PREVALENCE OF PATHOGENS AND INDICATORS IN FOODS ORDERED FROM ONLINE VENDORS

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ABSTRACT OF THE THESIS

Prevalence of pathogens and indicators in foods ordered from online vendors.

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The widespread availability of the Internet has fostered the emergence of a new business sector: online sales of perishable foods. While there is an abundance of information available on safe food handling practices in homes, retail and foodservice establishments, the same can not be said for the handling of 'mail-ordered foods'. This project used microbial techniques to identify bacterial foodborne pathogens and indicator organisms in foods ordered online.

Randomly chosen food items were ordered from different online vendors. On arrival of the package containing the food, details such as the packaging materials used, temperature of food, presence of coolants etc. were recorded. Frozen samples were thawed at 2-5°C for no more than 18 hours. Food samples were enumerated for the presence of indicator organisms including total plate count, coliforms and generic *E. coli*, and tested for the presence of pathogens including *Salmonella*, pathogenic *E. coli*, *Listeria monocytogenes*, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, and *Vibrio parahemolyticus*. Testing protocols were based on US Food and Drug Administration (FDA) Bacteriological and Analytical Manual (BAM).

A total of 341 samples were tested (196 meat, 34 poultry, 111 seafood). Of these, 18.7% were positive for generic *E. coli*, 9.9% for pathogenic *E. coli*, 10.2% for *Salmonella*, 50.3% for *B.*

cereus, 38.7% for *V. parahemolyticus* and 18.2% for C. perfringens. Also, on comparing incidence rates of food pathogens it was found that prevalence of *Salmonella*, *B. cereus* and *V. parahemolyticus* was higher in most of the mail-ordered foods than foods from other retail sources and prevalence of *E. coli* and *C. perfringens* was lower in most mail-ordered foods than foods from other retail sources. The temperature recorded on arrival of samples showed that majority of the coliforms*, B.cereus, C. perfringens* and *L. monocytogenes* positive samples were received at acceptable temperatures while *E. coli, Salomonella* and *V. parahemolyticus* were not at an acceptable temperature. Amongst the 21 Ready-to-eat (RTE) samples tested, *L. monocytogenes* was detected in 4 samples and none of the samples tested positive for *S. aureus*. In conclusion, this study illustrates the risks associated with online purchase of foods.

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1.1 MAIL ORDERED FOODS THROUGH ONLINE VENDORS

In the past few years, small to large sized companies have set up websites for online sales of meats and seafood products. A survey estimates over 500 such online vendors (Hallman 2011), making this a large business. Big companies such as Omaha steaks claims to ship over 4 million coolers annually to nearly 3 million active customers (Omaha steaks fact sheet). At the same time there are small companies that operate based on individual farms and ranches or single fishing boats and smoke houses.

Online vendors provide a variety of exotic and high end products such as antelope steaks, yak roasts, zebra haunches, giant scallops, filet mignon and others. They offer consumers the option to purchase live or fresh seafood (such as lobsters, clams, oysters), smoked fish, specialty meats from a popular farm or ranch or a local region or state (for example: www.wisconsinmade.com) or grass-fed and organic foods (for example: www.localharvest.org). At the same time, they also help in addressing religious preferences such as Kosher and Halal meats and seafood.

They operate on direct marketing to consumers by offering potentially better and fresher foods due to time elapsed between production and consumption of food via overnight or 2-day delivery.

The option of purchasing food online without having to visit supermarkets not only provides ease but also 24 hours online ordering flexibility. Also making payments using credit cards could be a convenient option for some consumers.

Another benefit associated with online purchase of foods is the availability of the option for delivery to any state or city at even the most remote locations. Plus, scheduled delivery on specific dates for special occasions could be useful.

1.2 POTENTIAL RISKS ASSOCIATED WITH MAIL-ORDERED FOODS

There are a few risks associated with online sales of foods, which questions the safety of such operations.

1. Safety regulations

Though detailed recommendations and regulations have been laid down by FDA and USDA regarding food transportation safety, no extensive set of rules have been enforced. Details have been provided regarding HACCP and GMP for production, processing and packaging of foods along with distribution and transportation (FSIS, 2005; FDA, 2005). This will be discussed in detail in section 1.6.

2. Packaging may or may not be effective

Based on recommendations from FSIS for mail ordered foods, packaging for refrigerated or frozen foods should use an insulated box with dry ice and/or refrigerated gel packs

(USDA, 2008). The ice packs or dry ice does not always last long enough before the consumer can refrigerate the food. Also packaging abuse could render it ineffective.

3. Shipping issues

Most companies ship foods using courier services like FedEx and UPS. The temperature during transit can fluctuate a lot causing temperature abuse. FedEx clearly states that aboard the aircrafts carrying the packages, temperature can fluctuate between 0 °F to 90 °F (FedEx, 2010) and likewise for UPS states that "Temperature extremes globally can range from -80 °F to 160 °F" (UPS, 2005).

Shipping companies are advised by International Air Transport Association (IATA) not to name the contents or put the name of the company in order to prevent thefts (except for mentioning the use of dry ice) (FedEx, 2010). This makes it difficult for the courier companies to distinguish perishable from non-perishables. This can be an especially serious issue where products are sent as gifts, and the intended recipient may not know what a package contains.

4. Drivers release

To reduce the cost of shipping, the companies sign up for 'Drivers Release'. This is a document that authorizes the courier companies to deliver the packages without the need to take signature from the recipient. Such released parcels can be left at exterior door locations. Research has revealed that amongst more than 50% of married couples, both the spouses work outside their home (Bureau of Labor Statistics, 2008) meaning such

package may sit at their doorstep for hours before refrigerating. This poses greater problems in case of shipments received at offices that may be claimed the next day or after 2-3 days in case of weekends.

5. General lack of food safety knowledge by consumers

USDA's Food Safety and Inspection Service (FSIS) recommends that the foods not stay in the temperature danger zone (40 to 140 °F or 4 to 60 °C) for more than 2 hours (FSIS, 2013), yet some consumers may wait for more than two hours (up to 8 hours) before refrigerating or freezing perishables (Kain, 2002). A study on consumer behavior mentions the "cellophane effect" (Hallman, 2009), when consumers assume that food spoilage is due to exposure of foods to external contaminants and that it is assumed safe and clean until exposed to some external source of contamination (Rozin and Fallon, 1987). Consumers may also fail to understand that a product's natural flora can also cause spoilage.

Moreover, some consumers, unaware of recommendations, feel confident about judging key aspects of food safety such as temperatures by touching the product (Godwin, 2005). Though spoilage bacteria like *Pseudomonas, Xanthomonas* and others do produce unpleasant taste, odor and texture they may not necessarily cause foodborne illness (Blackburn, 2006). On the other hand, foodborne pathogens like *L. monocytogenes, Salmonella, Shigella, V. parahaemolyticus, Camphylobacter* may not always affect the sensory aspects of food.

6. Inherent vulnerabilities of perishable foods

The natural constituents and microbial flora of foods make render them perishable. No extent of refrigeration or heating can keep them safe forever. Spoilage and pathogenic organisms will act on the food and make them unfit for consumption.

7. False reassurance by the companies

Some companies, (e.g. Kansas City Steak Company), state that the consumer should not be alarmed if the dry ice is gone by the time they open the containers and that if the product is still cool to the touch, food quality is not diminished (Handling, thawing and refreezing, Kansas city steaks). People's perspective of 'cool to touch' (Godwin, 2005) can vary and so it isn't a scientific manner to judge the safety or quality of food.

8. Lack of advise/support from vendors

In a preliminary unpublished survey just one out of 60 online vendors specified the importance of storing perishable foods at temperatures of 4 °C or less. Companies may avoid specifying exact storage requirements to avoid consumer complaints from knowledgeable customers. While some companies may reship out of temperature products at their cost, many others may not.

1. 3 MARKET TRENDS

In todays' world, the use of the Internet is an integral part of much of our society. Data published by Internet World Stats in 2012 shows that Internet use has increased 152.3%

from 2000 to 2012 (Internet world stats, 2012), and 78.6% of North Americans were using the Internet as of June, 30, 2012 (Internet world stats, 2012). This is a trend that can be expected to increase in future.

A similar trend can be observed in case of online sales of goods (i.e. e-commerce). A study performed on shopping trends revealed that online shopping is challenging traditional retailing (Ward, 2001). This is in agreement with the survey performed by United Postal Service (UPS) which found that since 2012, the online shopping business has risen by 15% to a market of $186 billion (UPS, 2013a).

This trend holds true specifically for online shopping for food products as well. In 2012, U.S. Grocery Shopper Trends conducted a survey on 1401 people. Fifty-four percent of those surveyed said that they at least occasionally shop online for groceries (FMI, 2012). While a majority (85%) of online shoppers more commonly purchase items such as electronics, books or music, 12% of those surveyed do purchase dry groceries and beverages, and 4% purchase fresh foods and produce (FMI, 2012).

According to the U.S. Department of Commerce, in 2011 online shoppers spent nearly $12 billon. Out of this, $4.4 billion was spent on food and beverages alone (Path to purchase institute, 2012) and in a 2013 annual survey by supermarket.com a boost in online grocery shopping was predicted (Supermarket news, 2013). This was based on the fact that e-commerce and grocery store giants such as Amazon and Walmart plan to expand the sector of online sales of foods further. Amazon Fresh (an initiative by

Amazon for home delivery of food products) plans to launch operations in 20 more urban locations after test market success in Seattle. Likewise, Walmart stores also plan to expand their online grocery offerings (Supermarket news, 2013).

1.4 REGULATIONS ON FOODS

In United States, food safety is addressed through surveillance and regulation by several national governmental agencies. The Centers for Disease Control and Prevention (CDC) closely monitors foodborne illnesses through its Foodborne Disease Outbreak Surveillance System. This includes both population-based (FoodNet) and laboratory based surveillance (PulseNet) to identify the genetic sequence of the organism. The Center for Food Safety and Applied Nutrition (CFSAN) under Food and Drug Administration (FDA) is responsible for the regulation of domestic and imported food with the exception of meat and poultry products. The US Department of Agriculture (USDA) agency called the Food Safety and Inspection Service (FSIS) is responsible for the regulation of meat, eggs, and poultry products.

1.5 FOOD INSPECTIONS IN UNITED STATES

Depending upon the type of food produced, either FDA or USDA would be responsible for the inspection of a given food-processing establishment. In some cases a single food product or production facility may be covered by multiple jurisdictions. In general, most

intrastate transportation of food is regulated by state or local regulatory agencies (like state health departments or department of agriculture). Food that enters into interstate commerce requires inspections by FDA or USDA (FDA, 2013). One exception is Ohio, which is the first state to gain approval to sell meat from small, state-inspected slaughterhouses across state lines (Bottemiller, 2012).

1.6 FOOD TRANSPORTATION SAFETY

Backhauling involves the practice of trucks transporting garbage/ chemicals/ other contaminants after delivery of foods, which came to public attention in 1989, due to several well-publicized food safety incidents (Keener, 2003). This led to the Sanitary Food Transportation Act (SFTA) of 1990. The SFTA has been criticized for being overly narrow since it did not incorporate transportation of food by modes other than motor and rail vehicles (Keener, 2003). In 1997, the primary responsibility of food transportation safety was transferred from U.S. Department of Transportation (DOT) to U.S. Food and Drug Administration (FDA) via provisions included in the National Economic Crossroads Transportation Efficiency Act (NEXTEA) (Keener, 2003).

Since 1997 USDA and FDA have had the primary responsibility of food transportation safety. USDA has authority over safety of meat and poultry under the Federal Meat Inspection Act and Poultry Product Inspection Act. Likewise, FDA regulates safety of seafood and others along with adulteration, misbranding and interstate commerce of food

under the Federal Food, Drug and Cosmetic Act (FD&CA) and Public Health Service Act. FDA acts based on the U.S. Code of Federal Regulations (Keener, 2003).

USDA FSIS gives comprehensive details on transportation and distribution of meat, poultry and egg products (FSIS, 2005). USDA FSIS help manufacturers and distributers to identify the vulnerable points, train food handlers, design storage and transportation vehicles and provide detailed instructions on loading, transit and unloading of food products (FSIS, 2005). In addition, FSIS has a two-page document on 'Mail Order Food Safety' (USDA, 2008), which gives tips to companies and consumers involved with selling and purchase of mail ordered foods. The document also gives recommendations regarding type of packaging to be used and storage of foods on arrival, and provides agency contact details if a consumer wishes to file a complaint or has query regarding safety of such operations (USDA, 2008).

FDA likewise, gives extremely detailed information on many aspects of transportation of foods. There is material available regarding transportation of such as fruits, vegetables, eggs and dairy products, specifications on equipment and vehicles involved, regulations on good manufacturing practices and establishment and maintenance of records (FDA, 2005).

FDA documents state (21 CFR 1.352, 2013) that it is the responsibility of the carrier service (such as FedEx, etc.) to take responsibility of and maintain records for packages containing food. But these carrier services require the shipper to sign a disclaimer stating

that the shipper takes over all the responsibilities (UPS, 2013b). While most shippers act on their responsibility to ensure delivery of safe and good quality food to consumers, others may not do so. So even though rules and regulations for food transportation safety exist, the ultimate assumption of liability is not clear.

1.7 NEED FOR RESEARCH

Not much is known about incidence rates of pathogenic foodborne microorganisms in mail-ordered food items. A literature search revealed only a single peer-reviewed article regarding microbial safety of cheese delivered after purchase online (Ramsey and Funk, 2009). A recall issued by FSIS as a result of *L. monocytogenes* being found in products ordered online (Gaffney, 2010) indicates the need for additional research on the safety of mail-ordered foods.

Literature survey also yielded a number of similar studies performed on prevalence of indicators and/or pathogens in various food types including fresh produce, meats, poultry, seafood, dairy, spices etc. Though these studies are not always the same as the current study and differ based on three major reasons. First is the difference in the geographical area where the research was conducted. These included Korea (Ryu, 2012), Australia (Barlow, 2006), Netherland (Giffel, 1996), Turkey (Guven, 2005), United Kingdom (Sooltan, 1987), South Africa (Nortje, 1999), Tiwan (Wong, 1999) etc. The second difference arose in the time period during which the research was carried out. Most of these studies were performed before the implementation on Pathogen Reduction Program in 1997 (Samadpour, 1994; Giffel, 1996; Sooltan, 1987; Strong, 1962; Hall, 1965;

Lammerding, 1988). Finally, unlike the current research, which used culture based methods (adopted from FDA's Bacteriological Analytical Manual) some researchers utilized other techniques such as PCR (Barlow, 2006; Miwa, 1999; Van, 2007; Chen, 2008) and DNA probe for detection of toxins (Mataragas, 2008). At the same time, more comparable studies were also available (FDA, 2010; Scheinberg, 2013; Zhao, 2001; Baffone, 2000; Jaksic, 2002; Yang, 2007; FSIS, 2001).

Given the rapid growth of online food vendors coupled and the wide range of issues discussed above, it becomes important to understand the risks posed by food obtained from such operations.

