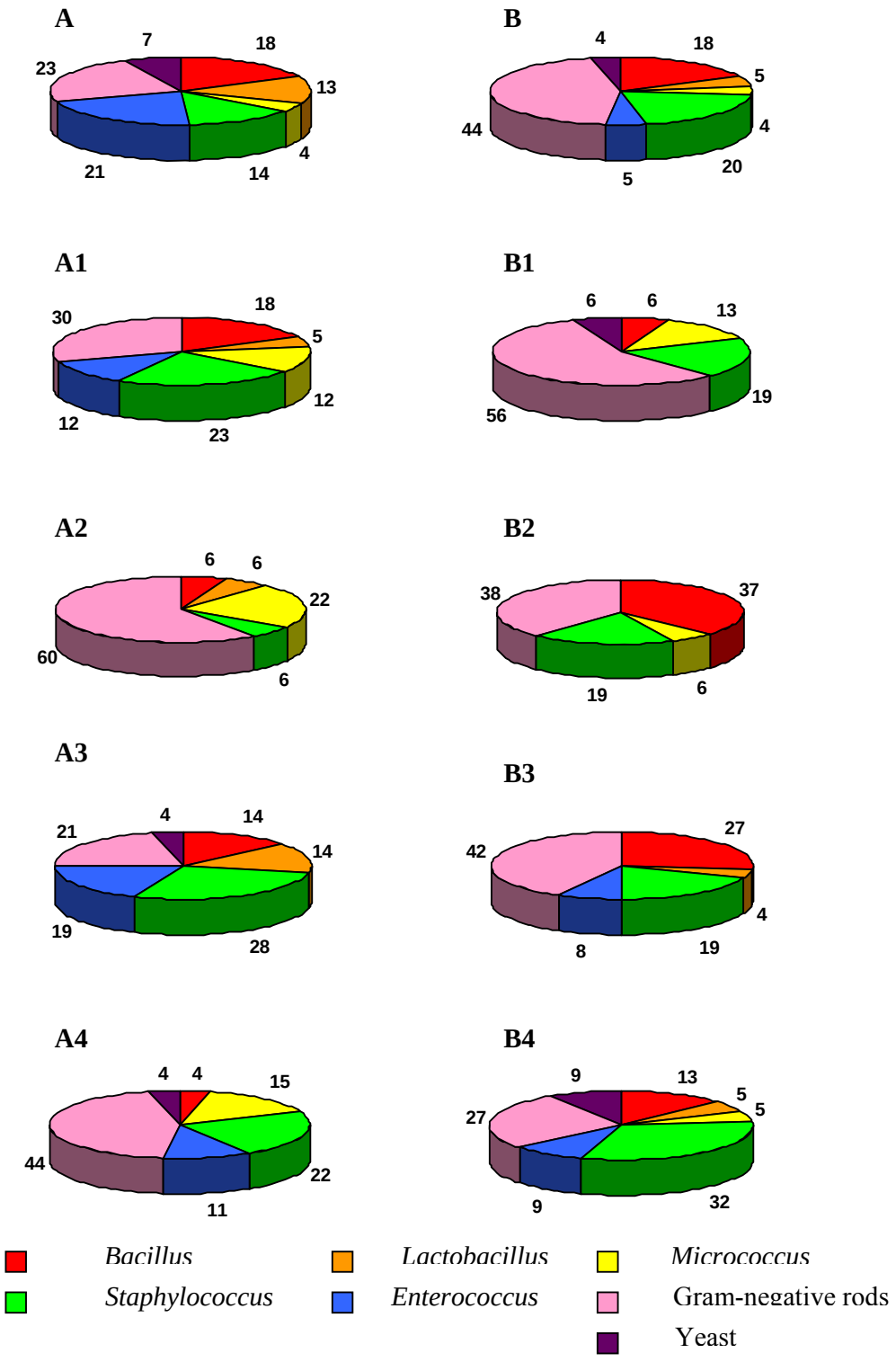


Figure 3.7: Percentage distribution of 648 predominant bacteria isolated from aerobic plate counts of filled baguettes (140 isolates) (A); assorted salads (140 isolates) (B); associated preparation knives (92 isolates) (1); preparation spoons (92 isolates) (2); plastic chopping boards (92 isolates) (3) and handlers' hands (92 isolates) (4) sampled from four retail delicatessens in Johannesburg.

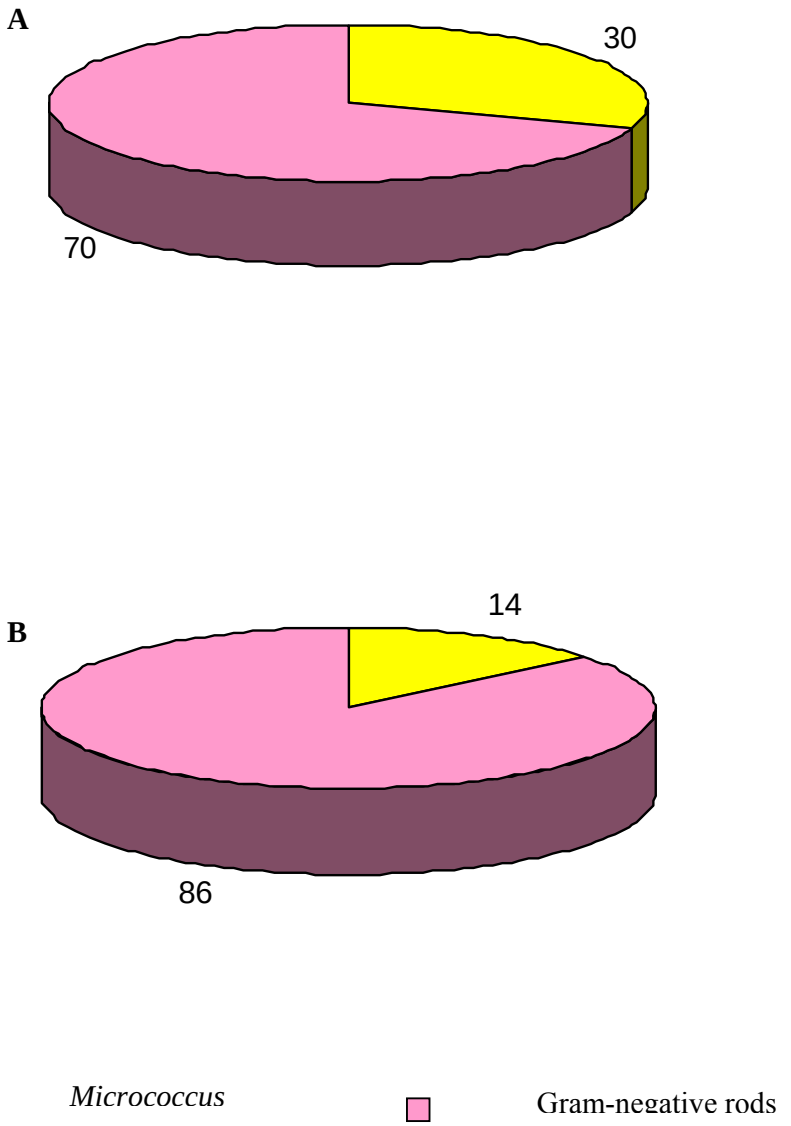


Figure 3.8: Percentage distribution of 64 predominant bacteria isolated from salad display ice (32 isolates) (A) and storage refrigerators (32 isolates) (B) sampled from four retail delicatessens in Johannesburg.

