**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
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MICROBIAL RISK ASSESSMENT OF MIXED VEGETABLE SALADS FROM SELECTED CANTEENS IN THE KUMASI METROPOLIS

BY
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DECLARATION

I, Douglas Amoah, hereby declare that except for the references to the literature, which have been duly cited herein, this thesis is the result of my own field and laboratory work towards the award of Master of Science (MSc) Degree in Food Quality Management under the supervision of Dr. F. C. Mills-Robertson of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology. I further declare that the research has not been submitted previously, either wholly or partially, for a degree in the Kwame Nkrumah University of Science and Technology or elsewhere, except where due acknowledgement has been made in the text.

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ABSTRACT

The aim of this study was to assess the microbiological quality of mixed vegetable salads and the risk associated with its consumption from food vendors on and around two university campuses in the Kumasi Metropolis. Microbiological quality of mixed vegetable salads from the vendors was determined using Aerobic plate count (APC) as well as enumeration and detection of *S. aureus* and *Salmonella* spp. using standard microbiological methods. A total of twenty seven (27) mixed vegetable salad samples were taken from nine (9) randomly selected vendors (three different times from each vendor), from 8th to 15th November, 2013. A survey was also carried out with structured questionnaire that had both observational and responsive questions to determine handling practices and consumption pattern that are critical to microbial quality and the risk of mixed vegetable salads. Monte Carlo simulation of *S. aureus* using the exponential model ($r = 7.64 \times 10^{-8}$) for 10,000 iterations for quantitative microbial risk assessment of three exposure scenarios was used. APC with range of 3.1 log CFU/g to 4.83 log CFU/g was obtained which was in agreement with both the Ghana Standards Board (GSB) and the UK Public Health Laboratory Services (PHLS) standard references of < 5 log CFU/g and 6 to < 7 log CFU/g, respectively while the *S. aureus* count ranging from 2.97 log CFU/g to 5.13 log CFU/g obtained was above both the GSB and PHLS acceptable standards of < 4 log CFU/g in majority (66.67%) of the test canteens. *Salmonella* spp. was, however, not detected in any of the samples. The survey conducted revealed that, storage temperature for vegetable salads during sales and frequency of consumption had critical effects on the microbiological quality and annual risk of vegetable salad consumed. The mean annual risks of *S. aureus* infection for the three exposure scenarios were 10.90×10^{-1}, 10.05×10^{-1} and 7.71×10^{-1} for frequent, average and occasional consumers respectively. This indicates approximately 11, 10 and 8 out of 10 frequent, average and occasional mixed vegetable salad consumers, respectively, could be infected with *S. aureus*. Thus, the study revealed the likelihood of a very high risk associated with the consumption of mixed vegetable salads from food vendors on and around the two university campuses in the Kumasi Metropolis.

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LIST OF ACRONYMS AND ABBREVIATIONS

AK	Adwoba's kitchen
ANOVA	Analysis of variance
APC	Aerobic plate count
APHA	American Public Health Association
AT	African taste
BC	Becky's corner
BPW	Buffered Peptone Water
CAC	Codex Alimentarius Commission
CFU	Colony forming Unit
FAO/WHO	Food and Agriculture Organization/ World Health Organization
FDA	Food and Drug Administration (USA)
FDA	Food and Drug Authority(Ghana)
FEHD	Food and Environmental Hygiene Department
FRNR	Faculty of Renewable Natural Resources
GD	Golden dish
GNA	Ghana News Agency
GSB	Ghana Standards Board
H	Helenus foods
ICMSF	International Commission on Microbiological Specification for Foods
IFF	Indece fast food
IFT/FDA	Institute of Food Technologists/ Food and Drug Administration
ISO	International Standards Organization
KNUST	Kwame Nkrumah University of Science and Technology
KMA	Kumasi Metropolitan Assembly

LRCS	Lordy's Restaurant and Catering Services
MLGRDE	Ministry of Local Government, Rural Development & Environment
MRA	Microbial Risk Assessment
MSA	Mannitol-salt Agar
ND	Not Detected
P1	Point 1
PCA	Plate Count agar
PHLS	Public Health Laboratory Services
QMRA	Quantitative microbial risk analysis
QRA	Quantitative risk assessment
SCB	Selinite Cystine broth
SCF	Scientific Committee on Food
SD	Standard Deviation
SSA	Salmonella-Shigella agar
THCS	Top hill catering services
UEW-K	University of Education Winneba, Kumasi campus
U.S.	United States
USD	United States Dollar
WHO	World Health Organization

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CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

Food safety issues have been given much attention in recent times because of increasing food related illnesses (WHO, 2002; Thurston, 2006; Peattie, 2006; Leech, 2005). Risk Analysis has been developed as a tool to help produce safe foods in order to reduce the incidence of food related illnesses (Collado *et al*., 2011). It is a useful tool that can be used to control microbial hazards in food by both the regulatory authorities and food processors to ensure that microbiologically safe foods are provided to unassuming consumers (Duffy *et al*., 2006). Risk assessment, one of the three components of risk analysis, employs scientific information and statistical probabilities to estimate the likelihood and severity of an adverse effect (illness or death) (Cassin *et al*., 1998; Duffy *et al*., 2006). Quantitative microbial risk assessment (QMRA), identifies microbial risks associated with the consumption of a particular food and provides estimates of the level of illness that a pathogen can cause in a given population exposed to the food concerned (Forsythe, 2002). According to Cassin *et al*., (1998), Quantitative Risk Assessment (QRA), can identify the contribution of each stage in the food supply chain (from production to consumption) to the risk of foodborne illnesses. This provides the benefit of ensuring that resources are purposefully directed to help minimize the risk posed by foodborne pathogens.