2. INDICATORS AND PATHOGENS OF INTEREST

2.1 INDICATORS

Indicator organisms are used to indicate the potential absence/presence of foodborne pathogens, and they are generally thought to reflect the safety or sanitary aspects of food and the conditions under which it has been processed and/or handled. According to Jay (Jay, 2006) a good indicator should be detectable in all types of foods tested, should provide a direct indication on food quality and/or safety, should be easy to identify and enumerate and differentiate from other microorganisms in food, should have quick enumeration method and should not be negatively affected by other food micro-flora. Traditionally, total plate count, coliform counts and generic *E. coli* counts have been used as indicators of microbial quality (Phillips, 2013), fecal contamination (Borrego, 1990) and presence of pathogens (Borrego, 1987), or health risk (Dudley, 1980).

Some studies did find one or more of the three above-mentioned effective indicators in meats (Pohlman, 2001), water (Borrego, 1987; Geldreich, 1970; Efstratiou, 1997) and seafood (Hood, 1983). But other research (e.g. Miskimin, 1976) found that neither total aerobic plate count, coliform count nor *E. coli* count were suitable indicators for food safety.

2.2 TOTAL PLATE COUNT

Total plate count (TPC) is also known as total aerobic plate count (TAPC) or aerobic plate count (APC). This test is used to get a general indication of the microbial content in a food sample. It measures all the culturable aerobic bacteria in the food able to grow on the nutrient rich agar without differentiating one species from another. It generally can not differentiate between microorganisms that are a natural part of the food's microflora, organisms deliberately added to ferment the food, spoilage microorganism or pathogenic microorganisms.

A high TPC, in non-fermented foods, can indicate unsanitary or unhygienic conditions, or temperature abuse, either or which may have occurred during processing, manufacturing, storage or transportation of foods. It may or may not indicate presence of pathogenic bacteria in foods.

2.3 COLIFORMS AND *E. COLI*

Work performed by Schardinger (Schardinger, 1892) and later Smith (Smith, 1895) described the use of *E. coli* as indicator of presence of pathogenic microorganisms. This use was based on the fact that *E. coli* was found in association with human and animal feces. *E. coli*'s ability to ferment glucose (later changed to lactose) made it easy to detect it and differentiate it from other gastrointestinal organisms. But this differentiation was complicated by presence of other lactose fermenting enteric organisms such as *Klebsiella, Pseudomonas, Citrobacter* and others. All these organisms were termed "coliforms".

Coliforms are defined as Gram-negative, facultative anaerobic rod-shaped bacteria that ferment lactose to produce acid and gas within 48 h at 35°C (Feng, 2013).

The observation of natural occurrence of coliforms in environment led to usage of fecal coliforms (now more properly termed thermotolerant coliforms (Doyle and Erickson, 2006), a subset of total coliforms, as a more accurate indicator of hygiene. Thermotolerant coliforms are coliforms that ferment lactose with gas production within 48 hours at 45.5 °C (44.5 °C for shellfish). This group mainly consists of *E. coli* and *Klebsiella Enterobacter*, and *Citrobacter* species (Doyle and Erickson, 2006).

E. coli are Gram negative, non-spore-forming rods that are part of a healthy human intestinal tract. Some, but not most, strains are pathogenic. CDC classifies pathogenic *E. coli* into six pathotypes:

1. Shiga toxin-producing *E. coli* (STEC) or Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC).
2. Enterotoxigenic E. coli (ETEC)
3. Enteropathogenic *E. coli* (EPEC)
4. Enteroaggregative *E. coli* (EAEC)
5. Enteroinvasive *E. coli* (EIEC)
6. Diffusely adherent *E. coli* (DAEC)

The most common pathogenic *E. coli* are the STECs. During 1998-2008, STEC alone caused 308 outbreaks leading to 1,271 hospitalizations and 22 deaths (Gould, 2013). In United States, seven serogroups containing O antigen are of major concern O157:H7 and

non O157:H7 (O26, O91, O103, O111, O128 and O145). Each year (in United States) 265,000 STEC infections occur. O157 is responsible for 36% of these infections and the rest is caused by non-O157 STEC (Gould, 2013).

Upon ingestion, STEC colonize the mucosa of the large bowel (Nataro and Kaper, 1998) followed by binding to the bacterial cell (Nataro and Kaper, 1998). In the bowel, STEC secretes one or more cyto-toxins: Stx1, Stx2, Stx2c and Stx2d (Friedrich, 2002; O'Brien, 1992). These toxins blocks tRNA binding leading to inhibition of protein synthesis. The pathophysiological changes that occur result in HUS (Mohamed, 2004).

Both O157 STEC and O111 STEC are thought to have a low median infectious dose (<100 organisms) (Paton, 1998). The infectious dose of other serogroups is not known (Paton, 1998). Pathogenic strains can cause severe stomach cramps, diarrhea, and vomiting with mild to no fever. Some STEC infections are very mild, but others are severe or even life threatening leading to hemolytic uremic syndrome (HUS). Incubation time is 1-10 days. The symptoms last for 5–8 days (1 week for HUS) (HHS, 2013).

Food sources include contaminated or undercooked food such as ground beef, unpasteurized (raw) milk and juice, soft cheeses made from raw milk, and raw fruits and vegetables (HHS, 2013).

2.4 *BACILLUS CEREUS*

B. cereus was first described by Frankland and Frankland in 1887 followed by isolation

from air in a cowshed. (Tajkarimi, 2007) Since 1950, there have been many *B. cereus* related outbreaks in Europe in foods such as meats, poultry, fish, soups, milk and ice cream. The first well-characterized *B. cereus* outbreak in the USA occurred in 1969 (Tajkarimi, 2007). In this case B. cereus count was 7 x 10^6 cells/g of meat loaf resulting in diarrheal symptoms in 15 people after 10 hours (Kramer and Gilbert, 1992).

B. cereus is a Gram-positive, facultatively anaerobic, endospore- forming, large rod. It is very similar to *B. weihenstephanensis, B. mycoides, B. pseudomycoides, B. thuringiensis* and *B. anthracis* (Lampel, 2012).

Food poisoning due to *B. cereus* usually occurs due to reheating of foods followed by inadequate refrigeration leading to germination of spores. *B. cereus* causes two types of foodborne illness: Emetic or Diarrheal. Emetic toxin called cereulide causes emesis or vomiting. It consists of a ring structure of three repeats of four amino acids or oxy-acids. It causes inhibition of mitochondrial activity. Emetic strains of *B. cereus* do not grow below 10 °C (Ehling-Schulz, 2005). The emetic toxin is hydrophobic, resistant to heat, pH and proteolysis and is not antigenic (Kramer and gilbert, 1989)

Diarrheal disease is caused by one or more enterotoxins. *B. cereus* produces three enterotoxins which are protein or protein complexes. The first enterotoxin is Hemolysin Hbl consisting of three proteins B, L1 and L2. B binds to the target cell and the other two cause osmotic lysis (Beecher, 1997). Hbl has been suggested to be the primary virulence factor in diarrhea (Beecher, 1995). The second enterotoxin is the Nonhemolytic enterotoxin (Nhe). More than 99% *B. cereus* strains produce this enterotoxin. It is most active when the three components (A, B and C) are in the ratio of 10:10:1. The third

enterotoxin is the single protein enterotoxin CytK and is similar to the β-toxin of *C. perfringens*.

The median infective dose is greater than 10^6 organisms/g (Lampel, 2012). Symptoms can be diarrheal (watery diarrhea and abdominal cramps) or emetic (nausea and vomiting). Incubation period is 6-15 hours for diarrheal and 30 minutes to 6 hours for emetic. Symptoms can last for 24 hours (HHS, 2013). *B. cereus* spores can survive cooking but the toxins are inactivated after 56 C for 30 minutes.

Food Sources include rice and leftovers, as well as sauces, soups, and other prepared foods that have sat out too long at room temperature (HHS, 2013). During 1998-2008, *B. cereus* caused 235 outbreaks leading to 17 hospitalizations and no deaths (Gould, 2013). Most of the outbreaks go unreported or misdiagnosed due to similarity to *C. perfringens* food poisoning or *S. aureus* intoxication (Lampel, 2012).

2.5 *CLOSTRIDIUM PERFRINGENS*

Clostridium perfringens (also know as *Clostridium welchii*) was discovered in 1892 by George Nuttall and William Welch. The first large-scale outbreak in the world was described by Knox and MacDonald in 1943 where *C. perfringens* in gravy caused food poisoning in school children in England (Knox and MacDonald, 1943).

C. perfringens is a Gram positive, non- motile rod. It is encapsulated and produces toxins. Short doubling time (<10 minutes) and environmental stress resistant spores favor the

ability of *C. perfringens* to cause foodborne disease (Labbe, 1989). It is generally considered to be anaerobic but studies show that it actually requires only low reduction in oxidation-reduction potential (E_h) for growth (Labbe, 1989). Food sources include: Beef, poultry and gravies (HHS, 2013). According to the 1998-2008 CDC MMWR, *C. perfringens* caused 5% of the 5,059 confirmed, single-etiology outbreaks. Also beef was found to be responsible for the highest percentage of outbreaks caused by *Clostridium perfringens* (41.3%), followed by poultry (30%), and pork (16.3%).

The median infective dose is greater than 10^6 vegetative cells or more than 10^6 spores/g of food (Lampel, 2012). Symptoms include: Diarrhea and abdominal cramps without fever or vomiting (HHS, 2013). Incubation period is usually 6-24 hours. The symptoms can last for less than 24 hours but may last for 1-2 weeks in case of severe cases (HHS, 2013). Chilling or freezing inactivates vegetative cells. Spores are more tolerant to temperature abuse and may actually tolerate 100 °C for 1 hour or more. *C. perfringens* was tested only in meats and poultry and not in seafood due to lower risks associated with seafood.

C. perfringens is known to produce 14 different types of toxins (McClane , 2001). β-toxin (CBP) produced by type C isolates of *C. perfringens*, causes necrotic enteritis (a life threatening disease) and *C. perfringens enterotoxin* (CPE) causes the type A food poisoning (a self limiting disease). Type A food poisoning begins with ingestion of food contaminated with vegetative cells of *C. perfringens* carrying cpe genes. Most of these cells die in the stomach due to its acidity, but the ones that survive transfer to the small intestine where they multiply and sporulate. The cells enter the intestinal lumen where they are lysed to release the endospores. CPE toxin binds to epithelial cells and induce

tissue damage that causes intestinal fluid loss and ultimately diarrhea (McClane, 1992). CBP toxin production is the major virulence factor in necrotic enteritis (Sayeed, 2008). The exact mode of action of CBP in the intestine is not fully understood (Miclard, 2009). A proposed mechanism suggests that CPB bind to the vascular endothelial cells in the small intestine during the early stages of the disease. This later leads to widespread vascular necrosis, hemorrhage and hypoxic tissue necrosis (Miclard, 2009).

2.6 *STAPHYLOCOCCUS AUREUS*

S. aureus was first associated with a wound infection in the 1880s by Sir Alexander Ogston (Ogston, 1883). *S. aureus* is the most common cause of infections in hospitalized patients and In 1941, Skinner and Keefer reported its 82% of mortalities in hospitals is caused by *S. aureus* (Skinner, 1941). It can affect any organ system due to its potent virulence factors and resistance to antibacterial agents (Beery, 1984).

S. aureus is Gram-positive, non-motile, catalase-positive, small, spherical bacterium (coccus). It exists in pairs, short chains, or bunched in grape-like clusters (Lampel, 2012).

S. aureus can be found in 32.4% healthy individuals in United States (Kuehnert et al, 2006). It can be carried asymptomatically for weeks or days on intact skin. It may get moved from the skin into a wound after which it can spread around the wound or gain access to blood stream. Finally resulting in endocarditis, osteomylytis, renal infection, and arthritis (Beery, 1984). When fed to rodents, staphlylococcal enterototoxins (SE) do

no enter the blood stream. Instead, they are rapidly removed by kidneys (Sugiyama, 1965). The emetic response arises due to stimulation of local neural receptors in the abdomen (Mengaud, 1996) that ultimately stimulate the medullary emetic center.

The infective dose for *S. aureus* is 100,000 cells in humnas (Schmid-Hempel and Frank, 2007). The symptoms of staphylococcal food poisoning include nausea, vomiting, diarrhea, loss of appetite, severe abdominal cramps, and mild to no fever (HHS, 2013). The incubation period is usually 1-6 hours. The symptoms can last for 24-48 hours or less (HHS, 2013).

The *S. aureus* organism is sensitive to heat and most sanitizers, but the toxin, once formed is highly heat resistant. It therefore poses a concern in pre-cooked or ready-to-eat (RTE) foods that are contaminated by food handlers, and held out of temperature control in foods that allow growth of the organism. *S. aureus* was tested only RTE foods and not in raw foods due to lower risks associated with the later.

Typical food sources include deli salads, bakery products, sandwiches, milk and dairy products, meat, poultry, eggs, and other RTE food items that do not require additional cooking.

According to the CDC (Gould, 2013), *S. aureus* caused 3% of the 5,059 confirmed, single-etiology outbreaks in 1998 to 2008. CDC data also showed that *S. aureus* was responsible for 458 (6%) of the total outbreaks causing 6,795 (3%) illnesses, 333 (4%) hospitalizations and 3 (2%) deaths.

2.7 *SALMONELLA SPP.*

Salmonella was first discovered in 1880 and isolated on culture medium in 1884 (Darwin, 1999). *Salmonella* serotype Enteritidis followed by *Salmonella* serotype Typhimurium are the two most implicated *Salmonella spp.* in foodborne outbreaks (Gould, 2013). The first documented case of salmonellosis occurred in a beef product in 1888 (Darwin, 1999) where 58 people were affected by gastroentritidis and one died.

Salmonella is a Gram- negative, non-spore forming bacteria belonging to the family *Enterobacteriaceae.* It is motile with exception of *S.* Gallinarum and *S.* Pullorum (Lampel, 2012). All species of *Salmonella* are pathogenic.

Salmonella can cause two types of disesase: nontyphoidal salmonellosis (a self-limiting condition) and typhoid fever, a more serious condition with high fatality rate (Lampel, 2012). The typhoidal fever and the gastroentritidis symptoms are a result of the local inflammatory response due to invasion if the intestinal mucosa.

The median infective dose is 15-20 cells, but even one cell has the probability to cause illness (Lampel, 2012). Symptoms of salmonellosis include diarrhea, fever, abdominal cramps, vomiting. It can have more severe outcomes in elderly, infants, and persons with chronic diseases. Incubation period is usually 12-72 hours and the symptoms last for 4-7 days (HHS, 2013).

Since *Salmonella* is a non-spore former, it is sensitive to heat and is killed by cooking and pasteurization. Food sources include contaminated eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables (alfalfa

sprouts, melons, leafy greens, tomatoes), spices, and nuts (HHS, 2013).

Every year, approximately 42,000 cases of salmonellosis are reported in the United States (Gould, 2013). The CDC estimates that that *Salmonella* is second most common cause of foodborne illnesses causing 1,449 outbreaks, 39,126 illnesses, 4034 hospitalizations and 60 deaths annually (Gould, 2013).

2.8 *LISTERIA MONOCYTOGENES*

In 1926, Murray first described *L. monocytogenes* based on a case of death of 6 rabbits (Murray, 1926). At that time he referred to the causative organism as Bacterium monocytogenes. In 1940 Pirie renamed the genus as *Listeria* (Harvey, 1940).