CHAPTER 4

BACTERIAL POPULATIONS ASSOCIATED WITH CLEANING TOOLS AND DISPOSABLE PLASTIC GLOVES FROM FILLED BAGUETTE AND ASSORTED SALAD PREPARATION IN RETAIL DELICATESSEN ENVIRONMENTS

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ABSTRACT

This study assessed the association of bacteria with cleaning tools such as floor mops (n = 25), cleaning cloths (n = 39) and disposable plastic gloves (n = 20), used by food handlers during filled baguette and assorted salad preparation in four retail delicatessens in Johannesburg. One-gram samples of cleaning tools or one glove from each pair were prepared for bacteriological analysis of aerobic plate (APC), coliform (CC), *Escherichia coli* (ECC) and *Bacillus cereus*, *Staphylococcus aureus* counts. A further five-gram sample of cleaning tools or the second glove from each pair was tested for the incidence of *Listeria monocytogenes* (LM) and *Salmonella* (SALM). Duplicate samples were prepared for scanning electron microscopy (SEM) by standard methods. Overall, floor mops yielded the highest APCs (5.7 Log CFU/g), CCs (4.1 Log CFU/g) and ECCs (3.0 Log CFU/g). Disposable plastic gloves yielded the lowest counts (3.6; 2.0; 1.0 Log CFU/cm^2, respectively). Counts of *Bacillus* and *Staphylococcus* associated with both floor mops and cleaning cloths were lower than APC, CC, ECC (*ca*. 1.2 Log CFU/g). Similarly *Salmonella* spp. was detected in only 8% of cleaning tool samples. The incidence of *Listeria monocytogenes* in cleaning cloths (10%) was double that for floor mops (4%). Disposable plastic gloves were only associated with low numbers of SAC (1.1 Log CFU/g). Characterisation of 336 predominant isolates from the APC plates of cleaning tools and disposable plastic gloves indicated that Gram-positive rods and cocci (66.7%) predominated on cleaning tools and disposable plastic gloves. The remaining isolates were comprised of Gram-negative rods (33.3%) and yeasts (6.4%). The latter results corresponded with observations on scanning electron micrographs (SEM) in which both rod- and coccoid shaped bacterial microcolonies were observed to be associated with all cleaning tool types. This is the first study to demonstrate bacterial contamination of retail delicatessen cleaning tools, especially with respect to floor mops. Results suggest that cleaning tools in retail delicatessens may act as reservoirs for the contamination of subsequently prepared RTE foods.

INTRODUCTION

Increases in the incidences of foodborne related illness worldwide has been attributed to changes in the production of foods, especially with regard to minimal processing (Ryan *et al*., 1996). Food contamination during processing is often the primary cause of food recalls, spoilage problems and most importantly foodborne illness outbreaks (Kornacki, 2000). Air, water, tools and workers may further contribute to bacteria in the food-processing environment (Faust and Gabis, 1988; Scott and Bloomfield, 1990).

The attachment of bacteria to food contact surfaces or inanimate objects during processing and their subsequent transfer to foods passing over these surfaces has been documented in previous reports (Roberts, 1982; Ryan *et al*., 1996; Zhao *et al*., 1998; Chen *et al*., 2001). Biotransfer of potential foodborne bacterial pathogens from food contact surfaces to ready-to-eat (RTE) foods during processing is undesirable as these foods are not further processed before consumption (Jay *et al*., 2005). In recent years it has been recognized that cleaning tools may play a role in the contamination of foods if strict hygiene practices are not adhered to. As a result, several studies have been conducted to assess the extent to which cleaning tools contribute to contamination of preparation surfaces and foods in domestic kitchens (Cogan *et al*., 1999; Barker *et al.,* 2003; Mitakakis *et al*., 2004; Rayner *et al*., 2004). Previous studies have suggested that raw ingredients are the primary source of contamination in a domestic kitchen, however, when preparing food, potential foodborne pathogens may be spread through cleaning cloths and sponges (Scott and Bloomfield, 1990; Olsen *et al*., 2000).

For example, previous reports have shown that bacteria may survive for hours or days on hands, sponges and cleaning cloths after initial contact with bacteria (Scott and Bloomfield, 1990; Rusin *et al*., 1998; Kusumaningrum *et al*., 2002). In addition, cross contamination from cleaning cloths, hands and sponges to food contact surfaces and or to RTE foods have been implicated in foodborne disease outbreaks in the USA (Bryan, 1998; Mitakakis *et al.,* 2004; Rayner *et al.,* 2004)

Studies have shown that *Salmonella* spp. on cleaning cloths associated with the preparation of raw chicken in a domestic kitchen were more difficult to remove after overnight storage, compared to cleaning cloths sampled on the day of use (Cogan *et al*., 1999). Furthermore, *Listeria* was found to be more prevalent on cleaning tools, such as cleaning cloths and washing-up brushes, than on equipment surfaces, such as kitchen sinks and vegetable refrigerator compartments (Beumer *et al*., 1996). Similarly, cleaning cloths were sampled from 250 domestic kitchens and investigated for the presence of potential foodborne pathogens (Beumer and Kusumaningrum, 2003). It was found that wet surfaces such as wet cleaning cloths harboured more bacteria than dry surfaces and *Listeria monocytogenes* was isolated from 10% of the cleaning cloths tested. The authors concluded that cleaning cloths, which harboured high numbers of bacteria, would be reservoirs for food contamination (Beumer and Kusumaningrum, 2003). However, few studies, have concentrated on the role that cleaning cloths may play in RTE food contamination in retail delicatessens.

Food handlers' hands also come into direct contact with RTE foods during preparation and have been implicated in the contamination of RTE foods with bacterial foodborne pathogens and subsequent foodborne disease outbreaks (Harrington, 1992). As a result, the use of disposable plastic gloves during RTE food preparation has been suggested as a method to decrease the risk of cross-contamination between food handlers' hands and RTE food (Montville *et al*., 2001). Moreover, studies have shown that the incorrect usage of disposable plastic gloves may exacerbate the contamination issue and that disposable plastic gloves may in fact act as reservoirs for food contamination (Michaels, 2001).

The contamination of cleaning tools and disposable plastic gloves with bacteria is undesirable as these bacteria may be transferred to food contact surfaces and subsequently to RTE foods prepared within the retail delicatessen environment. There have been few reports focusing on the contamination of cleaning tools and disposable plastic gloves with potential foodborne bacterial pathogens in retail delicatessens in South Africa. This study aimed to determine the level of potential foodborne bacterial contamination of cleaning tools and disposable plastic gloves in retail delicatessens in Johannesburg by standard bacterial counts and to observe bacterial populations associated with these tools by scanning electron microscopy (SEM).

MATERIALS AND METHODS

a) Sample collection

Cleaning tools utilized during the preparation of RTE foods were sampled aseptically from four retail delicatessens on four separate occasions. Cleaning cloths (*ca*. 15g) and floor mops (*ca*. 15g) were aseptically removed from the intact cleaning tool by cutting with sterile scissors and placed in 18oz Whirl Pak Bags (Nasco, California, USA). Where disposable plastic gloves were used, one pair was aseptically collected and placed into an 18oz Whirl Pak bag (Nasco). Unused floor mop, cleaning cloth and glove samples were collected in the same manner and used as controls. All samples were transported to the laboratory in a cooler box within 4 hours of sampling and analysed within 24 hours.

b) Bacterial counts

From each cleaning tool, 1g was aseptically removed and for disposable glove samples one glove from a pair was placed in 9ml sterile peptone saline solution (0.1% peptone, 0.85% NaCl) containing 20g sterile glass beads and shaken vigorously by hand for 10 minutes to detach bacterial cells (Lindsay *et al*., 1997; Lindsay *et al.,* 2002). The dislodged cell suspension was allowed to stand at room temperature for 20 minutes to allow for cell recovery (Lindsay *et al*., 1997). Serial ten fold dilutions in sterile peptone saline solution were prepared and plated in duplicate using standard pour- and spread-plate techniques, onto the relevant media for aerobic bacterial (APC) (Bell *et al*., 2005), coliform (CC), *Escherichia* (*E*.) *coli* (ECC)(International Standards Organisation 4832, 1991), *Staphylococcus* (*S*.) *aureus* (SAC) (International Standards Organisation 6888-1, 1992) and *Bacillus* (*B*.) *cereus* counts (BCC) (International Standards Organisation 7932, 1993). Plates were incubated and those containing between 30 and 300 colony-forming units (CFU) (or the highest number if below 30) were counted.