Food-borne illnesses, defined by WHO (2007) as infectious or toxic diseases caused by agents that enter the body through the ingestion of food, have become an important global issue. Bryan (1982) indicated that food alone is responsible for the transmission of over two hundred different

diseases. Globally, millions of people are affected by foodborne and waterborne diarrhoeal diseases each year and that outbreak of foodborne illnesses is responsible for 5000 and 500 deaths each year, in the USA, and England and Wales respectively (Adak *et al.*, 2002; Mead *et al.*, 1999). In Ghana, it is estimated that one out of every 40 individuals suffers from foodborne illnesses (GNA, 2010), a very daunting situation that requires collaboration of everyone to improve food safety.

According to WHO (1984), food safety measures must be in place during production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, wholesome and fit for human consumption. Food safety, thus, involves everyone in the food supply system, from production to processing and consumption and according to Ababio and Adi (2012), Governments, law makers, food manufacturers, caterers, food vendors, farmers, and all consumers have roles to play in making food safe.

Consumption of fresh fruits and vegetables is encouraged in recent years. According to James and Ngarmsak (2011), at least five servings per day have been recommended to American consumers. Salad vegetables, although rich in vitamins and minerals as well as other important nutrients including dietary fiber and phyto nutrients, are also good sources of pathogenic microorganisms. However, they are usually not subjected to any form of heat treatment or may be partially cooked before consumption. Additionally, adequate washing and peeling may not be employed in extreme cases making consumption of the commodity a potential vehicle for food borne diseases (Tambekar and Mundhada, 2006).

Pathogenic bacteria, viruses and parasites can contaminate vegetables at any stage from planting to consumption. The use of untreated wastewater and water supplies contaminated with sewage used for irrigation, post-harvest handling, and preparation in unhygienic environments in food services and home settings are among the commonly reported sources of vegetable contamination (Amoah *et al*., 2007; Beuchat, 2002; Simões *et al*., 2001). *Staphylococcus aureus*, *Enterobacter* spp., *Salmonella typhi*, *Pseudomonas aeruginosa* and *Shigella sonnei* are among the commonly isolated pathogens from vegetable salads (Poorna and Randhir, 2001). Microbial contamination of vegetables can have negative effect on the product including spoilage, decreased sensory appeal and decreased shelf life. According to Halablab *et al*., (2011), outbreaks of foodborne illnesses related to vegetable consumption can be large or small, ranging from few numbers of persons to thousands. For example, Meldrum *et al.*, (2009) reported two large outbreaks in the United Kingdom which were attributed to the consumption of contaminated vegetable salads.

Consumption of vegetable salad away from home can significantly compound the problem of vegetable related outbreaks of foodborne illnesses since food handlers play a critical role in the spread of pathogens during food preparation. Green and Selman (2005) reported that, although food contamination may occur at any point from production, processing, distribution and preparation, food handlers and other people responsible for food preparation have a critical role in the occurrence and spread of foodborne illnesses. Food workers knowledge on microbial growth and survival in food and food service environment plays a major role in foodborne outbreak situations. According to Sousa (2008), contamination can be transferred to and from workers through raw food, hands, clothing, food packages, as well as other environmental

sources. Pathogens, for instance, can survive for extended periods of time on many surfaces, including skin (Sousa 2008). Forsythe and Hayes (1988) also noted from their study, that cross contamination during food preparation contributes notably to the occurrence of foodborne diseases. Therefore, because salad consumption is highly patronized in recent times, mostly outside the home, continuous training and monitoring of food workers, especially those involved in vegetable salad preparation, is needed for microbiologically safe products.

Our understanding of foodborne microbes has increased tremendously in recent years and more stringent food safety regulations are in place to ensure substantial food safety, however, several factors including, large scale food production, adjustments in traditional methods of processing food, proliferation of heat-and-eat convenience foods and nationwide distribution with increased potential for mishandling, still hamper the efforts to ensure pathogen free products (Sousa, 2008). High risk foods with high moisture and nutrient value such as vegetable salads support the growth of pathogenic microorganisms (Wallace, 2006) and should be a major concern for all food handlers.

1.2. PROBLEM STATEMENT AND JUSTIFICATION

In recent years, eating habits have changed remarkably in Ghana with a lot of people eating away from home even though the food usually prepared and sold at various roadside restaurants and other places are mostly unhygienic (Newman, 2005). The factors that indicate why people eat away from home include; female participation in labour force, changing lifestyle, longer working hours, absence from home while travelling, urbanization, and quest for higher education (Tinker, 1997; Maxwell *et al.*, 2000). A study by Amoah *et al.*, (2007), indicated that fresh vegetables

have become a normal part of fast food served on the street, canteens and restaurants in Ghana. Rheinländer (2006) also reported that most food vendors in Kumasi served various types of salad made up of lettuce and a variety of toppings such as eggs, onions, cabbage, tomatoes and other raw vegetables. The reported nutritional benefits (James and Ngarmsak, 2011) associated with the consumption of fresh vegetables appear to be one of the main reasons for such a high patronage of mixed vegetable salad, especially among urban dwellers.

Foodborne illnesses related to the consumption of contaminated raw or partially processed vegetables have, however, been increasingly reported worldwide (Sousa, 2005; Altekruse and Swerdlow, 1996; Beuchat, 1996). A lot of researches have also confirmed that vegetables are easily prone to microbial contamination through contact with soil, contaminated water and by handling at harvest or during postharvest processing (Adu-Gyamfi and Nketia-Tabiri, 2007; Amponsah-Doku *et al.,* 2010). Thus, despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh or partially processed vegetables have been documented.