Listeria monocytogenes is a Gram-positive, rod-shaped, motile, facultative bacterium. It has 13 known serotypes, including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. Serotypes 1/2a, 1/2b, and 4b have been the most associated with outbreaks (Lampel, 2012). Both FDA and FSIS maintain a zero tolerance (absent in either of two 25g samples of food) for *Listeria monocytogenes* in RTE foods (intended to be eaten without prior heat treatment) (FSIS-USDA, 2003).

L. monocytogenes infects humans via the gastrointestinal tract. Internalin A on the *L. monocytogenes* interacts with the epithelial cadherin thus infecting the intestinal epithelial cells (Mengaud, 1996). From here, the bacteria enter the bloodstream and translocate to different organs such as liver and spleen. In liver and spleen, *L.*

monocytogenes cells are ingested by macrophages where they replicate. *L. monocytogenes* cells also are transported to lymph nodes via the blood. If the T-cell mediated immune response is not effective, they may spread to brain or placenta (in case of pregnant females). The organisms' ability to cross the placental barrier can result in death or permanent damage to fetus. *L. monocytogenes* is also known to cause septicemia, meningitis, or meningoencephalitis (Osfay-Barbe, 2004). Symptoms of listeriosis include fever, stiff neck, confusion, weakness, vomiting, sometimes preceded by diarrhea. Incubation period is usually 3-70 days and the symptoms last for days-weeks (HHS, 2013).

L. monocytogenes has very high tolerance to environmental stress factors. It can grow in salt concentration up to 10% NaCl, water activity down to 0.92 and from pH 4.4 to 9.4. Also it can grow in temperature range of -0.4 to 45 °C (National Advisory Committee on microbiological criteria for foods, 2010).

Food sources include: ready-to-eat foods, refrigerated foods, unpasteurized (raw) milk and dairy products, soft cheese made with unpasteurized milk, smoked seafood, deli meats deli salads and sprouts (HHS, 2013). *L. monocytogenes* was tested only in RTE foods and not in raw foods.

CDC estimates that approximately 1600 illnesses and 260 deaths due to listeriosis occur annually in the United States and that *L. monocytogenes* has the highest mortality rate and is second most common cause of deaths 48 out of 148 total deaths due to bacterial pathogens (Gould, 2013). It resulted in 216 hospitalizations and was responsible for seven multistate outbreaks.

2.9 *VIBRIO PARAHEMOLYTICUS*

V. parahemolyticus is a Gram-negative, curve-shaped rod isolated from the estuarine and marine environments of the United States and worldwide (Lampel, 2012). *V. parahaemolyticus* was first isolated (Fujino,1974) from clinical samples and dried sardines as a result of an outbreak of gastroenteritis in Japan in 1950. Since then it has been recovered from various other food products such as raw fish, shellfish, and cucumbers (Joseph, 1982; Miwatani, 1976). Foodborne disease outbreak sources include raw or undercooked shellfish, particularly raw oysters (HHS, 2013).

Not all *V. parahemolyticus* strains are pathogenic. It has been determined that only the strains with thermostable direct haemolysin (TDH) cause gasteroentritidis (Joseph, 1982). *V. parahemolyticus* attaches to the host cell and injects the virulence proteins via the Type III secretion system. This leads to disruption of host cell functions and finally cell death via apoptosis.

The median infective dose has been determined to be 100 million organisms. However, evidence from an outbreak in 2004 suggests an infectious dose >1,000-fold less than in the FDA risk assessment (Lampel, 2012). Symptoms of *V. parahemolyticus* infection include Diarrhea, vomiting, abdominal pain (in healthy individuals) and sudden chills, fever, shock, skin lesions (in high-risk individuals**)**. Incubation period is usually 2-48 hours and symptoms can last for 2-8 days (HHS, 2013).

V. parahemolyticus is halophilic and isolation media usually requires 3% salt to insure

recover of the organism. Temperatures of less than 5 °C and more than 65 °C greatly limit the growth and survival of the organism. *V. parahemolyticus* was tested in seafood samples and not meat and poultry foods due to lower risks associated with the later.

The CDC reports that *V. parahemolyticus* was responsible for 2 multistate outbreaks, 71 (1%) outbreaks and no deaths from 1998 to 2008 (Gould, 2013).

3. METHODS AND MATERIALS

All the methods were based on Food and Drug Administration's (FDA) Bacteriological and Analytical Manual (BAM). Slight modifications were made where needed, specifically the use of commercial test kits (e.g. Remel Rapid One Test kit for *V. parahemolyticus*) instead of individual biochemical tests (e.g. urease test).

3.1 FOOD SAMPLING

Food sampling was roughly broken down into 5 stages. The Food Policy Institute, Rutgers University handled stages one through 4 and the final stage was carried out at the Food Science Department, Rutgers University.

The first stage involved creation of an inventory of online purveyors of meat and seafood products. A pool of over 500 online purveyors was identified (Hallman, 2011). Specific details regarding each of these vendors were recorded. The details included company catalogue, shipping carrier used (FedEx, UPS, DHL, USPS, others), shipping options (overnight, 2-day ground, 2-day air etc.), food safety advice provided by the company to the consumers regarding the safe handling, storage, cooking, cooling, and reheating of the meat, fish, and seafood products.

The second stage involved selection of online vendors followed by ordering food from them. Assuming that the incidence of problems were one 1 in 10 shipments or less, a power analysis indicates that sampling 168 of 500 potential purveyors would permit a margin of error of +/- 4% at a 95% confidence level. Therefore, in stage 2, up to 168 of

the online purveyors of meat, fish, and seafood products were randomly selected from the list collected in the first stage. The products offered by each company were categorized (example: finfish, shellfish, beef, pork, chicken, deli meats etc.). To maximize the number and variety of products analyzed, giving us the broadest look at potential problems across the industry, the sampling strategy employs several criteria: a) No single order could exceed $200, including shipping; b) no single order could exceed more than 5 items randomly selected from among the categories of foods offered by a company; c) because of their presumed low level of risk, pasteurized, canned, and retorted products were excluded from the sample; d) because they are typically boiled or steamed by the consumer, live crabs, lobsters and mussels were also excluded, however because they are often eaten raw, live clams and oysters were included; e) because they are outside the scope of this study, prepared meals were also excluded. In addition, since it is particularly vulnerable to microbial contamination, if the purveyor sold ground meat, it was chosen as one of the purchased products by default. Testing this protocol on a random sample of 25 purveyors suggests that it would result in the availability of more than 600 products for microbial analysis. Samples of meat, fish, and seafood products offered by each selected purveyor were purchased and sent to Rutgers University and Tennessee State University (TSU) for analysis.

The third stage involved receiving the food packages and recording various details regarding the package and the food itself. The recorded details included: time of delivery and the size, type, construction, maker, integrity, and other conditions of the package, the ambient temperature of the interior package, temperature of the food products, total weight and overall condition of the products, date or lot code information, estimate of the

amount and form (block or pelletized) of dry ice or gel packs both originally included and remaining in the package.

The fourth stage involved reducing the size of the samples in sterile environment, using aseptic techniques and equipment before they were brought to the lab for analysis.
The samples were transported to the lab in styrofoam containers with dry ice or chilled gel packs based on the temperature at which the samples were initially received.
Unfrozen or refrigerated samples were stored in ice and frozen samples were stored at -4 °C before used for testing. To cut samples down to workable size they were thawed at 2-5 °C for no more than 18 hours.
The fifth stage involved preparation of samples for microbial testing. On the day of testing, the food sample was placed on a poly-ethylene plastic cutting board that had been thoroughly cleaned with soap and water and then sanitized with 70% ethanol. The knife used to cut the sample was sprayed with 70% ethanol and flame sterilized. Sterile plastic spoons were used to transfer 25g sample to a double-layered filter bag (Thermo Fisher Scientific, Labplas Inc, QC, Canada) as per FDA's BAM. The appropriate buffer or broth (see below) was added to the filter bag, then homogenized (for blending of sample to obtain uniform dilution or effective enrichment) for 2 minutes in a Stomacher (Stomacher lab blender 400, Cooke laboratory, Virginia, USA).

3.2 AEROBIC PLATE COUNT

Using the sample preparation method explained above, a 10^{-1} (1:10) dilution of sample was made by adding 225 ml of peptone water (Difco™, BD - Becton, Dickinson and

Company, Sparks, MD, USA) to 25g of sample followed by homogenization in a Stomacher. Next this was serial diluted to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. The serial dilutions were prepared by adding 1 ml liquid from the first dilution into a test tube containing 9 ml peptone water to obtain the next higher dilution. The process was repeated to get the subsequent dilutions.

To ensure uniform concentration, the test tubes containing the dilutions were vortexed for 7 seconds using the Fisher Vortex Genie 2™ (Scientific Industries, Inc., N.Y., USA). Dilutions were plated within 15 minutes from the preparation time.

Next, 100 μL of each of the dilution was added to total plate count agar (Difco™, BD, Sparks, MD, USA) (TPC). This was done in duplicate. The TPC plates were inverted and incubated at 35 °C for 24-48 hours.

Spreader-free (colonies that spread and cover part or whole of that agar in a petri dish thus making it difficult to get an exact count of microorganisms present, example: *B. cereus*) plates having 25 to 250 colonies were used for counting. All types of colonies were counted, including pin-point colonies (colonies as small as the size of a pinhead, formed by organisms such as *Staphylococcal epedermidis* and *Rhodospirillum rubrum*). The counts obtained outside the range of 25-250 colonies were reported as estimated aerobic plate counts (EAPC). Plates with very high counts were reported as too numerous to count (TNTC).

Computing counts

For plates with 25-250 colony forming units (CFU)

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)}$$

The APC was calculated as follows, where:

N = Number of colonies per ml or g of product

Σ C = Sum of all colonies on all plates counted

n1 = Number of plates in first dilution counted

n2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

Example:

1:100	1:1000
232, 244	33, 28

$$N = \frac{(232 + 244 + 33 + 28)}{[(1 \times 2) + (0.1 \times 2)] \times 10^{-2}}$$

= 537/0.022 = 24,409 ≈ 24,000

When counts of duplicate plates fell inside and outside the 25 to 250 colony range, only those counts that fell within the range were used.

When plates from both dilutions yielded fewer than 25 CFU each, actual plate count were recorded but the counts were recorded as less than 25 × 1/d where d is the dilution factor for the dilution from which the first counts were obtained.

When plates from both 2 dilutions yielded more than 250 CFU each (but fewer than 100/cm^2), the aerobic counts from the plates were estimated to the nearest 250 and multiplied by the dilution.

Media

Table 1. Composition of peptone water

Difco™ Peptone Water per L	
Content	g
Peptone	10
Sodium Chloride	5

Peptone water is a general-purpose buffer. In this procedure it was used for sample preparation and m dilutions. Peptone provides carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the o balance. Phosphates provide buffering action.

Table 2. Composition of plate count agar

Difco™ Plate Count Agar per L	
Content	g
Pancreatic Digest of Casein	5
Yeast Extract	2.5
Dextrose	1
Agar	15

Enzymatic or pancreatic digest of casein provides the amino acids and other nitrogen sources essential for growth of bacteria. Yeast extract supplies B-complex vitamins. Dextrose acts as the carbon or energy source.

3.3 COLIFORMS AND *E. COLI*

The procedure was based on FDA's Bacteriological Analytical Manual (Feng, 2013). Test tubes containing 9 ml of Lauryl tryptose (LST) broth (Difco™, BD, Sparks, MD, USA) were inoculated using the peptone dilutions from above. Triplicates of each dilution were used, giving a total of 15 LST tubes/sample. For each positive LST tube, one *E. coli* media (EC) (Difco™, BD, Sparks, MD, USA) and one Brilliant Green media (BG) (Difco™, BD, Sparks, MD, USA) tube was inoculated by dipping in a sterile loop and transferring 10 µl liquid. For each positive EC and BG tube, one plate each of Levine's eosin-methylene blue (EMB) (BBL™, BD, Sparks, MD, USA) agar and MacConkey agar (BBL™, BD, Sparks, MD, USA) was spiral streaked with 1 µl liquid. LST, EC and BG tubes contain a smaller tube (Durham tube) that trap the air bubbles produced by coliforms and *E. coli* during fermentation of sugars. A positive LST, EC or BG tube is the one with air bubble (small or big) in the Durham tube. On EMB media, positive colonies appear shiny green with metallic sheen. And on MacConkey, the lactose positive coliforms and *E. coli* appear pink.

Gas formation and/or effervescence in LST tube indicate presence of presumptive coliforms and *E. coli*. Likewise, Gas formation and/or effervescence in BG and EC tubes indicate presence of confirmed coliforms and presumptive fecal coliforms and *E. coli* respectively. Use of EMB and MacConkey agar help in further isolation of presumptive *E. coli*.

Some strains of *E. coli* have been found to be anaerogenic (non-gas producers). These are detected using the LST-MUG (Oxoid, Hampshire, England) test. It involves incorporating the substrate 4-methylumbelliferyl β-D-glucuronide (MUG) into LST

broth. MUG gets cleaved by the enzymatic activity of β-glucuronidase (GUD). Most (~95%) of *E. coli*, including anaerogenic strains, are GUD positive. Cleavage of MUG produces 4-methylumbelliferone (MU), which produces blue fluorescence on exposure to longwave (365 nm) UV light.

Enterotube II (BBL™, BD, Sparks, MD, USA) was used as a final step of identification of *E. coli*. It identifies *Enterobacteriaceae* and a variety of other oxidase negative Gram-negative rods involving a series of biochemical reactions. After inoculation, the Enterotube is incubated at 37 °C for 24 hours. Based on color change and production of gas and H_2S a 5-digit code is generated. Comparing this code with the database helps in conformation of presence of *E. coli*.

OXOID's dry spot latex test kit (Oxoid, Hants, UK) was used to identify pathogenic *E. coli*. Here two kits were used. The first kit identified *E. coli* as one of the 6 pathogenic strains (i.e. the seroscreen or Big6), O26, O91, O103, O111, O128 and O145. The second kit identified *E. coli* as O157:H7. Both these kits were based on the principle of antigen-antibody agglutination reaction. The antibody is present on the sensitized latex particles dried onto the test cards. Positive reaction was obtained in the presence of a specific cell wall antigen-O carried by most Verotoxin producing pathogenic *E. coli.*

Enumeration was done using Most Probable Number (MPN) technique, discussed in detail in section 1.4 below.

Media

Table 3. Composition of Lauryl Tryptose Broth

Difco™ Lauryl Tryptose Broth per L

Content	g
Tryptose Peptone	20
Lactose	5
Dipotassium Phosphate	2.75
Monopotassium Phosphate	2.75
Sodium Chloride	5
Sodium Lauryl Sulfate	0.1

Peptone provides nitrogen, carbon, sulfur and trace ingredients. Potassium phosphates provide buffering capacity. Sodium chloride maintains osmotic equilibrium. Lactose is a source of fermentable carbohydrate. Fermentation of lactose forms gas that helps in identification. Sodium lauryl sulfate inhibits organisms other than coliforms.