Confirmation of coliforms, *E. coli*, *B. cereus* and *S. aureus* was carried out using the methodology outlined in Chapter 2.

c) *Listeria monocytogenes* and *Salmonella* spp. detection

Examination for *L. monocytogenes* was carried out by a two step enrichment procedure in Fraser broth (Bio-Rad, Marnes-La Coquette, France) according to the EN ISO 11290 method for detection of *L. monocytogenes* (Scotter *et al*., 2001). Pre-enrichment for *Listeria* (*L*.) *monocytogenes* was conducted by homogenizing 5g of the cleaning tool sample (or one glove) in 45ml Fraser ½ Broth (Bio-Rad, Marnes-La-Coquette, France) for 2 minutes in a Colworth 400 Stomacher (Seward Medical) followed by incubation for 24 hours at 30°C. For secondary enrichment 0.1ml of the incubated Fraser ½ Broth was inoculated into 10ml Fraser 1 Broth (Bio-Rad) and incubated at 37°C for 48 hours. Fraser 1 broths showing black discoloration were streaked onto Rapid' *L. mono* agar (Bio-Rad) plates and incubated at 37°C for 48 hours. Blue colonies without yellow halos were indicative of the presence of *L. monocytogenes* (Scotter *et al*., 2001). Isolates which were Gram-positive, catalase positive rods were confirmed as *L. monocytogenes*.

Pre-enrichment for *Salmonella* spp., was conducted by homogenizing 5g of the cleaning tool sample (or one glove) in 45ml Buffered Peptone Water (BPW) (Oxoid, Basingstoke, UK) for 2 minutes in a Colworth 400 stomacher (Seward Medical) and incubating for 18 hours at 37°C. Aliquots (0.1ml) of the enriched culture in BPW were inoculated into 10ml Rappaport-Vassiliadis Broth (RVB) (Scharlau) and incubated at 41.5°C for 24 hours. A second aliquot (1ml) of the enriched culture in BPW was inoculated into 10ml Müller-Kauffmann Medium (MKM) (Scharlau, Barcelona, Spain) plus Brilliant Green and Novobiocin Selective Supplement (1vial / 500ml) (Scharlau) and 200µl Gram's Iodine, and incubated at 30°C for 24 hours. A loopful of the MKM and RVB were each streaked onto Xylose-Lysine Deoxycholate (XLD) agar (Scharlau) and Brilliant Green Modified Agar (BGAM) (Scharlau) (South African Bureau of Standards 6579:1993, 1993). Red colonies with black centres were counted as presumptive *Salmonella* spp. (South African Bureau of Standards 6579:1993, 1993). Isolates which were Gram-negative, oxidase negative rods and showed a fermentative reaction for the Oxidative-Fermentative test were confirmed as *Salmonella* spp. (South African Bureau of Standards, 6579:1993, 1993).

d) Statistical analysis

Counts for the cleaning tools and disposable plastic gloves were meaned over the four surveys and standard deviations between samples were calculated. In addition, colony count data was statistically analysed using the analysis of variance (ANOVA) test at the 95% confidence level (STATGRAPHICS 7.0, Manugistics Inc. and Statistical Graphics Corporation, USA) programme.

e) Isolation and characterization of predominant bacterial populations

Four colonies (von Holy and Holzapfel, 1988) for each of the floor mops (100 isolates), cleaning cloths (156 isolates) and disposable plastic gloves (80 isolates) were selected from APC plates of the highest dilution showing growth (von Holy and Holzapfel, 1988). A total of 336 predominant bacterial isolates were obtained in this way. The bacterial isolates were purified by standard methods (von Holy and Holzapfel, 1988) and characterized according to the dichotomous key of Fischer *et al*. (1986) (Chapter 3). In addition, isolates falling into the *Micrococcus* group were further divided into *Micrococcus* and *Staphylococcus* using a modified oxidase test (Faller and Schleifer, 1981). Gram-negative bacterial isolates were not further characterized. An incubation temperature of 30˚C was used for all biochemical tests.

f) Scanning electron microscopy

Approximately 1cm^2 samples of cleaning cloth and floor mop samples and the thumb area of disposable plastic gloves, were fixed overnight in 3% gluteraldehyde and dehydrated in an ethanol series (Lindsay *et al*., 1996; Lindsay *et al*., 2002). Samples were critically point dried, mounted, coated with carbon and gold /palladium and viewed under a JSM-840 Scanning Electron Microscope (Lindsay *et al*., 1996; Lindsay *et al*., 2002).

RESULTS

Results from the four replicate surveys are shown in Tables 4.1 and 4.2 and Figures 4.2, 4.3 and 4.4.

a) Bacterial counts associated with cleaning tools and disposable plastic gloves

Overall the highest bacterial counts were obtained from mop samples with highest APCs followed by CCs and ECCs (5.7; 4.1 and 3.0 Log CFU/g, respectively) (Fig. 4.2). Similarly APCs of *ca*. 5.5 Log CFU/g, were obtained from cleaning cloth samples, while CC and ECCs were *ca*. 1.5 Log CFU/g lower than corresponding counts obtained from floor mop samples (Fig. 4.2). No statistically significant differences ($P>0.05$) between counts obtained for cleaning cloths used during the preparation of filled baguettes or assorted salads were found. The lowest bacterial counts were obtained for disposable plastic gloves (3.6 and 2 Log CFU/g) for aerobic plate counts and coliform counts, respectively and *E. coli* counts were below the lower detection limit (1 Log CFU/g) (Fig 4.2).

B. cereus and *S. aureus* were associated with both floor mops and cleaning cloths in low numbers (*ca*. 1.2 Log CFU/g) (Fig. 4.2). *Salmonella* was detected in 8% of floor mops and cleaning cloth samples (Table 4.1), while *L. monocytogenes* was associated with 4% floor mops and 10% cleaning cloths. By contrast, disposable plastic gloves were only associated with low numbers of *S. aureus* (1.1 Log CFU/g).

b) Predominant bacterial populations associated with cleaning tools and disposable plastic gloves

The percentage distribution of predominant bacterial populations (336 isolates) associated with cleaning tools and disposable plastic gloves are shown in Fig. 4.3. Gram-positive cocci and rods (60.2%) collectively predominated on cleaning tools and disposable plastic gloves, while the remaining isolates comprised of Gram-negative (35.3%) rods and yeasts (4.5%) (Table 4.2).

Gram-positive and Gram-negative genera were isolated in approximately equal proportions from floor mops (100 isolates) (Table 4.2). *Bacillus* (21.1%), *Micrococcus* (10.5%) and *Staphylococcus* (21.1%) collectively comprised 52.6% (Fig. 4.3) whilst the remaining 42.1% of isolates were Gram-negative rods and 5.3% yeasts (Table 4.2). Interestingly, the proportions of bacterial isolates obtained from floor mops and cleaning cloths used during filled baguette preparation were proportionally similar (Fig. 4.3).

Gram-positive (58.3%) and Gram-negative (41.7%) bacteria were present on filled baguette cleaning cloths (80 isolates) in approximately equal proportions (Table 4.2 and Fig. 4.3). By contrast Gram-positive cocci and rods (65.2%) predominated on cleaning cloths (76 isolates) sampled during assorted salad preparation (Table 4.2 and Fig 4.3). In addition a small proportion of yeast were also isolated from the latter cleaning cloths (13.1%) (Table 4.2 and Fig. 4.3).

Predominant isolates associated with disposable plastic gloves (80 isolates) were Gram-positive and included *Bacillus* (16.7%), *Micrococcus* (16.7%) and *Staphylococcus* (33.3%), which collectively comprised 66.7% (Table 4.2 and Fig. 4.3). The remaining isolates were composed of Gram-negative rods (33.3%) (Table 4.2 and Fig. 4.3).

c) Scanning electron microscopy of cleaning tools and disposable plastic gloves

Scanning electron micrographs of unused (control) cleaning tools showed that floor mops and cleaning cloths were characterized by strands and fibers interlinking and crossing on different focal planes (Fig. 4.4A and 4.4F), whilst disposable plastic gloves showed smooth surfaces punctuated with micropores (Fig. 4.4I). No bacterial cells were attached to any of the controls. SEM qualitatively showed high densities of rod and coccoid shaped bacterial cells attached to the fibers of floor mops (Fig. 4B-D) and cleaning cloths (Fig. 4F-H). Predominantly coccoid-shaped bacteria were attached to the smooth surfaces of disposable plastic gloves (Fig. 4J-L).