S. aureus and *Salmonella* spp. are among the bacteria commonly reported to be associated with contamination of vegetable salads (Ameko *et al*., 2012; Myhara *et al.,* 2003; Fung *et al.,* 2011, Uzeh *et al.*, 2009). Previous researches have also confirmed that *S. aureus* and *Salmonella* spp. are transmitted into food mainly through improper food handling, temperature abuse and cross contamination during food preparation (Loir *et al.,* 2003; FDA, 2012). Kwame Nkrumah University of Science and Technology (KNUST) and University of Education, Winneba, Kumasi campus (UEW-K) have been identified to be among the areas in the Kumasi metropolis with

high population of food vendors and restaurants (Ababio and Adi, 2012). The research by Rheinländer (2006) indicated that most food vendors in Kumasi use the same knife and chopping board for meat and vegetables as well as bare hands for serving ready to eat vegetables and other foods. There is therefore the need to determine the contribution of canteen workers to the microbial contamination of ready to eat mixed vegetable salads in KNUST and UEW-K and their environs using *S. aureus* and *Salmonella* spp., pathogens that are very good indicators of improper food handling, temperature abuse and cross contamination.

Several studies have reported on the microbial contamination of vegetables and vegetable salads in Ghana including contamination by vendors during preparation (Ameko *et al.*, 2012; Mensah *et al.*, 2002). However, only a few of these studies have reported on quantitative microbial risk assessment of vegetable salad (Ackerson and Awuah, 2012; Seidu *et al*., 2008). If in the future we wish to effectively encourage food vendors to improve vended mixed vegetable salads and thereby minimize the dangers of food borne diseases, it is vital to gain insight into the actual health risk associated with the consumption of mixed vegetable salads and how this is linked with post preparation handling practices and consumption patterns. This thesis therefore, seeks to determine the microbiological health risk posed to consumers of mixed vegetable salads from canteens on the campuses of the two public universities, KNUST and UEW-K, all in the Kumasi Metropolis using *Staphylococcus aureus* and *Salmonella* spp., two highly infective pathogens that can easily be transmitted by food and food workers.

1.3. AIM

The aim of this study was to assess the microbial contamination levels and evaluate the microbiological risk associated with the consumption of ready to eat mixed vegetable salads from canteens on the campuses of the two public universities in the Kumasi Metropolis in reference to the presence or absence of *Staphylococcus aureus* and/or *Salmonella* spp.

1.4. SPECIFIC OBJECTIVES

- To determine total aerobic mesophiles of mixed vegetable salads from canteens on the two public university campuses in the Kumasi Metropolis
- To determine the presence of *Staphylococcus aureus* and *Salmonella* spp. in the mixed vegetable salads from the canteens on the two public university campuses in the Kumasi Metropolis
- To determine the handling practices employed by canteen workers during sales of salad and establish salad consumption profile
- To determine the microbiological risk ready-to-eat mixed vegetable salads pose to consumers using predictive models (exponential and/or beta-poisson) and Monte Carlo simulation

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. GLOBAL RELEVANCE OF FOOD SAFETY AND FOODBORNE ILLNESS

Food is very necessary for human survival. It is a source of energy and also provides the nutrients required by the human body to withstand diseases but can also be the source of many human illnesses. In recent years, foodborne diseases have become a significant issue and the foodservice industry is mostly linked with a large number of occurrences (Sheppard *et al.*, 1990; Cavalli and Salay, 2004). Olsen *et al.*, (2000), indicated that over 40% of foodborne disease outbreaks that were reported in the United States (US) between 1993 and 1997 involved retail foodservice establishments. The astronomical increase in the foodservice sector worldwide with a corresponding increase in the number of people eating away from home (Nyarango *et al.*, 2003) indicate a significant contribution of the foodservice industry to the rising incidence of foodborne diseases.

The prevalence of foodborne illnesses makes food safety a global issue. Almost all countries are putting in place measures to enhance food safety issues, however, despite the positive progress made by many countries in this direction, significant number of people continue to be ill from consumption of contaminated food. Deaths related to foodborne diseases and economic losses still affect all countries worldwide (Thurston, 2006). Food safety must, therefore, continue to be one of the topmost priorities of the food industry and governments worldwide.

Symptoms of illnesses associated with the ingestion of foodborne pathogens or their toxins are mostly gastrointestinal symptoms of nausea, vomiting, stomach pains, diarrhoea, fever and chills.

The symptoms can range from mild to serious and can last from few hours to several days. According to Newman (2005), the severity of the symptoms of foodborne illnesses may depend on the causative agent and can lead to dehydration and death. Usually, very old or very young people, pregnant women or people who are very weak for some other reasons are particularly susceptible to foodborne illnesses. These classes of vulnerable people are more likely prone to fatalities of diarrhoeal diseases. Cancer patients and other immuno-compromised individuals are also subject to foodborne bacterial infections. It is estimated that foodborne and waterborne diarrhoeal diseases together cause over 2 million annual deaths worldwide (WHO, 2001) with Ghana alone contributing to an estimated 65,000 of these deaths (GNA, 2010).

Foodborne diseases do not only significantly affect people's health and well-being, but they also have economic consequences for individuals, families, communities, businesses and countries. These diseases impose a substantial burden on healthcare systems and markedly reduce economic productivity. Affected individuals may lose income through assessment of health care and inability to work; businesses may lose out completely due to loss of consumers or may close down their businesses. Additionally, regulatory agencies will have to spend extra resources to investigate outbreaks. According to FAO/WHO (1983), the contribution of foodborne illness to reduced economic growth is global with an estimated annual cost to the US economy alone approximating $152 billion. In Ghana, about 69 million USD is spent annually on this problem (Ghana News Agency, 2010), thus, no doubt that the issue of foodborne illness must be considered a pressing national issue that calls for an all-inclusive approach to urgently deal with it.