Table 4. Composition of EC Medium

Difco™ EC Medium per L	
Content	g
Tryptose	20
Lactose	5
Bile Salts No. 3	1.5
Dipotassium Phosphate	4
Monopotassium Phosphate	1.5
Sodium Chloride	5

Peptone provides nutrients, lactose provides fermentable carbohydrate an bile salts inhibit Gram-positive bacteria. Potassium phosphate provides buffering action. Sodium chloride maintains the osmotic balance.

Table 5. Composition of Brilliant Green Bile Broth

Difco™ Brilliant Green Bile Broth per L	
Content	g
Peptone	10

Oxgall	20
Lactose	10
Brilliant Green	0.0133

Peptone provides nutrients, lactose provides fermentable carbohydrate and Oxgall and brilliant green dye inhibit both Gram-positive and selected Gram-negative organisms.

Table 6. Composition of Levine Eosin Methylene Blue Agar

BBL™ Levine Eosin Methylene Blue Agar per L	
Content	g
Pancreatic Digest of Gelatin	10
Lactose	10
Dipotassium Phosphate	2
Eosin Y	0.4
Methylene Blue	0.065
Agar	15

Pancreatic digest of Gelatin provides nutrients, lactose is a carbohydrate source and Dipotassium phosphate provides buffering action. The eosin Y and methylene blue dyes inhibit Gram-positive bacteria and also differentiate between lactose fermenters and lactose non-fermenters due to the presence or absence of dye uptake in the bacterial colonies.

Table 7. Composition of MacConkey Agar

BBL™ MacConkey Agar per L	
Content	g
Pancreatic Digest of Gelatin	17

Peptones (meat and casein)	3
Lactose	10
Bile Salts	1.5
Sodium Chloride	5
Agar	13.5
Neutral Red	0.03
Crystal Violet	0.001

Peptones and yeast extract provide nitrogen, trace elements, vitamins, amino acids and carbon. Lactose is a fermentable carbohydrate. Magnesium sulfate is a source of divalent cations. Sodium chloride maintains osmotic balance in the medium. Bile salts, bile salts no. 3, oxgall and crystal violet inhibit growth of Gram-positive organisms.

3.4 MOST PROBABLE NUMBER (MPN)

MPN uses a set of serial dilutions to estimate the concentration of the target organism. This method is based on following assumptions: within the sample, bacteria are evenly distributed, the bacteria exist as individual cells and not in chains or clusters, the bacteria do not repel each other, presence of even one cell will lead to visualization of positive results, the result obtained in each tube are independent of one another.

The solution for the concentration, λ, can be obtained using the equation,

$$\sum_{j=1}^{k} \frac{g_j m_j}{1-\exp(-\lambda m_j)} = \sum_{j=1}^{k} t_j m_j$$

where:

exp(x) means ex, and *K* denotes the number of dilutions,

gj denotes the number of positive tubes in the *j*th dilution,

mj denotes the amount of the original sample put in each tube in the *j*th dilution

tj denotes the number of tubes in the *j*th dilution.

An MPN calculator (http://www.i2workout.com/mcuriale/mpn/), based on the above formula, was used to estimate the probable concentration of the target bacteria. Only three (out of 5) dilutions were chosen for calculation purposes, as per rules in the FDA's BAM manual. The procedure was based on FDA's Bacteriological Analytical Manual (Blodgett, 2010).

3.5 *BACILLUS CEREUS*

B. cereus is very similar to 5 other Bacillus species (*B. mycoides, B. thuringiensis, B. megaterium, B. anthracis and B. weihenstephanensis*), hence, it is essential to perform a series of confirmatory test to eliminate false-positives.

The procedure was based on FDA's Bacteriological Analytical Manual (Tallent, 2012). On day 1, 25g of sample and 225 ml of peptone buffer were added to the double-layered filter bag. This was homogenized in a stomacher at low speed for 2 minutes. 0.1 ml of this was spread plated on Mannitol-egg yolk-polymyxin (MYP) agar (Difco™, BD, Sparks, MD, USA). These plates were incubated at 30 °C for 18-24 hours.

The plates were checked after 24 hours of incubation for presumptive *B. cereus* colonies. Presumptive colonies appear pink (since *B. cereus* is mannitol-negative) with a zone of precipitation around the colony due to lecithinase activity. Gram stain was performed on

presumptive colonies from MYP. *B. cereus* would appear as large Gram-positive rods under the microscope.

Using a sterile plastic loop, a presumptive *B. cereus* colony was picked up from the MYP media and streaked onto Blood agar plates (containing 5% sheep blood) (Remel Inc., KS, USA). After incubation at 35 °C for 24 hours, a positive reaction leads to formation zone of clearance surrounding the colonies or growth. Since *B. cereus* is strongly hemolytic, it produces 2-4 mm zones. Since some other *Bacillus* spp. are hemolytic-negative, they are screen out by this test.

A few presumptive colonies from blood agar plates were added to 10 ml peptone water and incubated for 18-24 hours. One µl of this suspension was streaked onto nutrient agar. After the liquid was absorbed into the agar, it was incubated at 30 °C for 48-72 hours. Formation of galaxy-like growth (colonies growing in spiral winding shape usually in elliptical form) serves as further confirmation of *B. cereus*.

A loopful of the peptone water suspension above was used to inoculate nutrient agar slant followed by incubation at 30 °C for 24 hours and then at room temperature for 2-3 days. A smear of colonies from nutrient agar was prepared on a microscope slide and heat fixed by passing over a Bunsen burner. Methanol was poured on the slide and after 30 seconds, the methanol was poured off and the slide air-dried. The slide was then stained with 0.5% basic fuchsin stain by flooding the stain and then passing the slide over flame. This procedure was repeated after 1-2 minutes and then slide was rinsed with tap water. Observation under oil immersion showing free spores and darkly stained tetragonal (diamond-shaped) toxin crystals indicates presumptive colonies of *B. thuringiensis* (not *B. cereus*).

The peptone suspension used for above tests was also streaked on two Tryptic Soy Agar (TSA) plates (Difco™, BD, Sparks, MD, USA). One was incubated at 6°C for 28 days and the second at 43°C for 4 days. If grow is observed at 6°C but not at 43°C this indicates *B. weihenstephanensis*, while *B. cereus* grows at 43°C but not at 6°C.

Media

Table 8. Composition of Mannitol-Egg Yolk-Polymyxin

Difco™ Mannitol-Egg Yolk-Polymyxin per 900ml	
Content	g
Beef Extract	1
Peptone	10
D-Mannitol	10
Sodium Chloride	10
Phenol Red	0.025
Agar	15

Beef extract and peptone provide carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source and it plays a role in differentiating mannitol fermenters from non-fermenters, which give the colony a yellow or a pink color respectively. Phenol red is the pH indicator. Egg Yolk Enrichment 50% is responsible for lecithinase activity that produces a precipitation zone around presumptive *B. cereus* colonies. Antimicrobic Vial P inhibits the growth of most non-*B. cereus* bacteria.

Table 9. Composition of Blood agar

Remel Blood Agar	
Content	%
TSA	95

Sheep Blood agar	5

The presence of 5% sheep's blood helps test for presence of hemolytic bacteria that lyse the blood cells to form a zone of clearing around the colony.

Table 10. Composition of Nutrient agar

Difco™ Nutrient Agar per L	
Content	g
Beef Extract	3
Peptone	5
Agar	19

This is a relatively general media that facilitates growth of most of the bacteria. Beef extract and peptone provide carbohydrates, vitamins, organic nitrogen compounds and salts.

Table 11. Composition of Tryptic Soy agar

Difco™ Tryptic Soy Agar (Soybean-Casein Digest Agar) per L	
Content	g
Pancreatic Digest of Casein	15
Papaic Digest of Soybean	5
Sodium Chloride	5
Agar	15

This medium can be used for a variety of purposes such as aerobic plate count, maintenance of stock cultures etc. Presence of both casein and soy peptones makes the medium rich in organic nitrogen, particularly amino acids and longer-chained peptides.

Sodium chloride maintains the osmotic equilibrium.

3.6 *CLOSTRIDIUM PERFRINGENS*

C. perfringens is anaerobic microorganism that forms spores and is ubiquitously present in the environment. Both identification and enumeration of *C. perfringens* was done using a procedure based on FDA's Bacteriological Analytical Manual (Rhodehamel, 2001).

Twenty five g of sample and 225 ml of peptone buffer were added to the double-layered filter bag. This was homogenized in a stomacher at low speed for 2 minutes. One-tenth ml of the homogenate was spread plated on Tryptose Sulfite Cycloserine (TSC) (Oxoid, Hampshire, England) agar. Five minutes post inoculum absorption, the plate was layered with more TSC agar to create a sandwich of TSC agar with the inoculum in the center in an anaerobic environment. These plates were incubated at 35 °C for 18-24 hours in an anaerobic chamber along with a Gas-Pak™ EZ anaerobe container system with indicator (BD, Sparks, MD, USA) to remove oxygen.

The plates were checked after 24 hours of incubation for presumptive *C. perfringens* colonies. Presumptive colonies appear black due to conversion of sodium disulfite to sulfite in presence of H_2S producing bacteria.

Gram stain was performed on presumptive colonies from TSC agar. *C. perfringens* appear as Gram-positive rods. Change in color of lactose agar (Difco™, BD, Sparks, MD, USA) slants (after inoculation by stabbing) from yellow to red due to acid production indicated presence of *C. perfringens*.

Plate count agar slant was stabbed with needle picked from presumptive *C. perfringens* colonies on TSC agar. Non-motile bacteria grow only along the stab line. *C. perfringens* is motile, and positive slants had growth in a diffused pattern.

A slant of gelatin (Sigma, MO, USA) was stabbed multiple times with a needle containing presumptive *C. perfringens* colonies picked from TSC agar. The slant was incubated at 35 °C for 18-24 hours followed by chilling the tube at 5 °C for 1 hour. In case the gelatin did not liquefy, the tube was again chilled for one hour after 24 hours. Positive *C. perfringens* liquefied gelatin within 48 hours.

Media

Table 12. Composition of Perfringens agar base

Oxoid Perfringens agar base (TSC & SFP) per L	
Content	g
Tryptose	15
Soya peptone	5
Yeast extract	5
Sodium metabisulphite	1
Ferric ammonium citrate	1
Agar	19

Meat peptone, soya peptone and yeast extract provide nutrients and vitamins for the development of clostridia. Sodium disulfite helps in differentiating the H_2S-positive bacteria by formation of black salt with ammonium ferric citrate (FeS).

3.7 *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus was identified and enumerated. The procedure was based on FDA's Bacteriological Analytical Manual (Bennett, 2001). 25 g of sample and 225 ml of peptone buffer were added to the double-layered filter bag. This was homogenized in a stomacher at low speed for 2 minutes. One-tenth ml of this was spread plated on Baird Parker (BP) (Difco™, BD, Sparks, MD, USA) media. These plates were incubated at 35 °C for 24-48 hours.

After 24 hours of incubation, presumptive *Staphylococcus aureus* colonies were counted. Presumptive colonies appear as gray or black occasionally with off-white margin, an opaque zone and an outer clear zone. The colonies are usually circular, smooth, convex, 2-3 mm in diameter, moist with buttery/gummy consistency. Colonies from frozen samples may have lighter black color as compared to non-frozen samples.

A single isolated colony was transferred to a glass microscope slide for the catalase test. A drop of hydrogen peroxide (H_2O_2) was added. Production of bubble indicated presence of *S. aureus*.

Presumptive *S. aureus* colonies were transferred to a small tube containing 0.2-0.3 ml Blood Heart Infusion (BHI) (Remel Inc., KS, USA) broth. This was mixed well and incubated at 35 °C for 18-24 hours. Next, 0.5 ml of reconstituted BBL rabbit coagulase plasma with EDTA (BD, Sparks, MD, USA) was added to 1 ml BHI culture followed by incubation at 35 °C for 4-6 hours. A positive reaction involves formation of large clots, while small clots indicate false-positive reactions. This test is based on presence of enzyme coagulase in *S. aureus,* which on liberation from the cell acts on prothrombin to

form a thrombin-like product. This thrombin-like product acts on fibrinogen to form a fibrin clot.

BBL™ Staphyloslide™ Latex Test (BD, Sparks, MD, USA) was used for confirmation of presence of *S. aureus*. It differentiates staphylococci based on possession of clumping factor and/or Protein-A, which are present in 97% of *S. aureus*. Agglutination indicates presence of *S. aureus*.

Media

Table 13. Composition of Baird-Parker Agar

Difco™ Baird-Parker Agar base per 950ml	
Content	g
Pancreatic Digest of Casein	10
Beef Extract	5
Yeast Extract	1
Glycine	12
Sodium Pyruvate	10
Lithium Chloride	5
Agar	20

Pancreatic digest of casein, peptone, beef extract and yeast extract provide nitrogenous compounds, c sulfur, vitamins and minerals. Sodium pyruvate helps in growth of S. aureus without destroying selec Glycine and lithium inhibit organisms other than *S. aureus*.

Table 14. Composition of Brain Heart Infusion Agar

Oxoid Brain Heart Infusion per L	
Content	g
Calf brain infusion solids	12.5

Brain heart infusion solids	5
Proteose peptone	10
Glucose	2
Sodium chloride	5
Di-sodium phosphate	2.5

This is a general-purpose medium suitable for cultivation of a variety of microorganisms. Brain-heart infusion and peptone provide organic nitrogen, carbon, sulfur, vitamins and trace substances for growth. Glucose provides carbohydrate source. Disodium phosphate provides buffering action.

3.8 *SALMONELLA SPECIES*

The following protocol was used for identification of *Salmonella* species. The procedure was based on FDA's Bacteriological Analytical Manual (Andrews, 2011). Twenty-five g of sample and 225 ml of lactose broth were added to the double-layered filter bag for pre-enrichment. This was homogenized in a stomacher at low speed for 2 minutes. The bag was sealed and incubated at 35 °C for 24 ± 2.0 hours. One ml of the above homogenate was added to 9 ml of Tetrathionate broth (TTB) (Difco™, BD, Sparks, MD, USA). This was mixed well by vortexing and incubated at 35± 2 °C for 24 ± 2.0 hours. Following incubation, 1 µl of enriched liquid was spiral streaked onto three agars: Hektone enteric (HE), Xylose Lysine Tergitol-4 (XLT-4) and Bismuth Sulfite (BS) (Difco™, BD, Sparks, MD, USA). This was incubated at 35 °C for 24 ± 2.0 hours. BS agar was prepared one day in advance and covered with aluminum foil to prevent the action of light).
These plates were checked for presumptive *Salmonella* colonies as follows: HE presumptive *Salmonella* colonies are Blue-green to blue with or without black centers or

completely black. Some atypical colonies may appear yellow with or without black centers. XLT-4 presumptive *Salmonella* colonies are pink with or without black centers or completely black. Some atypical colonies may appear yellow with or without black centers. BS presumptive *Salmonella* colonies are brown, gray, or black, sometimes with a metallic sheen. A brown or black halo might be present around the colony. Some atypical colonies may appear green colonies with little or no halo.

A Gram stain was performed on presumptive colonies from HE, BS and XLT-4. *Salmonella* appear as Gram-negative rods under the microscope.