DISCUSSION

a) Bacterial counts associated with cleaning tools and disposable plastic gloves

Previous studies have shown that bacterial attachment to surfaces occurs more readily if the environment is moist (Allan *et al*., 2004). Within the retail delicatessen environment of this study, floor mops were used for several weeks to clean floors and soaked in dilute sanitizer solutions when not in use. Thus, an ideal environment was provided for bacterial attachment to and growth on floor mop fibres. The numbers of aerobic bacteria associated with floor mops was relatively high (5.7 Log CFU/g). In addition it has previously been noted that cleaning operations often result in aerosols (Burfoot *et al*., 2003) which may in turn contaminate food contact surfaces. Aerosol droplets generated from contaminated floor mops during cleaning in this study may have carried potential foodborne pathogens which in turn may have led to contamination of food contact surfaces. Thus, whilst floor mops do not come into direct contact with RTE foods they may contain potential foodborne pathogens. Results from the present study have shown that potential foodborne pathogens such as *S. aureus*, *B. cereus*, *L. monocytogenes* and *Salmonella* spp., are harboured on retail delicatessen floor mops. Therefore floor mops may act as potential reservoirs for the contamination of RTE foods with potential foodborne bacterial pathogens.

Furthermore, previous studies have shown that cleaning cloths become heavily contaminated with many genera of bacteria after only a short period of use in domestic kitchen environments. These cleaning cloths may subsequently carry and transfer bacteria to other food contact surfaces and thus indirectly contaminate foods (Scott and Bloomfield, 1990, Scott and Bloomfield 1993, Cogan *et al*., 1999 and Hilton and Austin, 2000). For example, the transfer of *S. aureus* from artificially contaminated kitchen sponges to stainless steel surfaces and subsequently to RTE food has been previously demonstrated (Kusumaningrum *et al*., 2003). In addition, *S. aureus* was recovered from these stainless steel surfaces for up to 4 days after initial contamination (Kusumaningrum *et al*., 2003). Cleaning cloths sampled from other retail kitchens also producing RTE foods have been found to harbour similar numbers of aerobic bacteria such as *Enterobacteriaceae*, *E. coli* and *S. aureus* compared to bacterial counts obtained from food contact surfaces (Sagoo *et al*., 2003).

Previous studies conducted by Beumer and te Giffel (1999), showed the presence of coliforms associated with domestic cleaning cloths. In our study, coliforms and *E. coli* were also isolated from cleaning cloths of each of the four retail delicatessens evaluated. In concurrence with previous studies (Beumer and te Giffel, 1999) *S. aureus* and *B. cereus* were present in low numbers on cleaning cloth samples which were soaked in dilute sanitizer.

In a laboratory test kitchen, *Salmonella* spp. were reportedly transferred during the handling of chickens to food contact surfaces and cleaning cloths (Cogan *et al*., 2002; Gorman *et al*., 2002; Barker *et al*., 2003). Reports have shown that *Salmonella* spp. are generally only detected on cleaning cloths if they are sampled during or directly after food preparation (Beumer *et al.,* 1996). In the present study, cleaning cloths were sampled during or directly after RTE food preparation and 8% were subsequently positive for *Salmonella* spp. Previous results have further shown that *L. monocytogenes* was detected in 10-37% of domestic cleaning cleaning cloth samples elsewhere (Beumer *et al*., 1996; Beumer and te Giffel 1999). Similarly *L. monocytogenes* was detected in 10% of cleaning cloths sampled from retail delicatessens in the present study.

The biotransfer potential shown for foodborne pathogens in previous studies (Beumer and te Giffel 1996; Gorman *et al*., 2002; Kusumaningrum *et al*., 2003) together with the presence of potential foodborne pathogens associated with cleaning cloths in the present study, suggested that the latter cleaning cloths may act as potential reservoirs for foodborne pathogen contamination of RTE foods prepared in the retail delicatessen.

Previous reports have shown that greater numbers of bacteria, especially coliforms and *E. coli*, are harboured on gloved hands compared to bare hands during the preparation of foods. Gloved hands may be problematic as it has been observed that food handlers tend to wear the same pair of disposable plastic gloves over extended periods of time (Lynch *et al*., 2005). Previous studies have therefore concluded that the wearing of gloves by food handlers may be counter-productive in preventing contamination as handlers may become complacent with respect to frequency of hand-

washing (Michaels, 2001; Lynch *et al*., 2005; Paulson, 2005). By contrast counts of aerobic bacteria, coliforms and *E. coli* associated with disposable plastic gloves in this study showed that good glove practices were used by the food handlers.

b) Predominant bacterial populations associated with cleaning tools and disposable plastic gloves

The predominance of Gram-positive (60,2%) genera on cleaning tools and disposable plastic gloves may be from the food being prepared. *Bacillus*, *Lactobacillus* and *Enterococcus* are naturally associated with bread, sliced processed meat and raw vegetables and fruit (Jay *et al*., 2005). In addition *Micrococcus* and *Staphylococcus* are reportedly associated with human skin (Jay *et al*., 2005) and as RTE foods are prepared by hand, their presence on cleaning tools and disposable plastic gloves may be expected. The Gram-negative populations corresponded with counts as coliforms and *E. coli* were found associated with the cleaning tools and disposable plastic gloves. In addition the predominance of *Micrococcus* (16.7%) and *Staphylococcus* (33.3%) collectively on disposable plastic gloves was not surprising as both bacteria are associated with human skin if hands are not adequately washed (Jay *et al*., 2005).

c) Scanning electron microscopy of cleaning tools and disposable plastic gloves

The different microcolony morphologies observed by SEM suggested the attachment of several groups of bacteria to floor mops and cleaning cloths. For example, observations of rod-shaped bacteria corresponded to counts of coliforms, *E. coli*, *B. cereus*, and incidences of *Salmonella* spp. and *L. monocytogenes.* Similarly, observations of coccoid-shaped cells particularly associated with disposable plastic gloves corresponded to counts of *S. aureus* (Jay *et al*., 2005).

In this study, cleaning cloths were used in practice for a maximum of 24 hours. However, if a cleaning cloth was visually soiled it was discarded and a new cleaning cloth used. Previous laboratory controlled experiments have shown that domestic kitchen sponges may transfer bacteria to food contact surfaces and hands within minutes of contamination (Kusumaningrum *et al.,* 2002; Rayner *et al*., 2004), thus

highlighting the potential for attached bacteria to be spread from cleaning tools to food contact surfaces, hands or directly to food.

CONCLUSION

Results from this study showed that good glove practices were used by the delicatessen staff as coliform, *E. coli* and *S. aureus* counts were low. However, cleaning tools harboured aerobic and potential foodborne pathogens. The presence of these bacteria on cleaning tools is undesirable as the cleaning tools may disseminate bacterial pathogens throughout the delicatessen. Therefore cleaning tools may potentially act as reservoirs for the contamination of ready-to-eat foods in retail delicatessens. Thus, to minimize the spread of bacteria in the delicatessen by the cleaning tools, we suggest improved and more frequent cleaning and sanitation regimes. In addition, training in the correct usage practices for the cleaning tools may decrease the bacterial numbers. SEM was a useful tool for the visualization of bacterial contamination on cleaning tools and disposable plastic gloves associated with filled baguette and assorted salad preparation in retail delicatessens.

Table 4.1: Incidence of *Salmonella* spp. and *Listeria monocytogenes* on floor mops (n=25), cleaning cloths (n=39) and disposable plastic gloves (n=20) sampled from four retail delicatessens during the preparation of filled baguettes and assorted salads.

Sample type	**Percentage (%) positive samples**	
	***Salmonella* spp.**	***Listeria monocytogenes***
Floor mop (n=25)	**8**	**4**
Cleaning cloth		
Filled baguette (n=20)	5	10
Assorted salads (n=19)	11	11
Total (n=39)	**8**	**10**
Disposable plastic glove (n=20)	**0**	**0**

Table 4.2: Percentage distribution of predominant microbial isolates from floor mops (100 isolates), cleaning cloths (156 isolates) and disposable plastic gloves (80 isolates) sampled from four retail delicatessens during the preparation of filled baguettes and assorted salads.