There are advance systems in place for collecting data on occurrences and causes of foodborne illness in recent years, however, only a fraction of the number of cases are actually captured. Foodborne illness outbreaks are mostly traced to domestic kitchens, involving only few people (Redmond and Grifith, 2003) and are hardly ever reported (WHO, 2007). Newman (2005) reported that, the incidence of food related infections is grossly under-reported in Ghana, because only the very serious episodes are taken to hospital, therefore, the situation might be more serious than what is actually known making it even more serious than it appears.

2.2. MICROBIOLOGICAL FOOD HAZARDS

Food chemicals (heavy metals, food additives, pesticide residues, environmental chemical contaminants and other toxic chemicals) have been shown to be one of the causes of foodborne illnesses (Khan *et al.*, 2008; Sivapalasingam *et al.*, 2004), however, microbiological contamination is responsible for majority of outbreaks of foodborne illnesses. It is estimated that the ratio of foodborne illness caused by pesticide residue to microorganism is 1: 100,000 (Adams and Motarjemi, 1999). Microorganisms that are mainly responsible for foodborne illnesses are bacteria, viruses, parasites and fungi. While all pathogenic microorganisms can cause foodborne illnesses, causes due to bacteria are very common with almost all reported cases of foodborne illness attributed to bacteria or by the toxins produced by them. *Salmonella, Shigella, Escherichia coli (E. coli), Clostridium, Staphylococcus, Campylobacter,* and *Vibrio* are examples of bacteria that can be transmitted through food and cause food borne illness.

Bacterial foodborne diseases may include tuberculosis, typhoid fever, cholera (Foskett *et al.*, 2003), dysentery, diarrhoea (Macleod and Douglas, 1999), pneumonia, meningitis, whooping

cough, hepatitis and sore throat (Gates, 1987). These illnesses may be caused by the presence of the bacteria in the food (infection) or by the toxins produced in the food by the bacteria (intoxication). Foodborne infection is caused by consumption of food contaminated with living bacteria in numbers large enough to overcome the acidity of the digestive system. The bacteria that are able to overcome the body's defense mechanisms are able to multiply and produce the disease condition. With foodborne intoxications, the bacteria produce toxins in the food which in turn causes the illness. The bacteria could be killed at the time of ingesting the food but the toxin produced already in the food may remain to produce the symptoms. Common bacteria that produce toxins include *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*. Besides bacteria, Newman (2005) indicated that viruses, including Hepatitis A, Calicivirus and Norwalk virus as well as parasites such as *Giardia*, *Trichinella* and *Taenia* are also common foodborne pathogens.

Amoah *et al.*, (2007), found unacceptably high levels of faecal coliforms in lettuce grown in urban cities of Accra and Kumasi. The sources of contamination identified in the study were irrigation water, manure application and already contaminated soil. Another study by Mensah *et al.*, (2001) also reported of *Shigella dysenteriae, Shigella flexneri, Shigella boydii, E coli* and *Salmonella* Group B isolated from tomatoes purchased from farm gates and open markets in Accra. Such studies confirm how the use of waste water for irrigation and animal manure has impacted on the microbiological quality of vegetables grown in urban cities in Ghana.

Even though there has been tremendous strides in food science and technology in recent years, man is still confronted with the problems of food borne microbial pathogens. Modern farming

practices, food processing and distribution have not provided adequate protection from food-related illnesses. Poor environmental sanitation, improper storage conditions and poor personal hygiene of especially food handlers also contribute greatly to contamination and multiplication of foodborne pathogens. Different studies have indicated that in sub-Saharan Africa, preservation of foods usually takes place at room temperature for extended period before consumption. Additionally, food workers frequently mishandle food with the sale of food mostly taking place in unsanitary condition and usually on the street (Agbodaze *et al.*, 2005; Muinde and Kuria, 2005; Ghosh *et al.*, 2007). Other researchers have also shown poor food preparation practices in domestic kitchen, use of contaminated equipment and food ingredients already contaminated as major sources of greater number of food borne pathogens (Medeiros *et al.*, 2001; Beumer and Kusumaningrum, 2003; Redmond and Grifith, 2003). Lynch *et al.*, (2006), as well, cited factors such as unsafe keeping of food (temperature and time), poor personal hygiene and food from unsafe sources as major contributing factors of foodborne diseases. These reports suggest how complicated factors that influence microbial quality of food could be and therefore integrated approach must be the inevitable option to ensure microbiologically safe food to consumers.

2.3. READY-TO-EAT PROCESSED FRUITS AND VEGETABLES

According to ICMSF, (1998), fruits are portions of seed bearing plants, and vegetables are components of plants that may include the leaves, stalks, roots, tubers, bulbs, flowers, and seeds. Plant products that are used as fruits and vegetables vary from place to place. Common examples of fruits are mangoes, melons, oranges, apples, pears and bananas while common vegetables include tomatoes, cucumbers, green beans, carrots, cabbages, lettuce, pepper and onions.

Most fruits and vegetables are ready to eat and may require no further processing before consumption. Fresh cut vegetables are usually washed, sliced, chopped, or shredded and consumed alone or in a mixture with similar products. They may either be consumed raw or with little heat treatment, with or without other ingredients such as milk or mayonnaise as salad dressing. Fresh-cut fruits and vegetables are perishable and potentially hazardous because of their high moisture content and other intrinsic factors capable of supporting microbial growth. Therefore, appropriate storage conditions are very critical for their extended shelf life. According to ICMSF (1998), preservation of fruits and vegetables can be achieved by drying, salting, freezing, refrigeration, canning, fermentation, irradiation, and packaging under vacuum or modified atmospheres. Fruits and vegetables are usually traded fresh, partially processed (cut, sliced, chopped, shredded, or peeled) and sometimes canned, frozen, juiced, or dried.