OXOID's *Salmonella* latex test kit (Oxoid, Hants, England) was used to identify presumptive *Salmonella* spp. Here, a single isolated colony from either one of the three selective media (BS, HE or XLT-4) was dispersed in a drop of latex suspension on a test card. The card was rocked for 2 minutes. A positive test showed agglutination based on antigen-antibody reaction. The latex particles are sensitized using polyvalent antisera in rabbits, which is produced with the help of a variety of *Salmonella* flagella antigens.

BBL Enterotube II was used as a final step of identification. It identifies *Enterobacteriaceae* and a variety of other oxidase negative Gram-negative rods involving a series of biochemical reactions. After inoculation from either one of the three selective media (BS, HE or XLT-4), the tube was incubated at 37 °C for 24 hours. Based on color change and production of gas and H_2S a 5-digit code was generated. Comparing this code with the database helps in conformation of presence of *Salmonella*.

Media

Table 15. Composition of Lactose broth

Difco™ Lactose broth per L	
Content	g
Beef extract	3
Peptone	5
Lactose	5

The peptone and beef extract provide essential nutrients for bacterial metabolism. Lactose provides a source of fermentable carbohydrate for Gram-negative *Enterobacteriaceae.*

Table 16. Composition of Lactose broth

Difco™ Tetrathionate broth base per L	
Content	g
Protease Peptone	2.5
Pancreatic digest of Caesin	2.5
Oxgall	1
Sodium Thiosulfalte	30
Calcium Carbonate	10

Enzymatic Digest of Casein provides nitrogen, carbon, vitamins, and amino acids. Sodium Thiosulfate provides selectivity by suppressing commensal intestinal organisms. On addition of the iodine and potassium iodide solution tetrathionate is formed which helps in growth of organisms containing the enzyme tetrathionate reductase. Calcium Carbonate neutralizes and absorbs toxic metabolites.

Table 17. Composition of Hektoen Enteric Agar

Difco™ Hektoen Enteric Agar per L	
Content	g
Proteose Peptone	12
Yeast Extract	3
Bile Salts No. 3	9
Lactose	12
Saccharose	12
Salicin	2
Sodium Chloride	5
Sodium Thiosulfate	5
Ferric Ammonium Citrate	1.5
Agar	14
Bromothymol Blue	0.065
Acid Fuchsin	0.1

Three carbohydrates (lactose, sucrose and salicin) help in differentiation of enteric pathogens based on color produced by the colony and surrounding media. Acid fuchsin and bromothymol blue indicators. Bile salts inhibit Gram-positive and some Gram-negative bacteria while allowing *Salmonella spp.* to grow. Ferric-ammonium citrate and sodium thiosulfate produce black color colonies in presence of hydrogen sulfide producing bacteria such as *Salmonella*.

Table 18. Composition of XLT4 Agar Base

Difco™ XLT4 Agar Base per L	
Content	g
Proteose Peptone No. 3	1.6
Yeast Extract	3
L-Lysine	5
Xylopse	3.75
Lactose	7.5
Saccharose	7.5

Ferric Ammonium Citrate	0.8
Sodium Thiosulfate	6.8
Sodium Chloride	5
Agar	18
Phenol Red	0.08

Peptone, yeast extract, sodium thiosulfate provide complex nitrogen compounds, vitamins, inorganic sulfur. Sodium chloride maintains the osmotic balance. Phenol red is added as an indicator of pH changes based on fermentation of xylose, lactose and sucrose and decarboxylation of lysine. Ferric ions help in identification of production of hydrogen sulfide. XLT-4 Supplement inhibits growth of non-Salmonella organisms.

Table 19. Composition of Bismuth Sulfite Agar

Difco™ Bismuth Sulfite per L	
Content	g
Beef Extract	5
Peptone	10
Dextrose	5
Disodium Phosphate	4
Ferrous Sulfate	0.3
Bismuth Sulfite Indicator	8
Brilliant Green	0.025
Agar	20

Beef extract, peptone and dextrose provide nitrogen, vitamins, minerals and energy source. Disodium phosphate helps in buffering action. Bismuth sulfite and brilliant green are indicators, which inhibit Gram-positive bacteria and members of the coliform group, while allowing *Salmonella* to grow. Iron from ferrous sulfate produce black color

colonies in presence of hydrogen sulfide producing bacteria.

3.9 *LISTRIA MONOCYTOGENES*

Testing for *L. monocytogenes* involved identification followed by enumeration, using MPN technique (as described in 3.4 above). The procedure was based on FDA's Bacteriological Analytical Manual (Hitchins, 2013). 25 g of sample and 225 ml of BLEB (Buffered Listeria enrichment broth) (Difco™, BD, Sparks, MD, USA) were added to the double-layered filter bag. This was homogenized in a stomacher at low speed for 2 minutes. The bag was sealed and incubated at 35 °C for 24-48 hours. After 24 hours of enrichment, one loopful (1 µl) of the homogenate was spiral streaked onto Oxford agar (Difco™, BD, Sparks, MD, USA). This was incubated at 35 °C for 24-48 hours. The same procedure was repeated by streaking the homogenate after 48 hours of enrichment. The plates were checked after 24 and 48 hours of incubation for presumptive *L. monocytogenes* colonies (black colonies with a halo).

The presumptive *L. monocytogenes* colonies were confirmed using the API Listeria kit (BioMerieux, France). This kit uses miniaturized tests based on enzymatic reaction and sugar fermentation to generate a 4-digit code. Comparing this code with the BioMerieux database identified the listeria species.

Media

Table 20. Composition of Listeria Enrichment Broth

Difco™ Listeria Enrichment Broth per L

Content	g
Pancreatic Digest of Caesin	17
Soytone	3
Dextrose	2.5
Sodium Chloride	5
Dipotassium Phosphate	2.5
Yeast Extract	6
Cycloheximide	0.05
Acriflavine HCl	0.015
Nalidixic Acid	0.04

Peptones, yeast extract and dextrose provide nutrients such as nitrogen, vitamins, minerals and carbohydrate. Sodium chloride maintains the osmotic balance. Phosphates provide buffering capacity. Growth of Gram-negative and Gram-positive bacteria is inhibited by nalidixic and acriflavine respectively and cycloheximide acid inhibits saprophytic fungi.

Table 21. Composition of Oxford medium base

Difco™ Oxford medium base per L	
Content	g
Pancreatic Digest of Caesin	8.9
Proteose Peptone No. 3	4.4
Yeast Extract	4.4
Beef Heart, Infusion from 500 g	2.7
Starch	0.9
Sodium Chloride	4.4
Esculin	1
Ferric Ammonium Citrate	0.5
Lithium Chloride	15
Agar	153

Yeast provides vitamin B complex and starch provides energy. Sodium chloride maintains the osmotic balance. Lithium chloride, cycloheximide, clutistin, cefotetan, fosfomycin and acriflavine inhibit growth of non-*Listeria* microorganisms. *Listeria* hydrolysis esculin to 6,7-dihydroxycoumarin. This reaction is detected by ferric ions, which turns the colony and its surrounding media black.

3.10 *VIBRIO PARAHEMOLYTICUS*

Since *V. parahaemolyticus* requires enrichment, only identification was performed. The procedure was based on FDA's Bacteriological Analytical Manual (Depaola Jr., 2004). 25 g of sample and 225 ml of Alkaline peptone water (Difco™, BD, Sparks, MD, USA) with 3% NaCl (Fisher Scientific, USA) were added to the double-layered filter bag. This was homogenized in a stomacher at low speed for 2 minutes. The bag was sealed and incubated at 35 °C for 24 hours. One µl of the enriched inoculum was spiral streaked on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) agar (Difco™, BD, Sparks, MD, USA), and incubated at 35 °C for 24-48 hours. Presumptive *V. parahemolyticus* colonies appeared green or blue, round, opaque and 2-3 mm in diameter.
Remel's Rapid NF Plus kit (Remel Inc., KS, USA) was used for confirmation of presence of *V. parahemolyticus*. This test is based on series of enzymatic tests and sugar fermentation. The kit differentiates oxidase-positive, Gram-negative bacilli, including *Vibrio* spp. within 4 hours.

Media

Table 22. Composition of Alkaline peptone water

Oxoid Alkaline peptone water per L	
Content	g
Peptone	10
Salt	20

Alkaline peptone water provides an alkaline pH of 8.6. This is required for growth of *V. parahemolyticus.*

Table 23. Composition of TCBC Agar

Difco™ TCBS Agar per L	
Content	g
Yeast Extract	5
Proteose Peptone No. 3	10
Sodium Citrate	10
Sodium Thiosulfate	10
Oxgall	8
Saccharose	20
Sodium Chloride	10
Ferric Ammonium Citrate	1
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15

Yeast extract, enzymatic digest of casein, and enzymatic digest of animal tissue provides the nitrogen, vitamins, and amino acids. Sucrose is source of carbohydrate. Bromthymol Blue and Thymol Blue are pH indicators. Sodium Citrate and Sodium Thiosulfate inhibit growth of Gram-positive bacteria and coliforms while promoting growth of *Vibrio spp.*

4. RESULTS AND DISCUSSION

4.1 Prevalence of indicator or pathogenic organism in various food types

Table 24 shows the presence of generic *E. coli* (≥ 1 cell of *E. coli*/g of food), in various food types. The first column represents the category of foods, the second column represents the various food items tested, the third column represents the total number of samples tested in each category and the fifth column contains the prevalence rate (in %) of total *E. coli* in each food type. It is evident that presence of *E. coli* is higher in poultry (at 34%) than in meats (21%) or seafood (10%). Also shellfish (13%) tend to harbor *E. coli* more than finfish (9%).

Table 24. Prevalence of generic *E. coli* in various mail ordered foods order over the Internet.

Category	Food Type	n*	Positives	%
Meat	Beef	108	19	18
	Bison/Buffalo	18	3	17
	Deer/Venison	13	5	38
	Lamb	14	5	36
	Rabbit	2	0	0
	Pork	25	5	20
	Mixed	12	3	25
	Total	192	40	21
Poultry	Chicken	19	5	26
	Duck	7	3	43

	Emu	3	1	33
	Guinea Foul	1	1	100
	Quail	2	1	50
	Turkey	3	0	0
	Pheasant	3	2	67
	Total	38	13	34
Seafood		111	11	10
Finfish	Cod	5	1	20
	Coho lox	1	0	0
	Grouper	3	0	0
	Haddock	4	0	0
	Halibut	10	1	10
	Roughy	1	0	0
	Snapper	2	0	0
	Rockfish	2	0	0
	Salmon	30	3	10
	Sea bass	4	0	0
	Sole	4	0	0
	Sword fish	1	0	0
	Tilapia	1	0	0
	Tuna	11	2	18
	Total	79	7	9
Shellfish	Crab	4	0	0

	Lobster	13	2	15
	Oyster	3	1	33
	Prawn	1	0	0
	Scallop	5	1	20
	Shrimp	6	0	0
	Total	32	4	13

* 'n' is the number of samples tested, a positive means presence of *E. coli*, and percent prevalence is number of (positives/n)*100.

Table 25 shows the prevalence of STEC *E. coli* O157:H7 and the "big six" (≥ 1 cell of pathogenic *E. coli*/g of food) in various food types. These data show that incidence of pathogenic *E. coli* is much lower in seafood (3%) than in meats (12%) or poultry (21%). Out of the 14 different types of finfish, only salmon seems to be associated with pathogenic *E. coli* and likewise, among 6 different types of shellfish, only lobster seems to be associated with pathogenic *E. coli*.

Table 25. Prevalence of pathogenic *E. coli* (shiga-toxigenic *E. coli* O157:H7 and the "Big Six" as determined by latex agglutination) in various mail ordered foods order over the Internet.

	Food Type	**n***	**Positives**	**%**
Meat	Beef	108	11	10
	Bison/Buffalo	18	3	17
	Deer/Venison	13	2	15

	Lamb	14	5	36
	Rabbit	2	0	0
	Pork	25	1	4
	Mixed	12	1	8
	Total	192	23	12
Poultry	Chicken	19	3	16
	Duck	7	2	29
	Emu	3	0	0
	Guinea Foul	1	1	100
	Quail	2	0	0
	Turkey	3	0	0
	Pheasant	3	2	67
	Total	38	8	21
Seafood		111	3	3
Finfish	Cod	5	0	0
	Coho lox	1	0	0
	Grouper	3	0	0
	Haddock	4	0	0
	Halibut	10	0	0
	Roughy	1	0	0
	Snapper	2	0	0
	Rockfish	2	0	0
	Salmon	30	2	7

	Sea bass	4	0	0
	Sole	4	0	0
	Sword fish	1	0	0
	Tilapia	1	0	0
	Tuna	11	0	0
	Total	79	2	3
Shellfish	Crab	4	0	0
	Lobster	13	1	8
	Oyster	3	0	0
	Prawn	1	0	0
	Scallop	5	0	0
	Shrimp	6	0	0
	Total	32	1	3

* 'n' is the number of samples tested, a positive means presence of pathogenic *E. coli*, and percent prevalence is number of (positives/n)*100.

Table 26 shows the prevalence of *B. cereus* in various food types. The data in Table 26 confirms the ubiquitous nature of *B. cereus* with a prevalence of 58% in seafood, 47% in both meats and poultry. Almost all food types surveyed contain *B. cereus* at levels greater than the detection limit.

Table 26. Prevalence of *B. cereus* in various mail ordered foods order over the Internet at levels >2 log CFU/g.

	Food Type	n*	Positives	%
Meat	Beef	108	52	48
	Bison/Buffalo	18	6	33
	Deer/Venison	13	4	31
	Lamb	14	9	64
	Rabbit	2	1	50
	Pork	25	14	56
	Mixed	12	4	33
	Total	192	90	47
Poultry	Chicken	19	11	58
	Duck	7	4	57
	Emu	3	0	0
	Guinea Foul	1	0	0
	Quail	2	2	100
	Turkey	3	0	0
	Pheasant	3	1	33
	Total	38	18	47
Seafood		111	64	58
Finfish	Cod	5	3	60
	Coho lox	1	0	0
	Grouper	3	3	100
	Haddock	4	3	75
	Halibut	10	4	40

	Roughy	1	1	100
	Snapper	2	2	100
	Rockfish	2	1	50
	Salmon	30	19	63
	Sea bass	4	2	50
	Sole	4	4	100
	Sword fish	1	0	0
	Tilapia	1	0	0
	Tuna	11	5	45
	Total	79	47	59
Shellfish	Crab	4	2	50
	Lobster	13	8	62
	Oyster	3	1	33
	Prawn	1	0	0
	Scallop	5	3	60
	Shrimp	6	3	50
	Total	32	17	53

* 'n' is the number of samples tested, a positive means presence of *B. cereus*, and percent prevalence is number of (positives/n)*100.

Table 27 shows the prevalence of *Salmonella* in various food types at a level ≥ 1 cell of *Salmonella*/g of food. In foods order over the Internet, *Salmonella* appeared to be more

prevalent in seafood (18%), especially in finfish (20%), compared to poultry (11%) or meats (6%).

Table 27. Prevalence of *Salmonella* in various mail ordered foods order over the Internet.