Sample type	Number of isolates	% Gram-positive	% Gram-negative	% Yeast	Total
Floor mops[a]	100 isolates	52.6	42.1	5.3	100
Cleaning cloths					
Filled baguettes[b]	80 isolates	58.3	41.7	0	100
Assorted salads[b]	76 isolates	65.2	21.7	13.1	100
Total	156 isolates	61.7	31.9	6.4	100
Disposable plastic gloves[c]	80 isolates	66.7	33.3	0	100
Mean	**336 isolates**	**60.2**	**35.3**	**4.5**	**100**

[a] Floor mops used to clean the delicatessen floor in all ready-to-eat food preparation areas

[b] Cleaning cloths used in areas dedicated to the preparation of one ready-to-eat food type

[c] Disposable plastic gloves used interchangeably between the preparation of different ready-to-eat foods



Figure 4.1: Aseptic collection of cleaning tools such as a floor mop (A) used to clean the delicatessen floor in both the filled baguette and assorted salad preparation areas, dedicated cleaning cloths (B) used to clean food contact surfaces in either the filled baguette or assorted salad preparation area and disposable plastic gloves (C) used as a barrier between the handlers' hands and food during the preparation of filled baguettes and assorted salads from four retail delicatessens.

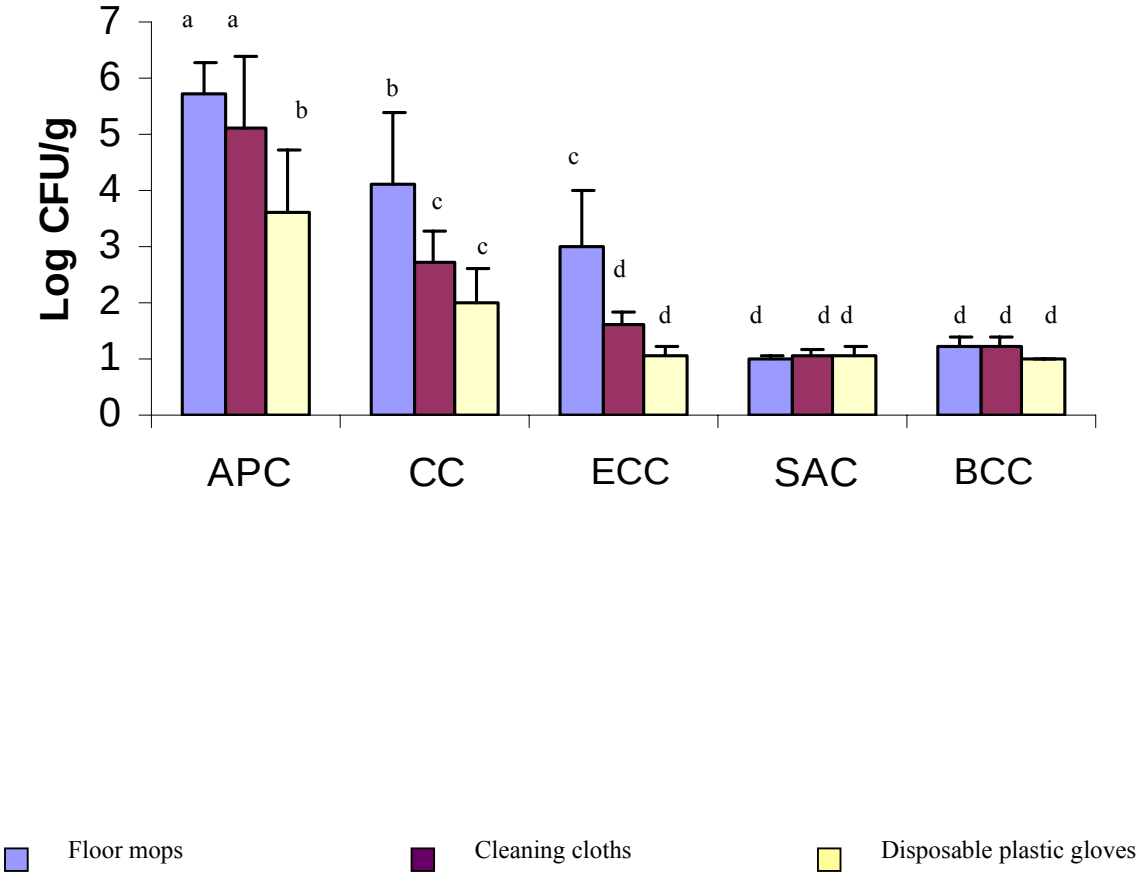


Figure 4.2: Mean aerobic bacterial (APC), coliform (CC), *E. coli* (ECC), *S. aureus* (SAC) and *B. cereus* counts (BCC) on cleaning tools such as floor mops, cleaning cloths and disposable plastic gloves sampled from four retail delicatessens during the preparation of filled baguettes and assorted salads. (Lower detection limit: 1 Log CFU/g)

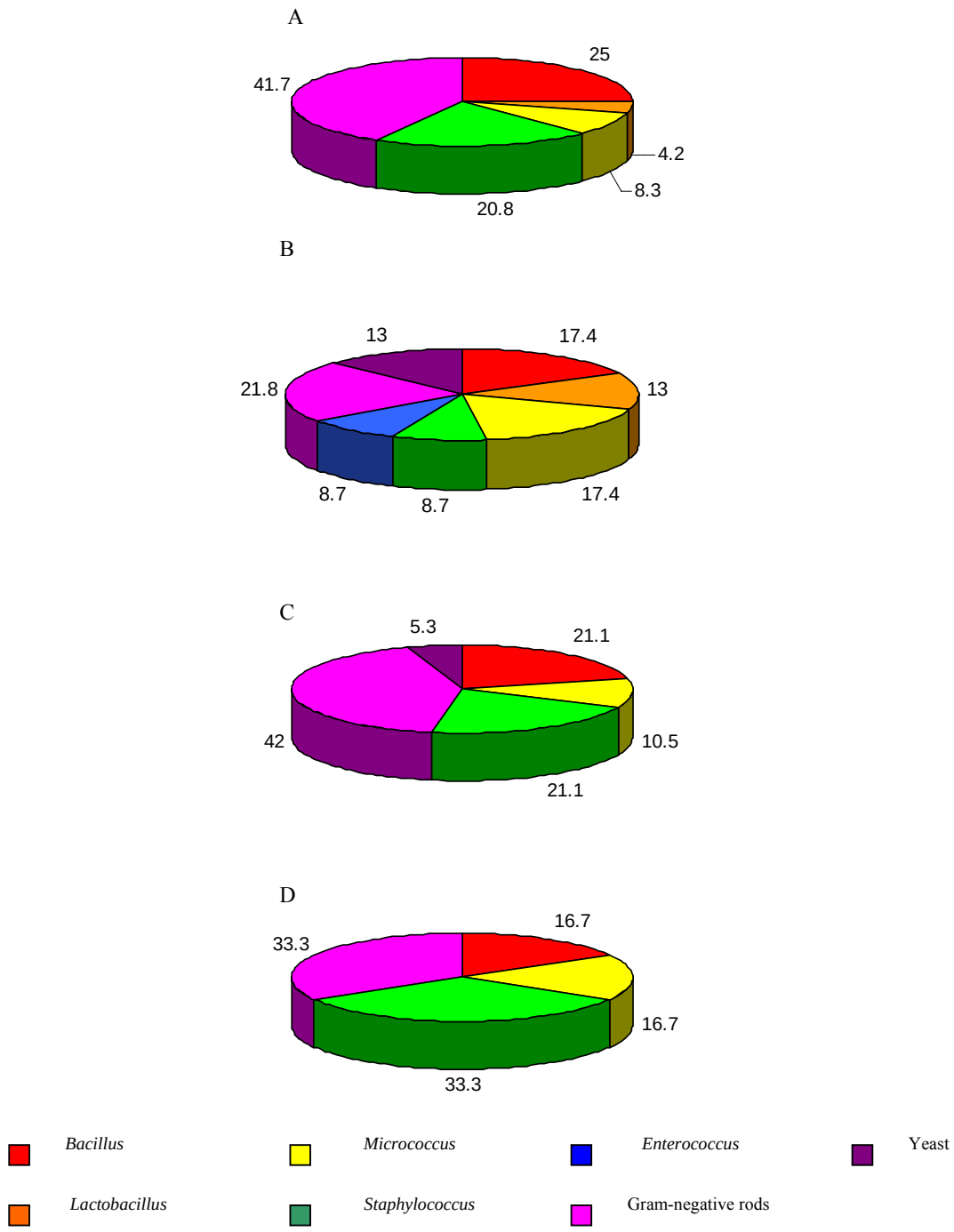


Figure 4.3: Percentage distribution of predominant microbial isolates from aerobic plate counts of floor mops (A) (100 isolates), cleaning cloths used during filled baguette preparation (B) (80 isolates), cleaning cloths used during assorted salad preparation (C) (76 isolates) and disposable plastic gloves (D) (80 isolates) sampled from four retail delicatessens during the preparation of filled baguettes and assorted salads.