The demand for organic fruits and vegetables has increased in recent years as a result of consumers increasing concern about the potential contamination of fruits and vegetables from the application of pesticides, chemical fertilizers and herbicides (European Commission, 2002). As a result, most farmers are using animal manure in the production of vegetables to meet the increasing demand of the commodity, however, according to Olayemi (2007) and Amoah *et al.*, (2009), the organic manure used for the production of vegetables in Africa is untreated, therefore, the scare of consuming unsafe vegetables by large number of people.

2.3.1. Vegetable Salads and Salad dressings

Salad refers to a food that is made of a mixture of raw vegetables and/or fruits (Uzeh *et al.*, 2009; Rajvanshi, 2010). It is usually made up of fresh-cut or minimally processed vegetables and/or

fruits with or without salad dressing. Across the globe, vegetables mostly used in salad preparation include cucumber, pepper, tomatoes, onions, carrots, spring onions and radishes. Some non vegetable ingredients that may also be used are olives, mushrooms, egg, green beans, cheese, herbs, nuts, poultry, meat and some sea foods. In Ghana, vegetables that are mainly used for salad preparation are lettuce, carrots and cabbage. Fresh salads are not part of the normal Ghanaian diets, but factors such as westernization of the Ghanaian culture have made it essential component of food served by fast food vendors, canteens and restaurants with about 200,000 consumers every day in Accra alone (Amoah *et al.*, 2007). According to Ameko *et al.*, (2012), in Ghana, mixed vegetable salads served in restaurants and canteens as well as by street vendors are usually prepared with fresh lettuce, tomato and onion, and sometimes with the addition of carrots or green pepper. In a qualitative study of local practices and perceptions of food quality, food hygiene and food safety in urban Kumasi, Rheinländer (2006) stated that salad sold by street vendors are usually made of lettuce and a variety of toppings such as eggs, onions, cabbage, tomatoes and other raw vegetables. Whereas most consumers patronize vegetable salads for the perceived nutritional benefits, restaurants and other food service centers usually serve them as appetizers.

Mayonnaise is a commonly known salad dressing (FEHD, 2002), however, other products that resemble mayonnaise are also available as salad dressings. In addition, substances such as garlic-in-oil, various herbs or spices-in-oil, and flavoured oils (IFT/FDA, 2001) are also available as salad dressings. According to FEHD (2002), salad dressings provide characteristic flavours and also offer certain preservative effects.

The production of salads typically involves purchasing and processing of raw materials as well as mixing of ingredients. The main ingredients, vegetables and/or fruits are usually washed, peeled, sliced, chopped and shredded and can be used fresh or partially processed. Some ingredients like poultry, meat, seafood and egg may require some cooking. Other already processed ingredients like canned products and dressings as well as herbs that require no cooking are just obtained for immediate use. Salads are usually served cold with or without dressing depending on consumers' preference.

2.3.1.1. Nutritional Information of Vegetable Salads

Consumption of fresh produce, mainly fruits and vegetables is increasing among consumers all over the world. According to a SCF (2002) report, consumption of pre-prepared minimally processed fruits and vegetables have become popular among European consumers in recent years. The situation is not different in Ghana. The rapid increase in large number of consumers interested in vegetables could be attributed to the well reported nutritional benefits associated with the product. It is reported that vegetables are good sources of vitamins, minerals, dietary fibers, antioxidants and phyto-nutrients such as flavonoids, carotenoids as well as phenolic compounds that can help reduce the risk of cancer, heart disease and others illnesses (Heo and Lee, 2006; Vrchovska *et al.*, 2006; James and Ngarmsak, 2011). According to Kalia and Gupta, (2006), adequate amount of fruits and vegetables in diet can help prevent vitamin C and A deficiencies and as well reduce the risk of several other diseases. Clearly, vegetables and vegetable salads are essential for good health and the promotion of its nutritional advantages has obtained greater success. It is, therefore, of little wonder that production of such commodities has sprang up in urban cities in Ghana where most consumers reside.

2.3.1.2. Sources of Microbial Contamination of Vegetables and Vegetable Salads

The risk of microbial contamination associated with vegetable salads is very high and can take place at any stage of production. Environment under which they are prepared and consumed, personal hygiene of handlers, processes of cultivation, handling at harvest or during postharvest processing, transportation of raw materials and the raw materials themselves are all implicated as potential sources of contamination (Martinez-Tomé *et al.*, 2000; Simões *et al.*, 2001; Cuprasitrut *et al.*, 2011; Taban and Halkman, 2011). Vegetable processing for salad preparation interferes with the protective surfaces resulting in the release of cellular fluids providing a medium for microbial growth. Microbes can also be distributed from contaminated areas to other parts during washing and mixing. It has been confirmed that exposing vegetable salad ingredients to various types of cutting has resulted in a six to seven-fold increase in microbial numbers (Garg *et al.*, 1990; O'Beirne, 1999). The cut surfaces expose inaccessible areas and increase the surface area for microbial contamination. Contamination of the raw vegetables during cultivation is also well documented as a major source of microbial contamination of vegetables. According to the works of Ray and Bhunia (2007) and Ofor *et al.*, (2009), the soil, animal manures, sewage or irrigation water are some of the sources that contribute to differences in microbial profile of vegetables.

Post harvest handling also plays a significant role in microbial numbers of vegetables. Usually vegetable trade involves transportation in trucks to far away distances and finally end on the floor and tables in open markets. The water used for rinsing and sprinkling to ensure vegetables are still fresh during sales also contribute significantly to microbial contamination of the product (Mensah *et al.*, 2002). Moreover, microbial infiltration of the raw vegetables and their end products are usually high due to the very high moisture content, the optimum pH and nutrient

composition, little or no heat treatment during salad preparation (to ensure full nutritional benefit), and in most cases, time and temperature abuse during preparation, distribution and storage. Thus, vegetables, the main ingredient of vegetable salads, by their very nature increase the microbial hazards of the product. Other ingredients included in salad preparations are also potential sources of microbes, for example, raw eggs are a known source of *Salmonella* (Olsen *et al.*, 2000; Gantois *et al.*, 2009) and can contaminate the final product if used in salad preparation or as part of salad dressing.