	Food Type	n*	Positives	%
Meat	Beef	108	6	6
	Bison/Buffalo	18	0	0
	Deer/Venison	13	0	0
	Lamb	14	2	14
	Rabbit	2	0	0
	Pork	25	2	8
	Mixed	12	1	8
	Total	192	11	6
Poultry	Chicken	19	2	11
	Duck	7	0	0
	Emu	3	0	0
	Guinea Foul	1	0	0
	Quail	2	0	0
	Turkey	3	1	33
	Pheasant	3	1	33
	Total	38	4	11
Seafood		111	20	18

Finfish	Cod	5	2	40
	Coho lox	1	0	0
	Grouper	3	1	33
	Haddock	4	1	25
	Halibut	10	4	40
	Roughy	1	0	0
	Snapper	2	0	0
	Rockfish	2	0	0
	Salmon	30	6	20
	Sea bass	4	0	0
	Sole	4	1	25
	Sword fish	1	0	0
	Tilapia	1	0	0
	Tuna	11	1	9
	Total	79	16	20
Shellfish	Crab	4	1	25
	Lobster	13	2	15
	Oyster	3	1	33
	Prawn	1	0	0
	Scallop	5	0	0
	Shrimp	6	0	0
	Total	32	4	13

* ‘n’ is the number of samples tested, a positive means presence of *Salmonella*, and percent prevalence is number of (positives/n)*100.

Table 28 shows the prevalence of *C. perfringens* in meats and poultry. The incidence rate of *C. perfringens* was higher in meats (19%) than in poultry (13%). Also, interestingly, among poultry, only chicken and turkey samples had *C. perfringens* present at levels greater than the detection limit. Note than seafood samples were not surveyed for *C. perfringens*.

Table 28. Prevalence of *C. perfringens* in various mail ordered foods order over the Internet at levels >2 log CFU/g.

	Food Type	**n***	**Positives**	**%**
Meat	Beef	108	19	18
	Bison/Buffalo	18	5	28
	Deer/Venison	13	3	23
	Lamb	14	3	21
	Rabbit	2	1	50
	Pork	25	2	8
	Mixed	12	4	33
	Total	192	37	19
Poultry	Chicken	19	4	21
	Duck	7	0	0
	Emu	3	0	0

	Guinea Foul	1	0	0
	Quail	2	0	0
	Turkey	3	1	33
	Pheasant	3	0	0
	Total	38	5	13

* 'n' is the number of samples tested, a positive means presence of *C. perfringens*, and percent prevalence is number of (positives/n)*100.

Table 29 shows the prevalence of *V. parahemolyticus* in seafood. Both shellfish and finfish had *V. parahemolyticus* present at levels ≥ 1 cell of *V. parahemolyticus*/g of food. *V. parahaemolyticus* has a higher prevalence in shellfish (47%) than finfish (35%).

Table 29. Prevalence of *V. parahemolyticus* in various mail ordered foods order over the Internet.

	Food Type	**n***	**Positives**	**%**
Seafood		111	43	39
Finfish	Cod	5	3	60
	Coho lox	1	0	0
	Grouper	3	2	67
	Haddock	4	2	50
	Halibut	10	2	20
	Roughy	1	0	0
	Snapper	2	1	50

	Rockfish	2	2	100
	Salmon	30	7	23
	Sea bass	4	3	75
	Sole	4	2	50
	Sword fish	1	0	0
	Tilapia	1	0	0
	Tuna	11	4	36
	Total	79	28	35
Shellfish	Crab	4	2	50
	Lobster	13	7	54
	Oyster	3	1	33
	Prawn	1	1	100
	Scallop	5	1	20
	Shrimp	6	3	50
	Total	32	15	47

* 'n' is the number of samples tested, a positive means presence of *V. parahemolyticus*, and percent prevalence is number of (positives/n)*100.

4.2 Results for comparison of pathogen prevalence in meats, poultry and seafood ordered over the Internet with results obtained in similar published surveys.

Table 30 shows the comparison between the results obtained for *E. coli* during this study and results obtained by others who have done similar work related to incidence of

indicators and foodborne pathogens in foods from conventional sources like grocery stores, farmers markets, fish markets etc. In most of the cases, the prevalence rate of generic *E. coli* in foods ordered from online vendors is the same or lower (except for pork) than the foods obtained from the conventional sources. In the Zhao et al, 2001 study nearly 200 samples (from 4 supermarkets of Washington DC) were tested for each food type: beef, pork, chicken and turkey. The higher incidence rate could be attributed to the fact that there has been a decrease in generic *E. coli* since 1999-2000 when this study was conducted. In case of the seafood comparison, Ryu, 2012 collected over 2500 samples from wholesale and retail markets in Korea. Biochemical tests, similar to the tests used in the current research, showed that incidence rate of *E. coli* in seafood is greater in US foods ordered from online vendors compared to the foods obtained from conventional sources in Korea.

Table 30. Results for comparison of Generic *E. coli* prevalence in meats, poultry and seafood ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Beef	18	19; 58-74	Zhao, 2001; FDA, 2010
Pork	20	16.3; 40-50	Zhao, 2001; FDA, 2010
Chicken	26	38.7; 72-88	Zhao, 2001; FDA,

			2010
Turkey	0	11.9; 74-92	Zhao, 2001; FDA, 2010
Seafood	10	6.7	Ryu, 2012

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

Table 31 shows the comparison between the results obtained for pathogenic *E. coli* during this study and results obtained by other researchers who have done similar work related to incidence of indicators and foodborne pathogens in foods from conventional sources like grocery stores, farmers markets, fish markets etc. Wherever available, multiple references have been added for purpose of comparison. In case of Barlow, 2006, 285 and 275 samples (from Australia) of beef and lamb cuts were analyzed. The higher incidence rates of pathogenic *E. coli* in this study could be due methodological differences (Barlow, 2006 used PCR methods) or geographical differences between the US and Australia. In case of Samadpour (1993), 294 samples of beef, lamb, pork and poultry were obtained from local grocery stores of Seattle. The higher incidence rates of pathogenic *E. coli* in their study could be due to methodological differences (use of PCR), or declines due to the implementation of HACCP in the US meat industry in 1997. In case of Mataragas, 2008, differences may be due to the data sources used to compile prevalence estimates used in their risk profiles.

Table 31. Results for comparison of pathogenic *E. coli* prevalence in meats, poultry and seafood ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Pork	4	0.9; 18	Mataragas, 2008; Samadpour, 1994
Poultry	21	0.5	Mataragas, 2008
Beef	10	23; 16	Samadpour, 1994; Barlow, 2006
Lamb	36	48; 40	Samadpour, 1994; Barlow, 2006
Chicken	16	12	Samadpour, 1994
Turkey	0	7	Samadpour, 1994
Finfish	3	10	Samadpour, 1994
Shellfish	3	5	Samadpour, 1994

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

Table 32 shows the comparison between the results obtained for *B. cereus* during this study and results obtained by other people who have done similar work. Giffel, 1996 analyzed incidence rates of *B. cereus* in food surveyed in The Netherlands. Application of isolation techniques similar to those used in the current study showed a large difference in the incidence rate of *B. cereus* in meats (90% as compared to 47% as obtained in the current study). Guven, 2005, Sooltan, 1987 and Nortje, 1999 tested 100, 102 and 51 samples respectively from Turkey, UK and South Africa using the similar methods for isolation of *B. cereus* that were employed in the current study. All three showed a much lower prevalence rates in the foods surveyed compared to what we observed probably due to geographical differences between US and Turkey, UK and South Africa (as discussed in section 1.7) and/or due to smaller number of samples analyzed.

Table 32. Results for comparison of *B. cereus* prevalence in meats, poultry and seafood ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Meat	47	90; 22.4	Giffel, 1996; Guven, 2005
Poultry	47	6.9	Sooltan, 1987
Chicken	58	2	Nortje, 1999
Beef	48	0	Nortje, 1999

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

Table 33 shows the comparison between the results obtained for *C. perfringens* during this study and results obtained by others who have done similar work related to incidence of *C. perfringens* in foods from conventional sources. Data from Strong et al., 1962 and Hall, 1965 both generally show much higher prevalence rates of *C. perfringens* than we observed in foods ordered from online vendors. This is likely due to the fact that both these studies are more than 40 years old and the incidence of *C. perfringens* in US meats in the 1960s has declined significantly. A more recent study by Miwa, 1999 showed a much lower incidence that our study and the two studies from the 1960's. Differences here may be due to the techniques used by Miwa, 1999 which included both MPN and nested PCR.

Table 33. Results for comparison of *C. perfringens* prevalence in meats and poultry ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Beef	18	70; 17.2; 6.8	Strong, 1962; Hall, 1965; Miwa 1999

Pork	8	37; 25.9; 4.3	Strong, 1962; Hall, 1965; Miwa 1999
Lamb	21	52	Hall, 1965
Meats	19	18.52	Strong, 1962
Chicken	21	58; 22.4; 0	Strong, 1962; Hall, 1965; Miwa 1999

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

Table 34 shows the comparison between the results obtained for *Salmonella* during this study and results obtained by others who have done similar work related to incidence of *Salmonella* in foods from conventional sources. In most cases *Salmonella* prevalence in foods ordered from online vendors was higher than the results reported by others. Two exceptions are Lammerding, 1988, which was published more than 20 years ago and Van, 2007, which employed a different technique (PCR).

Table 34. Results for comparison of *Salmonella* prevalence in meats, poultry and seafood ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Beef	6	3.4; 1.9; 2.6; 1	Zarei, 2012; Jaksic,

			2002; Rahimi, 2010; FDA, 2010
Pork	8	3.3; 8.5; 17.5; 64; 1	Zhao, 2001; Mataragas, 2008; Lammerding, 1988; Van, 2007; FDA, 2010
Chicken	11	15.7; 4.2; 4.3; 9.6; 60.9; 53; 10-20; 90	FSIS 2001; Zhao, 2001; FSIS-USDA, 2012; Duarte, 2009; Lammerding, 1988; Van, 2007; FDA, 2010; Scheinberg, 2013
Turkey	33	29.2; 2.6; 2.2; 69.1; 8.2; 11-19	FSIS 2001; Zhao, 2001; FSIS-USDA, 2012; Lammerding, 1988; Beli, 2001; FDA, 2010
Fish	20	30.5; 2.9	Kumar, 2008; Zarei, 2012
Oysters	33	7.4; 1.5	Brands, 2005; DePaola, 2010
Poultry	11	9.6; 53	Mataragas, 2008; Van, 2007
Shellfish	13	18	Van, 2007

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

Table 35 shows the comparison between the results obtained for *V. parahaemolyticus* during this study and results obtained by other who had done similar work on *V. parahaemolyticus* prevalence in seafood from conventional sources. Our results showed that in most cases *V. parahemolyticus* prevalence in foods ordered from online vendors was higher than the data reported by others. Some of them (e.g. Baffone, 2000, Jaksic, 2002, Kampelmacher, 1972, Yang, 2007) used similar isolation techniques as those used in the current study. While the others (e.g. Zarei, 2012, Messelhausser, 2010, Rahimi, 2010, Rosec, 2008 used PCR techniques. The two exceptions with prevalence rate lower in foods ordered from Internet as compared to foods obtained from conventional sources are Wong, 1999, which was carried out in Taiwan and Chen, 2008, which employed a different technique (PCR).

Table 35. Results for comparison of *V. parahemolyticus* prevalence in seafood ordered over the Internet with results obtained in similar published surveys.

Food type*	Prevalence (%)*	Prevalence in similar work (%)*	References*
Fish	35	2.9; 2.6; 29.3; 1.6; 6.7	Zarei, 2012; Baffone, 2000; Wong, 1999;

			Messelhausser, 2010; Jaksic, 2002
Lobster	54	44.1	Wong, 1999
Crab	50	73.3	Wong, 1999
Shrimp	50	7.1; 75.8; 7.1; 9.3	Zarei, 2012; Wong, 1999; Jaksic, 2002; Rahimi, 2010
Shellfish	47	5.2; 6.5	Rosec, 2008; Messelhausser, 2010
Oyster	33	0; 89.3	Kampelmacher, 1972; Chen, 2008
Prawn	100	32.2	Messelhausser, 2010
Seafood	39	33.4; 9.4	Yang, 2007; Jaksic, 2002

* where the first column contains the specific food type, the second column shows the positive results in percentage obtained in this research, the third column contains the results obtained by other people who have done similar work and the fourth column contains the references for each of the results stated in the third column.

4.3 **Frequency distribution of measured food temperatures (°C) on opening of packages at the delivery location**

Figure 1 shows the frequency distribution of measured food temperatures on opening of packages at the delivery location (as discribed in section 3.1 under food sampling). The x-axis is binned into 4 °C intervals. The y-axis shows the number of times a food arrived at that particular temperature. From Figure 1 it is evident that a large number of samples (35.7% or 122 out of a total of 341 samples) were received at temperatures greater that 4 °C.

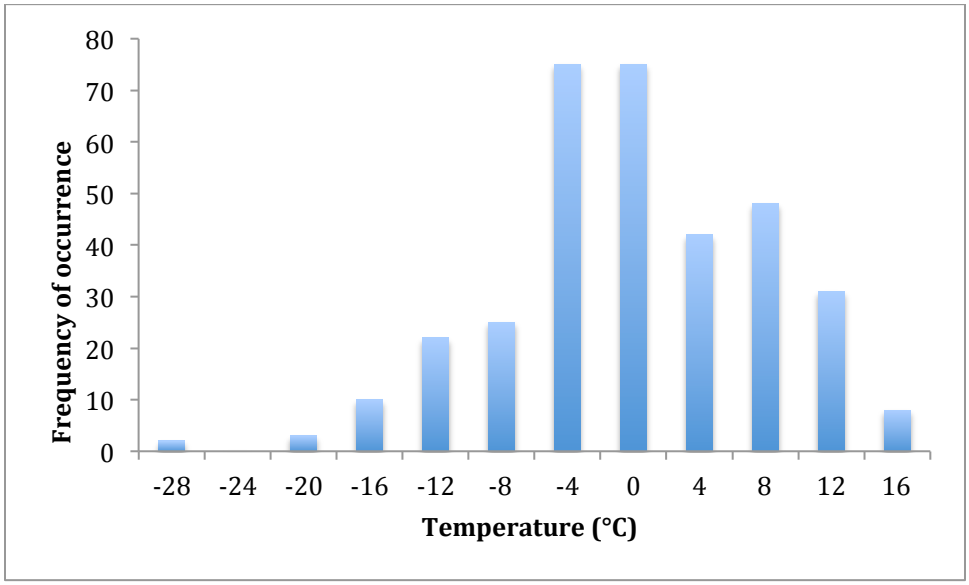

Figure 1. Frequency distribution of measured food temperatures on opening of packages at the delivery location (Bin= 4 °C)

4.4 **Results for association of temperature (recorded on arrival of samples) with occurrence of indicator and pathogenic bacteria (Safe: ≤4 °C; Danger: >4 °C)**

Figure 2 shows the relationship between the occurrence of coliforms, *E. coli, B. cereus* and *C. perfringens* in food samples and their association with foods received above and 4

°C. The graph shows that a majority of the coliform positive samples were received at acceptable temperatures. A similar pattern is seen with *B. cereus* and *C. perfringens*, where most positive samples were received at an acceptable temperature. A different situation is seen with *E. coli* where more positive samples were received at unacceptable temperatures. These reults show that coliforms, *B. cereus* and *C. perfringens* risk are generally not amplified by high temperature upon receipt, while *E. coli* prevalence is being amplified in foods received at elevated temperatures.