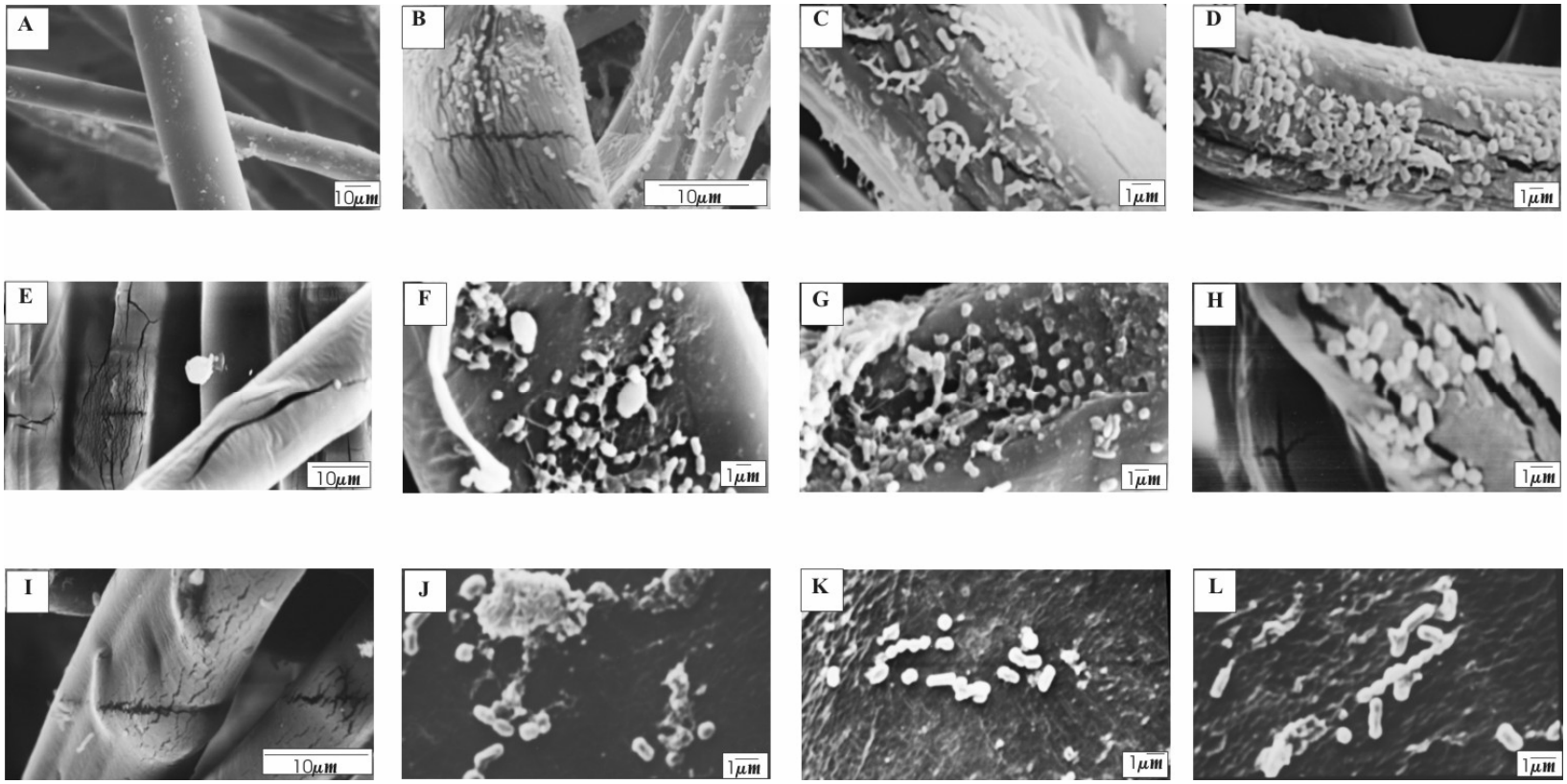


Figure 4.4: Scanning electron micrographs of an uncolonised, (control) floor mop (A), cleaning cloth (E) and disposable glove (I), and used floor mops (B-D), cleaning cloths (F-H) and disposable gloves (J-L) sampled from four retail delicatessens during filled baguette and assorted salad preparation.

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CHAPTER 5

SUMMARIZING DISCUSSION AND CONCLUSION

The consumer demand for ready-to-eat (RTE) foods, which are minimally processed and consumed without further processing, has increased in recent years. As a result of increased demand a larger variety of RTE foods have become available, each reportedly with their own quality and food safety constraints. There is little published data on the microbiological safety of RTE foods sold by retail delicatessens in South Africa, especially with respect to the sources of bacterial contamination during the preparation of these RTE foods.

Microbiological survey of retail delicatessens (A pilot study)

As a result of limited microbiological data on RTE delicatessen foods in South Africa, an initial microbiological survey of four RTE foods (filled baguettes, assorted salads, sliced processed meats and hot meals) sold by four retail delicatessens in Johannesburg was conducted. In addition, a pilot study was conducted in order to set the parameters for the remainder of the study. Results showed that filled baguettes and assorted salads had the highest mean bacterial counts and incidences of potential foodborne pathogens. It was concluded that further investigation was required in order to determine the full microbiological load on these RTE food types. Sliced processed meats had similar aerobic bacterial counts to the filled baguettes and assorted salads. However sliced processed meats are reportedly associated with lactic acid bacteria, which may account for a large portion of the aerobic bacteria associated with sliced processed meats (Jay *et al*., 2005). Lactic acid bacteria are generally associated with spoilage and as this study focused on the safety of delicatessen RTE foods, the sliced processed meats were not further investigated. Interestingly, results showed that vacuum packaging of the sliced processed meats did not statistically significantly ($P<0.05$) alter the bacterial counts and incidences of potential foodborne pathogens. This may be attributed to the short storage time period of the vacuum package sliced processed meats. Previous reports have mentioned that hot meals may be subject to post processing contamination by food handlers' and from the delicatessen environment. By contrast, the hot meals sampled in this study contained low aerobic bacterial counts and the incidences of potential foodborne pathogens were below the lower detection limit. These results indicated that good manufacturing practices such as adequate cooking and holding temperatures of the hot meals were implemented by the delicatessen staff.

Furthermore, previous surveys of domestic kitchens and school canteens have shown that food contact surfaces within the preparation areas may harbour bacteria and subsequently shed these bacteria onto the food passing over these surfaces. Therefore the food contact surfaces within the retail delicatessens were surveyed so as to determine their bacteriological status and identify possible sources of RTE contamination. It has been documented that the method of swabbing food contact surfaces does not provide the exact numbers of bacteria associated with surfaces (Moore and Griffith, 2002), however, for ease of use the swabbing method was used in this study as a method for monitoring the cleanliness of food contact surfaces within the retail delicatessens. Results showed that overall, the food contact surfaces such as preparation knives, spoons, plastic chopping boards and handlers' hands exhibited low numbers of aerobic bacteria. The presence of low numbers of coliforms and no *Escherichia (E.) coli* suggested that adequate cleaning and sanitation of the food contact surfaces was practiced.

In addition, the bacterial load on cleaning tools such as floor mops and cleaning cloths was investigated. The general aim of cleaning tools is to remove food soils and reduce bacterial contamination on food contact surfaces. However, previous findings have implicated cleaning tools in the harbourage and transfer of bacteria within food preparation environments. Interestingly high numbers of aerobic bacteria were associated with the floor mops and cleaning cloths in this study and whilst these bacteria may not necessarily be potential foodborne pathogens, the spread of these bacteria within the retail delicatessen is undesirable. Furthermore the isolation of coliform and *E. coli* on both floor mops and cleaning cloths suggested that potential foodborne bacteria may be harboured within the cleaning tools. It was concluded that further floor mop and cleaning cloth samples be analysed for potential foodborne pathogens.

Low numbers of aerobic bacteria and *Staphylococcus (S.) aureus* on the disposable plastic gloves indicated that good glove practices were employed by delicatessen staff. In addition it was observed that through effective training all food handlers' were well aware of correct glove usage.