All classes of microorganisms, bacteria, fungi, viruses and parasites, including plant and human pathogens and spoilage microorganisms (Nguyen-the and Carlin, 1994; Dunn *et al.*, 1995; Carmo *et al.*, 2004) can be associated with vegetables and vegetable salads. Bacteria commonly associated with vegetables or their environments such as the soil include; *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, *Listeria monocytogenes* and *E. coli* (De Rover, 1998). Other Pathogens including *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Shigella sonnei* have also been isolated from vegetable salads (Poorna and Randhir, 2001).

Contamination by enteric pathogens is common as a result of human and animal faecal waste used for cultivation. According to Ministry of Health and Welfare of Japan, (1997), *E. coli* O157:H7 was the cause of the vegetable borne outbreak in 1996, considered the world's largest which affected 11,000 people of which 6,000 were cultured, killing three school children. The prevalence of microorganisms in vegetables varies greatly according to different studies, however, *E. coli* O157:H7, Listeria *monocytogenes*, *Salmonella*, *Shigella* and *Campylobacter* are among the highest reported (SCF, 2002).

2.4. FOOD SERVICE AND FOOD CONSUMPTION TRENDS

2.4.1. Definitions of food service establishment

A food service establishment is a place where food is prepared and intended for individual portion service and includes the site at which the individual portions are provided, whether consumption occurs on or off the premises (Environmental Health Service, 1999). According to FDA (2006), this industry includes:

- Bakeries
- Bed and breakfast operations
- Cafeterias
- Restaurants
- Fast food
- Commissaries
- Snack bars
- Road-side stands
- Vending machines
- Meal services for home-bound persons
- School and hospital cafeterias
- Catering operations, and many other formats

2.4.2. Consumption Patterns

It is estimated that world-wide over 2.5 billion people regularly support food sold outside the home (Nyarango *et al.*, 2003). The reasons for such a high interest in the food vending business vary but in the Ghanaian setting, one of the main reasons for this trend is reduced prices of foods

and drinks sold by vendors. According to Maxwell (1998) and FAO/WHO (2003), in Ghana and elsewhere, food vendors are noted for selling foods and drinks at reduced prices and thus provide more affordable means to obtain nutritionally balanced meals outside the home. Rheinländer (2006) also indicated that, in Kumasi, a meal of 'waakye' and fish can cost as low as two Ghana Cedis fifty pesewas (Gh₵ 2.50 approximately 0.25 USD) while a meal of fried rice and chicken costs minimum of ten to fifteen Ghana Cedis (Gh₵ 10.00-15.00 approximately 1.5 USD). He further stated that the same meals could cost approximately twenty to thirty Ghana Cedis (Gh₵ 20.00 - 30.00 approximately 2 - 3 USD) to buy in a restaurant and a little less to buy the materials from markets and cook at home. Tinker (1997) and Maxwell *et al*., (2000), have also identified other factors that contribute to increase in number of people eating away from home in Ghana, including changes in life styles due to urbanization, longer distances from homes to work places, longer working hours for urban workers, young men, usually bachelors, with no cooking skills and/or kitchen facilities.

As a results of these and many other factors that have made a significant number of urban population dependent on food vendors, fast food services, restaurants and chop bars have sprang up tremendously in major cities in Ghana. It is estimated that food vendors make up 6-25% of the entire labour force in developing countries (Dawson and Canett, 1991) whereas in Ghana, Afele (2006) reported that Accra alone has about 60,000 food vendors of ready-to-eat foods. In Kumasi over 10,000 licensed vendors are estimated to operate (Olsen, 2005) while a large number operate without any appropriate license.

Consumption of vegetable salads prepared outside the home has also become popular. According to Olsen (2005) there is increasing awareness of the benefits of consuming fresh vegetables, therefore, people out of high expectations for health benefits consume them. In Ghana, it is common to see fast foods served with lettuce and other green vegetables usually regarded as a western practice and connected with affluent life style. According to Rheinländer (2006), traditional vendors even make use of lettuce as an attractive side dish to traditional Ghanaian meals. Thus, perceived nutritional values associated with salad consumption have contributed to eating vegetable salad by many Ghanaian consumers usually outside the home.

2.4.3. Factors Contributing to Contamination by Food Workers

Microbiological hazards can be introduced into food service operations through employees, the food itself, equipment and cleaning operations. Common sources of outbreaks of food borne illness in retail and food service establishments include inadequate food manipulation, improper holding temperatures, inadequate cooking, contaminated equipment and poor personal hygiene. According to Sousa (2005), sometimes foods are prepared a day or more before serving with improper holding and reheating, cross contamination from raw to cooked products as well as addition of contaminated ingredients to already cooked food; all contribute to contamination of food in food service establishments.

Food handlers play a major role in ensuring food safety throughout the chain of production, processing, storage and preparation. Mishandling and disregard for hygiene measures on their part may result in food contamination and its attendant consequences. Researchers have indicated that inadequate knowledge on transmission and growth of pathogens has significantly

contributed to cases of contamination and outbreaks in food (Holmberg and Blake, 1984; St. Louis *et al.,* 1990; Swerdlow *et al.,* 1997). Some of the factors that contribute to contamination of food by food workers include poor hygiene practices, cross-contamination as well as contamination through hand, fingernails and food workers apparel.