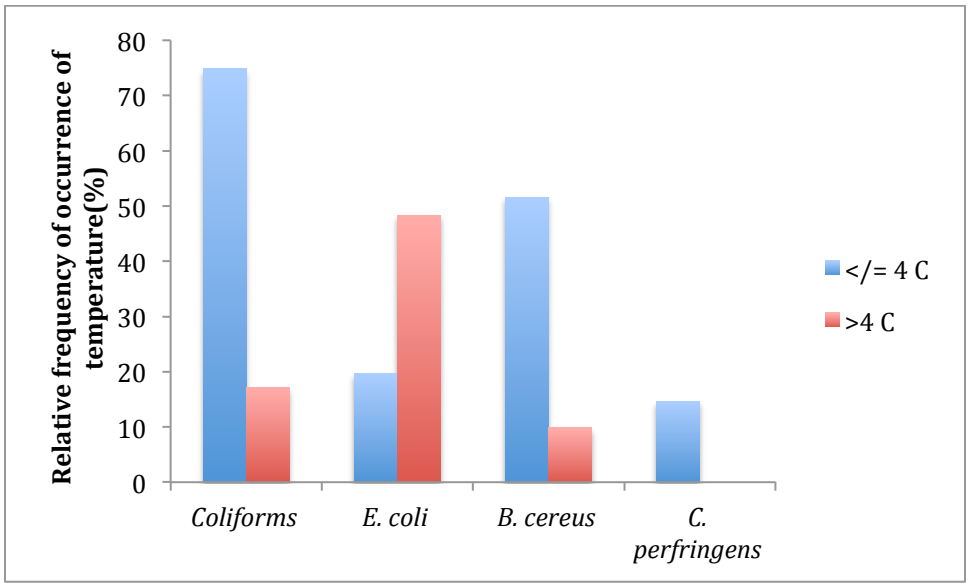

Figure 2. Association of temperature with occurrence of Coliforms, *E. coli, B. cereus and C. perfringens* positives in samples

Figure 3 shows the relationship between the occurrence of *Salmonella, V. parahemolyticus* and *L. monocytogenes* in food samples and their association with foods received above and 4 °C. The graph shows that a majority of the *Salmonella* positive samples were received at unacceptable temperatures. A similar pattern is seen with *V.*

parahemolyticus, where most positive samples were received at an unacceptable temperature. A different situation is seen with *L. monocytogenes* where more positive samples were received at acceptable temperatures. These results show that *L. monocytogenes* risk is generally not amplified by high temperature upon receipt, while *Salmonella* and *V. parahaemolyticus* are being amplified in foods received at elevated temperatures.

Also it was found that majority of the meat and poultry (68%) and seafood (56%) samples were obtained at safe tempratures whereas majority of RTE foods (57%) were not received at safe temperatures.

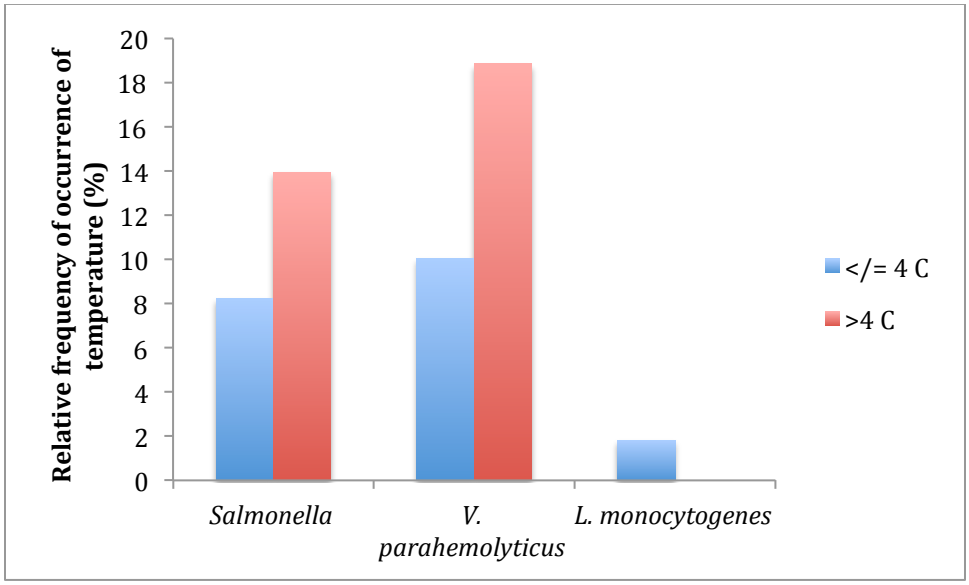

Figure 3. Association of temperature with occurrence of *Salmonella, V. parahemolyticus* and *L. monocytogenes* positives in samples

4.5 Results for Ready-to-eat (RTE) foods

Amongst 341 total samples tested, 21 were in the RTE category. The total plate count varied from 0 to 8 log CFU/g in RTE foods, including 3 samples with ~0 log CFU/g TPC and 2 samples above 6 log CFU/g TPC. Those two samples with high plate counts were both beef products. Four RTE food samples tested positive for *L. monocytogenes* including 1 cook bison meat sample (3.7 log CFU/g) and 3 cooked salmon (1.7, 2 and 2 log CFU/g) samples. No *S. aureus*, *E. coli* or *V. parahemolyticus* were detected in any of the RTE food samples. *Salmonella* was found in 1 cooked turkey RTE sample. Out of the 21 RTE samples tested, 6 had detectable coliforms including 1 cooked bison patty, 2 beef sausages, 1 cooked shrimp sample, 1 chicken-pork sausage and 1 beef-pork sausage. Ten RTE samples had detectable *B. cereus* (1 beef hotdog, 1 cooked shrimp product, 1 cooked haddock product, 4 smoked salmon samples and 3 beef-pork sausages). The *B. cereus* count ranged from 2.2 to 4.2 log CFU/g including 2 samples (Beef and Salmon) with >4 log CFU/g of *B. cereus*. Two samples had detectable *C. perfringens* including 1 venison sausage sample and 1 beef-pork sausage with 1.7 and 2.5 log CFU/g of *C. perfringens* respectively.

4.6 Results for TPC as an indicator

Relative Frequency (RF) of bacteria v/s TPC count (BIN=2 log CFU/g)

Figure 4 depicts the efficiency of TPC as an indicator for coliforms. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of coilforms. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of coliforms too.

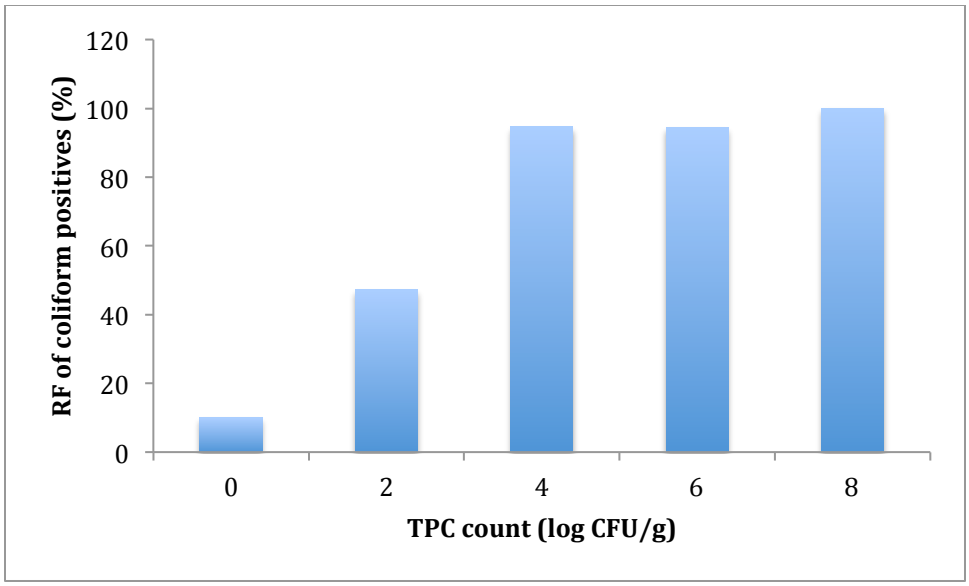

Figure 4. TPC as indicator for coliforms

Figure 5 depicts the efficiency of TPC as an indicator for *E. coli*. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *E. coli* positives. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of *E. coli* too.

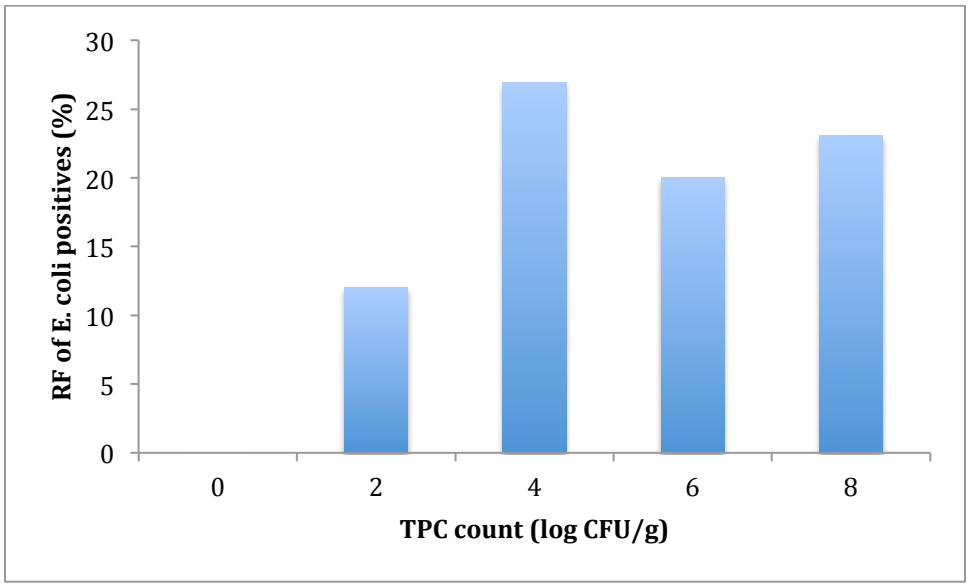

Figure 5. TPC as indicator for *E. coli*

Figure 6 depicts the efficiency of TPC as an indicator for *B. cereus*. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *B. cereus* positives. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of *B. cereus* too.

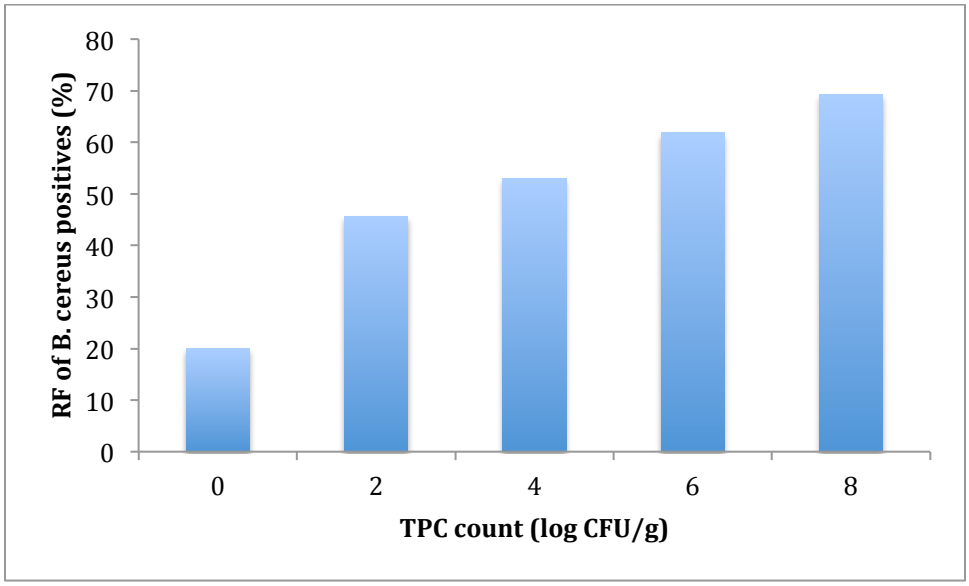

Figure 6. TPC as indicator for *B. cereus*

Figure 7 depicts the efficiency of TPC as an indicator for *C. perfringens*. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *C. perfringens* positives. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of *C. perfringens* too.

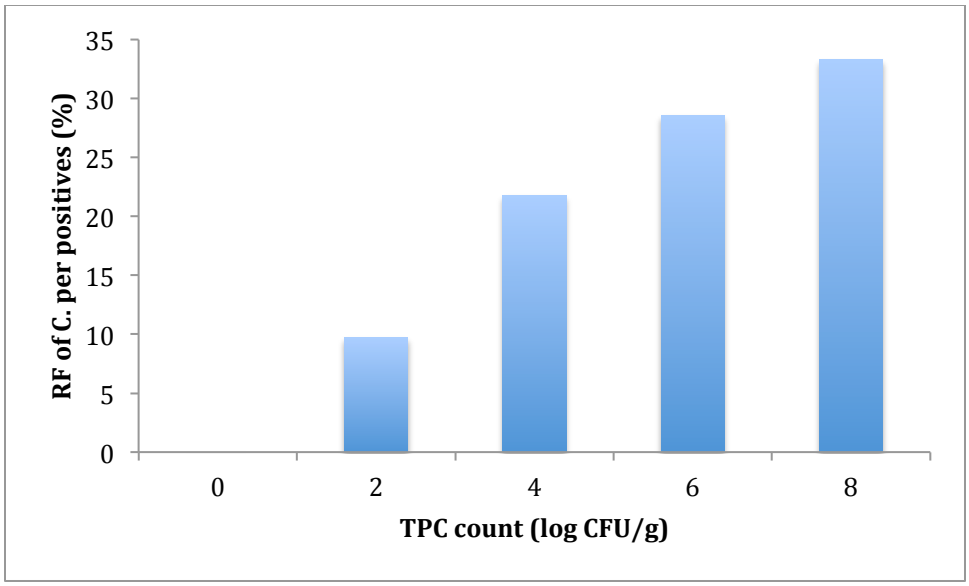

Figure 7. TPC as indicator for *C. perfringens*

Figure 8 depicts the efficiency of TPC as an indicator for *Salmonella*. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *Salmonella* positives. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of *Salmonella* too.