The preliminary incubation (PI) technique has been shown in a previous study to increase the numbers of low levels of bacterial contaminants such as *E. coli*, *Enterococcus* and coliforms in commercial yeast (O'Brien *et al*., 2004). Bacterial foodborne pathogens are often present in foods in low numbers and their detection is imperative for ensuring food safety. Therefore a modification of the PI method was used as a method to simulate mild temperature abuse of the RTE foods. Results showed no statistically significant ($P<0.05$) increase in bacterial counts when RTE food samples were incubated at 15°C for 18 hours. Interestingly, the detection of *Listeria* (*L*.) *monocytogenes* and *Salmonella* spp. decreased after PI. The PI study therefore showed that the RTE foods prepared by the four retail delicatessens can withstand mild temperature abuse and confirms that the 1-day shelf life of the foods is adequate.

The delicatessen is a unique food preparation environment as the environmental conditions of the delicatessen are not as controlled as those in a factory setting. In addition, the food handlers' not only prepare RTE foods but also interact with customers. Of the RTE foods selected for this study, the greatest demand was shown for the hot meals. As a result of this demand there were generally food handlers' dedicated to serving the customers and different food handlers' preparing the hot meals. Interestingly, the aerobic bacterial numbers of the hot meals were low and the incidence of potential foodborne pathogens were below the lower detection limit. By contrast, the demand for the filled baguettes, assorted salads and sliced processed meats was generally lower than the hot meals and the food handlers' served customers in between preparing these RTE food types. It was observed that the food handlers' seldom washed their hands when moving from food preparation to customer service and back to food preparation. Communal areas such as the scale used to weigh and price the salads and sliced processed meats may act as additional sources of bacterial contamination because the food handlers' seldom washed their hands before or after using this machine. Similar practices were observed when food handlers' used the delicatessen telephone. Therefore the importance of hand washing when moving between food preparation and customer service may be emphasized during delicatessen staff training. Alternatively, as a result of the time constraints of hand washing, which would keep customers waiting, the delicatessen staff could be separated into those which provide customer service and those dedicated to RTE food

preparation so as to minimize potential cross contamination between RTE foods within the delicatessens.

Microbiological status of filled baguettes and assorted salads

From the microbiological survey conducted on RTE foods, it was observed that filled baguettes and assorted salads showed the highest bacterial counts and incidences of potential foodborne pathogens. Upon further investigation it was determined that on average, 60% of the filled baguette and assorted salad samples (n=70) complied with the microbiological guidelines recommended by the retailer involved in this study and the South African Department of Health. Whilst more than half of the samples complied with the microbiological guidelines, 40% of the samples contained potential foodborne pathogens in greater incidences than those recommended. A large portion of the South African population is immunocompromised either due to pregnancy, cancers, terminal illnesses or HIV infection. Therefore the percentage of compliant samples should be increased.

No distinct trend was observed between bacterial counts obtained between meat and non-meat filled baguettes and assorted salads. It was observed that fruit salads generally contained the lowest bacterial counts and incidences of potential foodborne pathogens. This may in part be attributed to the low pH of the fruit salads from ingredients such as pineapples. This low pH is inhibitory to many bacterial species (Jay *et al*., 2005). Coliforms are reportedly associated with fresh produce and as the *E. coli* counts for filled baguettes and assorted salads were generally low, results do not suggest faecal contamination of these RTE foods. Future studies may establish whether the coliforms associated with these products are in fact derived from the fresh produce themselves.

Previous reports have shown that *L. monocytogenes* may be harboured in retail equipment surfaces such as processed meat slicing machines (Humphrey and Worthington, 1990; Aguado, *et al*., 2001). In the present study the incidence of *L. monocytogenes* was only detected in cheese based baguettes (7%), sliced processed meat baguettes (7%) and assorted salads containing sliced processed meats (8.3%). All sliced processed meats used to prepare these RTE food commodities were sliced

on an in-store processed meat-slicing machine. By contrast, no *L. monocytogenes* was determined for the other RTE food commodities such as chicken baguettes, fruit salads and vegetable salads, which were sliced with a hand held knife. These results suggest that the processed meat-slicing machine may act as a potential reservoir for RTE contamination with *L. monocytogenes.* Therefore adequate cleaning and sanitizing of the in-store slicing machine would be imperative to minimize cross contamination from the meat slicer to the processed meat

Two factors which have been highlighted in literature as impacting on the microbiological status of RTE foods are the microbiological quality of the ingredients and more importantly contamination of RTE foods during food preparation. Firstly the microbiological load of the raw ingredients used to prepare the filled baguettes and assorted salads may be evaluated. Future studies may focus on determining the numbers of bacteria associated with the ingredients compared to the finished RTE food. This may be achieved by conducting supplier audits. Secondly, whilst the microbiological load of the raw ingredients is important, previous reports have shown that contamination of RTE foods often occurs during the preparation of these foods. Therefore the latter factor was further investigated in this study by determining the bacteriological status of the delicatessen food contact surfaces and cleaning tools so as to determine possible sources of contamination for these particular types of foods.

Microbiological status of food contact surfaces

Results from the swabbing of preparation knives, spoons, plastic chopping boards and handlers hands' showed that the plastic chopping boards contained the highest bacterial numbers. Therefore these boards may be the most likely source of bacterial contamination for both filled baguettes and assorted salads. Observed cut marks on the plastic cutting boards (grooves for potential bacterial attachment and biofilm formation) and stains suggested that the plastic chopping boards may act as potential reservoirs for the contamination of RTE foods. Furthermore, indirect evidence was provided by the predominant populations isolated from the plastic chopping boards. Similar trends of predominating bacteria such as *Bacillus* (*ca*. 20%), *Staphylococcus* (*ca*. 20%), *Enterococcus* (*ca*. 6%) and Gram-negative rods (42%) were isolated from the plastic chopping boards used during filled baguette preparation and the actual

filled baguettes. A similar trend was observed between the plastic chopping board used during salad preparation and the actual assorted salads. Therefore more frequent cleaning of the plastic chopping boards may reduce the numbers of associated bacteria. However, knife scarred plastic chopping boards are reportedly more difficult to clean and sanitize (Gough and Dodd, 1998). It has been reported elsewhere that wooden and plastic chopping boards retain bacteria (Gough and Dodd, 1998). It has also been shown that glass harbours the least amount of bacteria due to the smoothness of its surface (Verran *et al*., 2000). Therefore the use of glass chopping boards in retail delicatessens may reduce the contamination potential of the chopping boards. However, the glass breakage hazard will outweigh the benefits of such boards.

Additional problem areas during filled baguette preparation may be from the handlers' hands. Despite the handlers' hands being swabbed after washing and sanitizing, the aerobic bacterial counts from the hands were the highest counts obtained for the food contact surfaces associated with filled baguette preparation. Bacterial counts from swabs of the hands generally showed low coliform and *E. coli* counts suggesting adequate personal hygiene of the food handlers. It has been reported elsewhere that the hand counts of workers are often higher than of the gloves. It has been suggested that this may be from inadequate hand washing as workers reportedly become complacent when they wear gloves (Jumaa, 2005). Additional training on correct hand washing procedures may need to be reiterated so as to minimize the cross-contamination potential of the food handlers' hands.

An additional problem area during salad preparation and display was associated with the spoons. The numbers of aerobic bacteria from the spoons were the highest count associated with the salad food contact surfaces. In addition, the predominant populations such as *Micrococcus* (*ca*. 6%), *Staphylococcus* (*ca*. 20%) and Gram negative rods (*ca*. 40%) which were isolated from the spoons correlated with those isolated from the assorted salads themselves. Bacterial contamination of the spoon may occur during the serving of the salads and therefore frequent cleaning and sanitizing of the spoons should be practiced.

Lower numbers of aerobic, coliform and *E. coli* bacteria were obtained from the preparation knives in this part of the study compared to the counts obtained during the

pilot study. This may be as a result of more awareness of knife hygiene by the delicatessen staff. Thus adequate cleaning and correct sanitizing of the knives resulted in lower counts in this study.

Overall the *E. coli* counts for the assorted salad surfaces were statistically ($P<0.05$) significantly higher compared to filled baguette surfaces. Previous studies have shown that foods contaminate food contact surfaces (Beumer and Kusumaningrum, 2003). Fresh fruit and vegetables reportedly contain low numbers of *E. coli* which may be transferred to food contact surfaces. If the surfaces are not adequately and frequently cleaned and sanitized, the number of *E. coli* cells may increase through repeated contamination and or multiplication by the bacterium itself. Thorough washing of all assorted salad ingredients may further decrease the numbers of contaminating *E. coli*.