2.4.3.1. Poor Hygiene Practices

Rheinländer (2006) observed that, most vendors in Kumasi preferred adjusted hygiene practices that suite their practical everyday work routines. He also observed that many of the vendors employed inadequate food hygiene practices such as flash frying of rice, washing vegetables very briefly and with little amounts of salt or vinegar, use of the same knives and chopping boards for meat and vegetables, use of hands for serving some ready-to-eat meals such as fried chicken, yam, spaghetti, or salads as well as use of unwashed hands when preparing meat and vegetables. These practices indicate great risk associated with the consumption of food away from home since contamination factors are numerous in such food facilities.

2.4.3.2. Cross-Contamination

Cross-contamination during food preparation has been identified as an important factor associated with foodborne illnesses (Wanyenya *et al.,* 2004). Cross-contamination from hands or contaminated surfaces to ready-to-eat-foods is common in food service establishments. According to Gerner-Smidt and Whichard (2007), cross contamination in the kitchen accounted for one-third of U.S. foodborne disease outbreaks from 1998 to 2002. Wachtel *et al*., (2003) also indicated that *E. coli* O157:H7 were transferred from hamburger patties to hands, to cutting boards and to lettuce subsequently put on the boards. In another study by Gorman *et al.,* (2002),

Campylobacter, *E. coli*, *Salmonella*, and *Staphylococcus aureus* from chicken were found on dish-cloths, refrigerator handles, oven handles, counter tops, draining boards, and preparers' hands. Dharod (2007) also reported of *Salmonella*, *S. aureus* and *Listeria* spp. from various food ingredients and contact surfaces during chicken salad preparation in Puerto Rico. The same study indicated that, 13% of the food workers used the same knife for both chicken and vegetables without cleaning the knife between uses. Other researchers have also identified that cleaning food preparation surfaces and hand washing could reduce *Salmonella* and *Campylobacter* contamination (Cogan *et al.,* 1999) and *E. coli* infections (Mead *et al.*, 1997). Therefore, it is very necessary to properly instruct and encourage food workers to apply basic hygiene practices to ensure that transmission of pathogens as result of cross contamination and other related factors are controlled.

2.4.3.3. Hands, fingernails and workers apparel

Contamination of food products by the hands of food workers plays a major role in the transfer of pathogens to food. Food manipulation practices and foods that require direct contact with the hand can be potential agents of microbial proliferation in food. For most ingredients, including vegetables that are used in vegetable salad preparation, activities such as slicing, peeling and tearing of raw vegetables as well as mixing of ingredients are involved, all of which require contact with the hand. Research carried out by Greig *et al*., (2007) revealed that 40% of the 816 outbreaks associated with food workers involved hand contact including 1.3% of the cases associated with food workers not wearing gloves. The report from the Conference for Food Protection (2002) also indicated that 31% of outbreaks that occurred in Washington State from 1990 to 1999 resulted from inadequate hand washing by food workers. Handling practices in the

food service establishment from preparation to point of service must, therefore, be of critical concern to food workers and consumers since it can provide great opportunity for cross contamination of microbes from raw products to ready-to-eat foods.

Fingernails, workers clothing and jewelry are also potential sources of contamination by food workers. Keeping long fingernails and wearing of artificial nails are common practices lately. Levy *et al*., (1975), reported that long and artificial fingernails can trap faecal matter and food particles thereby increasing overall microbial counts on hands and thus increasing the risk of microbial contamination by the hands. It has also been found that bacteria and viruses can persist under fingernails (Pereira *et al.,* 1994, Samadi *et al.,* 1983). Isolation of *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis*, *Pseudomonas* and *Coryneforms* beneath the fingernails has been reported in a study by McGinley *et al*., (1988). During salad preparation, fingernails may be involved in activities such as removal of boiled eggs shells, tearing of vegetables and picking up chopped vegetables from a chopping board. Therefore, as far as possible, fingernails of food workers should always be neatly cut and cleaned coupled with proper hand washing and sanitization during food preparation to ensure adequate food safety to consumers.

Contamination of food by food workers clothing is also a common practice in food service establishments. According to Maguire *et al*., (2000), a food worker at a London hospital who had been caring for a sick child and thus contaminated the cloth she wore to work, re-contaminated turkey sandwiches resulting in salmonellosis among some staff members and one patient. It has also been confirmed by a number of studies that rings and other jewellery worn by food workers

also contribute significantly to the total microbial counts on hands (Hoffman *et al*., 1985; Jacobson *et al*., 1985; Salisbury *et al*., 1997).

Fischer *et al.,* (2007), stated that although most consumers are knowledgeable about the importance of preventing cross-contamination and using adequate heating to prevent foodborne illness, this knowledge is not necessarily translated into behavior. The same can be said of workers at food service facilities. This suggests a more serious problem which requires pragmatic measures to address them. One of the best ways this can be addressed is to take steps to train the people involved in food-handling (Gilling *et al*., 2001). Special attention should also be given to cleaning procedures and equipment used for food preparation in food service facilities. Humans, rodents, insects and other agents (FDA, 1978) have the potential to introduce pathogens to food and food preparation utensils and should be carefully controlled by ensuring that the entire food service facility and its environment are thoroughly cleaned. FDA (1978), recommended that, as much as possible, food preparation should be of least manual contact preferably with appropriate utensils and on thoroughly cleaned and sanitized surfaces to prevent any possible cross-contamination. Microbiologically safe food measures should, therefore, be purposefully enforced by managers of food service facilities to ensure that workers do not compromise any food served to consumers.