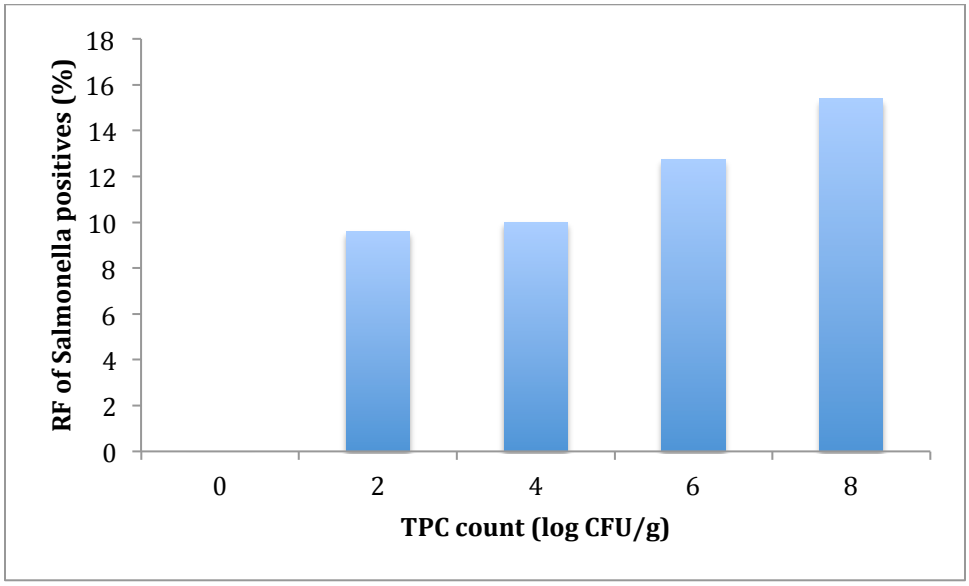

Figure 8. TPC as indicator for *Salmonella*

Figure 9 depicts the efficiency of TPC as an indicator for *V. parahemolyticus*. The x-axis has TPC count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *V. parahemolyticus* positives. In this graph, with increase in TPC count, there was an increase in relative frequency of occurrence of *V. parahemolyticus* too.

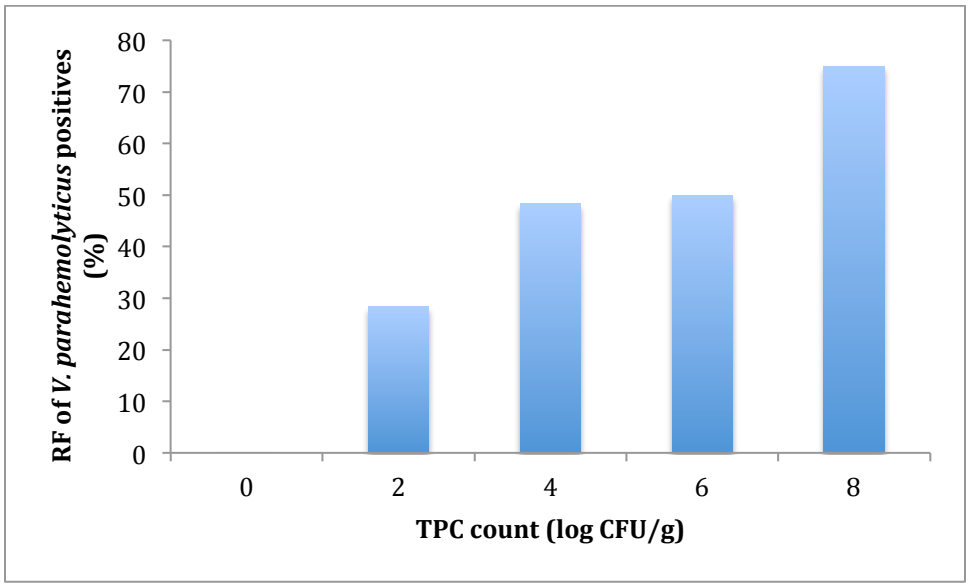

Figure 9. TPC as indicator for *V. parahemolyticus*

4.7 Results for Coliform as an indicator

Figure 10 depicts the efficiency of coliforms as indicator for *E. coli*. The x-axis has coliform count broken into bins of 2 log CFU/g and the y-axis shows the relative

frequency (in %) of occurrence of *E. coli* positives. In this graph, with increase in coliform count, there was an increase in relative frequency of occurrence of *E. coli* too.

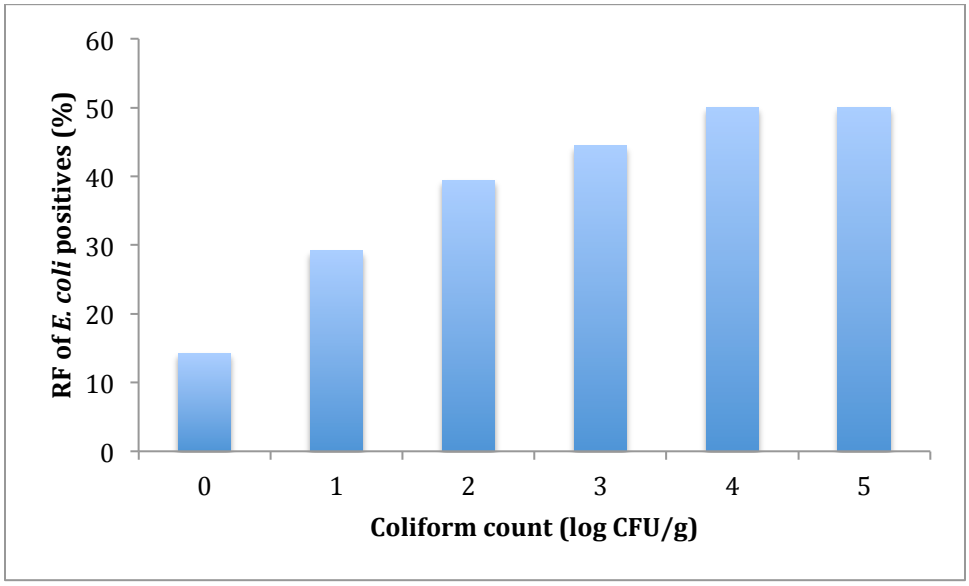

Figure 10. Coliforms as indicator for *E. coli*

Figure 11 depicts the efficiency of coliforms as indicator for *B. cereus*. The x-axis has coliform count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *B. cereus* positives. In this graph, with increase in coliform count, there was an increase in relative frequency of occurrence of *B. cereus* too.

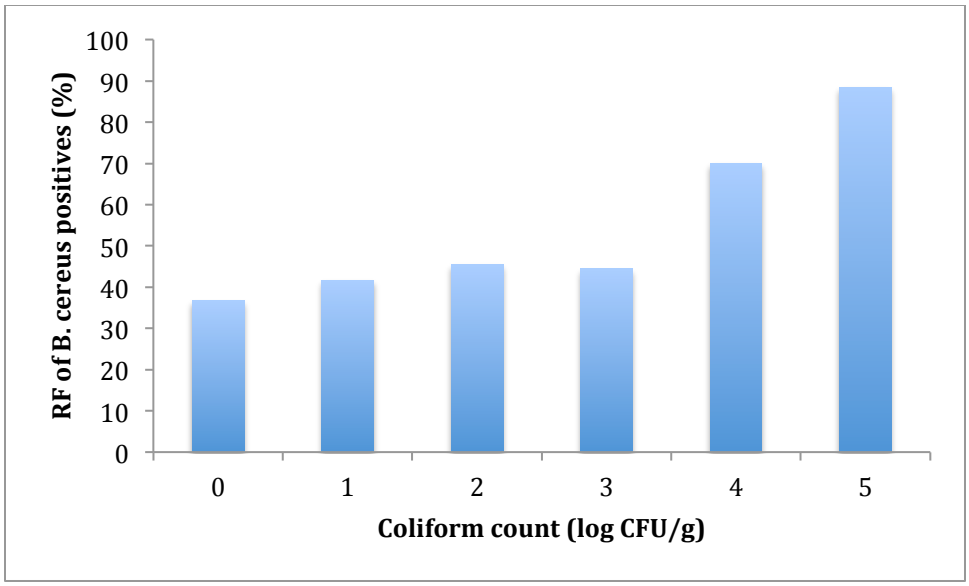

Figure 11. Coliforms as indicator for *B. cereus*

Figure 12 depicts the efficiency of coliforms as indicator for *C. perfringens*. The x-axis has coliform count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *C. perfringens* positives. In this graph, with increase in coliform count, there was an increase in relative frequency of occurrence of *C. perfringens* too.

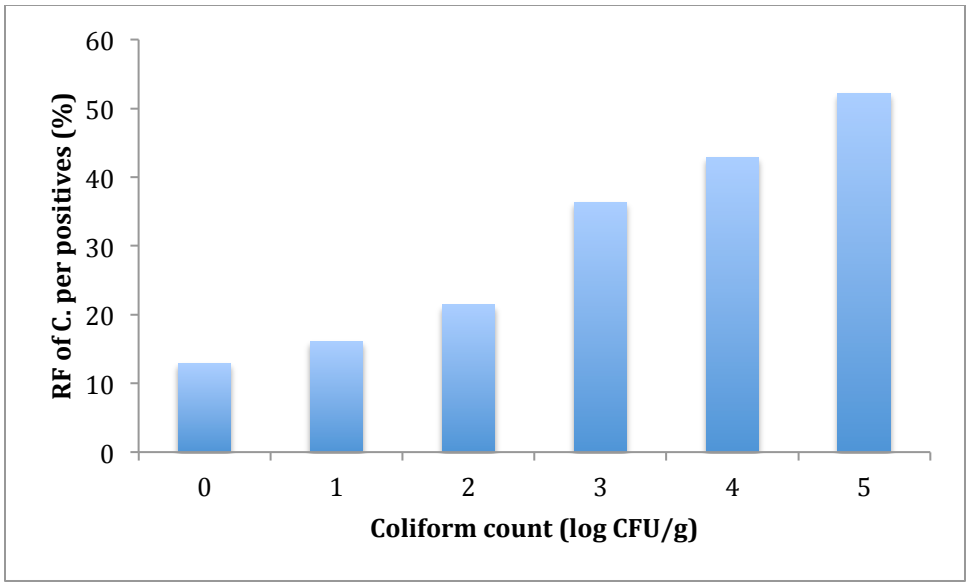

Figure 12. Coliforms as indicator for *C. perfringens*

Figure 13 depicts the efficiency of coliforms as indicator for *Salmonella*. The x-axis has coliform count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *Salmonella* positives. In this graph, with increase in coliform count, there was a uniform decrease in relative frequency of occurrence of *Salmonella* till 3 log CFU/g with a sudden increase at 4 log CFU/g.

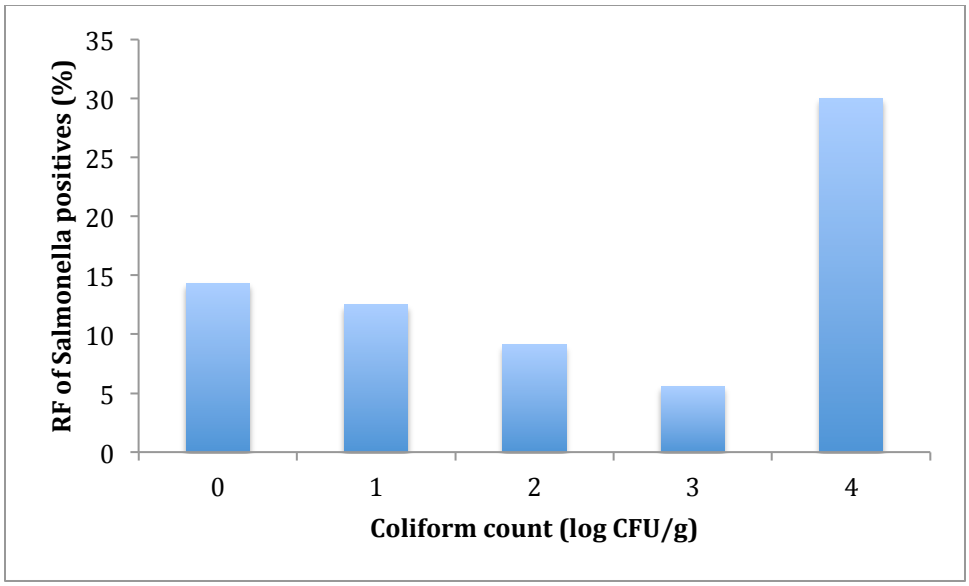

Figure 13. Coliforms as indicator for *Salmonella*

Figure 14 depicts the efficiency of coliforms as indicator for *V. parahemolyticus*. The x-axis has coliform count broken into bins of 2 log CFU/g and the y-axis shows the relative frequency (in %) of occurrence of *V. parahemolyticus* positives. In this graph, with increase in coliform count, there was an increase in relative frequency of occurrence of *V. parahemolyticus* too with the exception of positives at 4 log CFU/g which did not follow the trend.

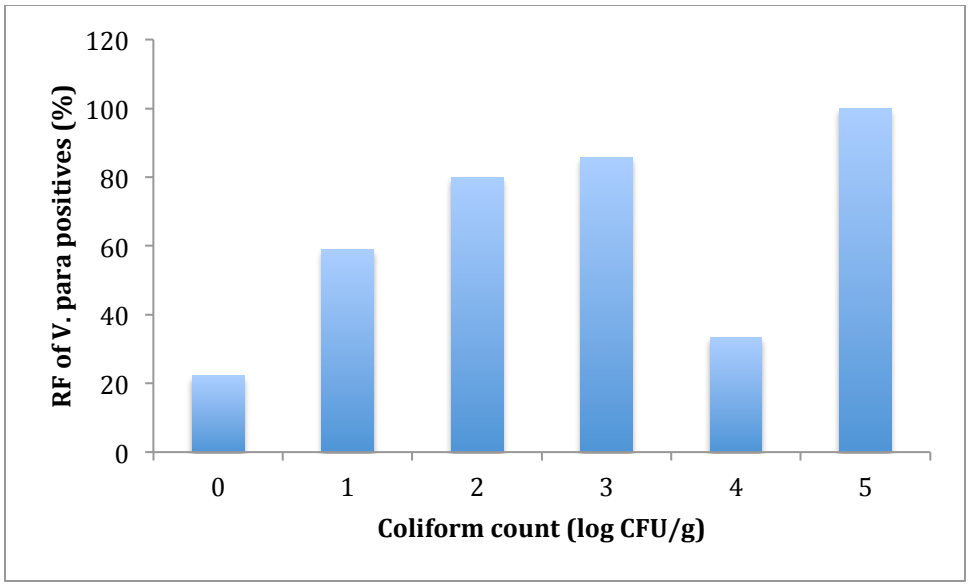

Figure 14. Coliforms as indicator for *V. parahemolyticus*

5. CONCLUSION

A total of 341 samples were tested (192 meat, 38 poultry, 111 seafood). Of these, 18.7% were positive for generic *E. coli*, 9.9% for pathogenic *E. coli*, 50.3% for *B. cereus*, 18.2% for C. perfringens, 10.2% for *Salmonella* and 38.7% for *V. parahemolyticus*. The prevalence of *Salmonella*, *B. cereus* and *V. parahemolyticus* was higher in most of the foods ordered from the Internet compared to other published surveys of foods from other retail sources. The prevalence of generic *E. coli*, pathogenic *E. coli* and *C. perfringens* was higher in some but not all mail-ordered foods compared to other published surveys of foods from retail sources. Amongst the 21 Ready-To-Eat (RTE) samples tested, *L. monocytogenes* was detected in four samples and none of the samples tested positive for *S. aureus, E. coli or V. parahemolyticus*. The results of this survey indicate that foods ordered online are often (35.7% of the time) received at temperatures greater that 4 °C. Coliforms prevalence, and *B. cereus* and *C. perfringens* risk are generally not amplified by high temperature upon receipt, while *E. coli* prevalence is amplified in foods received at elevated temperatures. *L. monocytogenes* risk is generally not amplified by high temperature upon receipt, while *Salmonella* and *V. parahaemolyticus* are being amplified in foods received at elevated temperatures.

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