Microbiological status of cleaning tools and disposable plastic gloves

Floor mops and cloths had high counts of aerobic bacteria when sampled during the pilot study. Results from further samples showed that both the floor mops and cleaning cloths were associated with potential foodborne pathogens. Floor mops and cloths are used to clean up food soils and therefore may easily become contaminated with potential foodborne bacteria from the foods. However, the growth and multiplication of these potential foodborne pathogens attached to the cleaning tools themselves is undesirable as they may potentially recontaminate food contact surfaces and RTE foods with higher numbers of these pathogens. Indirect evidence highlighting the potential of cleaning tools to transfer bacteria to food contact surfaces and subsequently to RTE foods was observed from the predominant populations.

Gram-positive populations from the salad cleaning cloths were similar to the plastic chopping boards and those isolated from salad. Similarly the predominant populations isolated from the hands were similar to salad cloths. This may be expected as handlers' hands come into direct contact with the cloths, thus reiterating the importance of both hand and cleaning tool cleanliness.

In order to prevent the growth and multiplication of potential foodborne pathogens in the cleaning cloths, they should first be washed in hot soapy water. The dilute sanitiser in which they are soaked when not in use should be changed more frequently (for example, hourly) or alternatively a stronger more effective sanitizer may be used. The cloths should be dried in between use as the drying of cloths has reportedly been shown to decrease the numbers of associated bacteria (Beumer and te Giffel, 1996). Previous studies have shown that 100% cotton cloths transfer less bacteria than microfibre cloths (polyester and polyamide fibres are split during production resulting in randomly arranged sharp-edged microfibre strands) which in turn transfer less bacteria to food contact surfaces than polycotton (50% cotton and 50% polyester) cloths (Sattar *et al*., 2001; Cogan *et al*., 2002; Moore and Griffith, 2006). Therefore using cloths which carry less contamination potential may reduce bacterial contamination in the retail delicatessen. Additional training on the importance of cleaning cloth sanitation may aid in reducing the bacterial counts. Future studies may include an *in situ* study on the efficacy of different sanitizers under everyday conditions within the delicatessen and establish the time taken for the cleaning cloths to become heavily soiled.

Mops are used to clean food spills on floors but the spread of potential foodborne pathogens over the delicatessen floor is undesirable due to possible aerosol production (Burfoot *et al*., 2003). The predominant populations isolated from the floor mops were similar to those isolated from the assorted salads and salad spoons. These results suggest that floor mops may indirectly contribute bacteria to the RTE foods. More work is required in order to establish the full extent to which aerosol production from floor mops during cleaning contribute to RTE food contamination.

Floor mops dedicated to the cleaning of the delicatessen floors shows good cleaning practices are in place. However, to minimize the numbers of contaminating foodborne pathogens, the floor mops should be washed in clean, hot soapy water, rinsed in freshly made sanitizer solutions and dried in between use. In addition, mops should be replaced when they show discoloration.

Good glove practices were generally observed which correlated to the low bacterial counts obtained in both the pilot and microbiological survey studies. These results confirmed that effective in-house training can indeed reduce the contamination potential by the disposable plastic gloves through increased awareness. By contrast less emphasis has been placed on good cleaning tool practices, however, with improved training in this area the contamination potential of the cleaning tools will be significantly reduced.

It is speculated that cleaning and handling tools may act as reservoirs for contamination of RTE foods with bacterial foodborne pathogens. Bacterial counts on the food contact surfaces and prevalence of bacterial foodborne pathogens on cleaning tools mentioned above already provide indirect preliminary evidence for this supposition. However, preliminary 16S rDNA sequence analysis carried out on one *S. aureus* strain from glove origin and one similar isolate from filled baguettes (results not shown) indicate a 100% genetic homology between the two isolates tested. The latter results provided further evidence for a link between cleaning and handling tools and RTE food contamination. Further work should encompass the comparison of the other potential foodborne pathogens isolated in this work.

Scanning electron microscopy (SEM) of cleaning tools and disposable plastic gloves associated with the preparation of filled baguettes and assorted salads showed the presence of large numbers of rod shaped and coccoid bacteria associated with the floor mops and cleaning cloths. This cell morphology corresponded to the bacterial populations characterized from the APC plates, where both rod shaped and coccoid bacteria were recovered. Scanning electron micrographs showed predominantly coccid shaped bacteria associated with the disposable plastic gloves, which is in concurrence with bacteria characterized from the APC plates as the percentage proportion of *Micrococcus* (16.7%) and *Staphylococcus* (33.3%) collectively predominated. SEM results also correlated with general count data as high numbers of bacteria (*ca*. 5.7 Log CFU/cm^2) were associated with cleaning tools. By comparison a lower bacterial count was obtained from APC plates of the disposable plastic gloves and SEM also showed fewer bacteria associated with the disposable plastic gloves.

Bacterial counts may be influenced by seasons

An interesting trend was observed in that bacterial counts in the RTE foods and on the food contact surfaces were greater during the summer months compared to the winter months. The initial pilot study of the retail delicatessens was conducted during May-October 2005 (predominantly winter months) (Chapter 2) whilst the microbiololgical survey of the filled baguettes and assorted salads was conducted during October 2005-May 2006 (predominantly summer months). In comparison the bacterial counts for the filled baguettes and assorted salads showed a 1.5-4 Log CFU/g increase in the summer months. The increase in atmospheric temperatures during summer provides optimal conditions for bacterial growth. Thus refrigeration becomes paramount in summer. The core temperatures of the filled baguettes and associated salads during display were *ca*. 15^{o}C. Therefore decreasing the core temperatures of these RTE foods may increase their microbiological quality and safety. It is suggested that the filled baguettes and assorted salads are displayed in closed refrigerators where the temperatures are more easily controlled. A similar trend was observed for the food contact surfaces. During the pilot study (winter) the preparation knives and spoons showed the highest counts whilst the chopping boards and hands showed negligible counts and *E. coli* was below the lower detection limits. By contrast, in the microbiological survey of filled baguettes and assorted salads (summer) the plastic chopping boards and hands showed higher APC, CC and ECCs. These results show that the sanitation regimes are adequate during the winter months when temperatures are cold but in summer once the temperatures increase the cleaning and sanitation regimes need to be increased. Secondly, the second study fell over the festive season where the increased demand for RTE foods resulted in casuals being employed to help keep up with demand. Therefore improved training for casual staff and more stringent cleaning and sanitation regimes may be required to cope with increased product flow.

Future studies

This work has contributed to new knowledge in the field of food microbiology, especially with regard to the bacteriological status of RTE foods sold in retail delicatessens in South Africa. The results from the present study show that the RTE foods evaluated were of higher microbiological quality compared to surveys done in

other countries. Particularly noteworthy was the absence of *Clostridium* spores and *E. coli* O157:117. In addition, problem areas for potential RTE food contamination within the delicatessen have been identified.

Future studies may include establishing quantitatively the rates of bacterial transfer between RTE foods, food contact surfaces and cleaning tools. This has been done in laboratory settings (Kusumaningrum *et al*., 2003) and the true transfer of bacteria during normal delicatessen operations should be established. Furthermore the sequencing of 16S rDNA from bacterial foodborne pathogens isolated in this study would provide further evidence for the transfer of bacteria within retail delicatessens. In addition, further studies would be required to establish whether the coliforms associated with these products are in fact derived from the fresh produce themselves. Similarly, future studies may include an *in situ* study on the efficacy of different sanitizers under everyday conditions within the delicatessen and establish the time taken for the floor mops and cleaning cloths to become heavily soiled.

On a more practical note, it was observed that through effective training good glove practices were employed by the food handlers as bacterial counts were low. Therefore further training of the delicatessen staff on the importance of cleaning and sanitizing food contact surfaces, especially cleaning tools, would decrease the bacterial numbers. Similarly the display of filled baguettes and assorted salads in closed refrigerators would allow for better temperature control of these RTE foods and therefore increase their safety. Microbiological testing of ingredients and supplier audits would add to the microbiological integrity of RTE foods.