2.5. RISK ANALYSIS

According to Vose (2000), risk analysis is a qualitative or quantitative assessment of the potential impact of risk. It consists of three related components: risk assessment, risk management and risk communication (Collado *et al.*, 2011). The risk assessment component of risk analysis characterizes and estimates potential adverse health effects associated with

exposure to hazardous materials and situations (Hoppin 1993). The risk management component employs the results of risk assessment to implement appropriate control measures while risk communication share information and views on risk and risk management among risk assessors, risk managers, consumers and other interested parties (WHO 1999). Risk analysis is therefore, an integrated food safety system which can ensure the understanding and management of risk and provide precautionary actions and the protection for individuals or populations at risk.

Risk analysis is a useful food safety tool which according to Zwietering and Nauta (2007) has been used for seeking solutions in commercial litigation to assess the risk that a food has to the consumer. It can also be useful in the management of microbiological food safety issues and can provide help for regulatory authorities and the food industry to ensure that potential risk due to pathogens in a given food product is controlled (Duffy *et al.*, 2006). Microbial risk analysis focuses on the health risks that occur due to exposure to harmful microbes. According to Nauta (2000), the aim of microbial risk analysis is to statistically model the transmission of a specific pathogen through a chain of processes.

2.5.1. Risk Assessment

Risk assessment determines the risk associated with biological, chemical, or physical hazards in food. It estimates the type of hazard and the probability of harm due to human exposure to the agent in food. Risk assessment broadly can be qualitative or quantitative depending on the availability of data. Qualitative risk assessment is mostly applicable as a preliminary process to ascertain if further investigation is necessary. It is often used when there is lack of data on consumption pattern, dose-response models, initial contamination, and survival of the

microorganism after treatment and until the time of consumption. The final risk is categorical in nature, mostly reported with descriptive ratings of probability and severity. They are usually expressed as insignificant, low, medium or high (FAO/WHO, 2008).

Quantitative risk assessment (QRA) can further be classified as deterministic (point estimate) or probabilistic (stochastic) (FAO/WHO, 2008). In deterministic QRA, individual values in the form of means or percentiles are used whereas probability distributions are used in probabilistic QRA.

2.5.1.1. Quantitative Microbial Risk Assessment (QMRA)

Risk assessments for food additives and chemical contaminants have been used for over 30 years now (Gaylor *et al*., 1997), however, the concept of microbial risk assessment (MRA) in food is quite recent, having been used since the mid 1990s (FAO/WHO, 2009). According to Fazil *et al*., (2005), MRA employs "a systematic analytical approach'' intended to enhance "the understanding and management of microbiological risk issues". MRA provides a scientific method to examine issues relating to food hygiene and foodborne diseases. It estimates the probability of some health effect (diarrhoeal disease, hospitalization or death) caused by specific food, pathogen, process, and distribution pathway. It is reported that quantitative risk assessment with respect to exposure to pathogenic microorganisms was first published in the 1980s (Haas, 1983). A number of dose-response relations for some pathogenic microorganisms have become available since that time (Haas *et al*., 1999; McBride *et al*., 2002). Quantitative microbial risk analysis (QMRA) gives quantitative estimate of microbial risk associated with exposure to harmful microbes in food. It also involves modeling the movement of pathogens concerned

through a food processing chain (Naata, 2000). The model can then provide an estimate of the probability of adverse health effects associated with consumption of the food concern.

Uncertainty and variability are associated with QMRA (Vose, 2000). Variability arises as a result of naturally occurring random heterogeneity within a population (Wu and Tsang, 2004; Pouillot *et al*., 2003) whereas uncertainty is associated with lack of knowledge about the system being investigated (Vose, 2000). According to Pouillot *et al*., (2003), the uncertainty and variability should be separated to ensure clear understanding of the system under study and make it possible for the total uncertainty to be reduced.

Risk assessment for microbiological hazards in food consists of four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (CAC, 1999). The results from the first three steps are put together to obtain a risk estimate or the probability of adverse health effect resulting from exposure to microorganisms associated with consumption of food. This can be a qualitative or quantitative estimate and must include a description of the uncertainties associated with these estimates.

2.5.1.1.1. Hazard Identification

In MRA, the hazard identification is a qualitative step that involves the identification of microorganisms or microbial toxins in food and the health effects (illness or death) related to the consumption of contaminated food (FAO/WHO, 2008). Information about the pathogens, its existence and adverse health effects related to the food concern must be sought at this stage. Data sources such as scientific literature, databases as well as expert opinion could be valuable sources of information at the hazard identification stage (Soller, 2006). According to WHO

(1999), information for the hazard identification is also obtainable through clinical studies, epidemiological and surveillance studies, laboratory animal studies, investigations of the characteristics of microorganisms, interaction between microorganisms and their environment, and studies of analogous microorganisms and situations. For new microbial hazards, hazard identification can be a very tedious process involving generation of firsthand information while the process can be simple and straightforward for already existing hazards, when information is readily available (FAO/WHO, 2008).

2.5.1.1.2. Exposure Assessment

This characterizes the actual or anticipated amount of microorganisms or their toxins consumed and thus provide a qualitative or quantitative estimate of the likelihood and the dose of pathogens in a given portion of food (Soller, 2006; FAO/WHO, 2008). Exposure assessment estimates individual or population exposure to microbial hazards and quantities that are likely ingested (Lammerding and Fazil, 2000). At this step the quantity of food consumed and frequency of consumption in a given period for a specified portion of the population is determined to evaluate the population's exposure to a microbiological hazard associated with the food concern. Exposure assessment should also estimate the probability that the microorganism concerned will be present in the food, the amount of the microorganism in the portion of food consumed and the contribution of food handling, processing and storage on the possible exposure (Dennis *et al.*, 2002). Moreover, the potential impact of environmental conditions (WHO 1999), frequency or duration of exposure and factors such as consumer preferences and seasonality that may affect the exposure patterns must also be considered at this step.

2.5.1.1.3. Hazard Characterization (Dose–response assessment)

The purpose of hazard characterization is to establish the adverse health effects that may result from ingestion of pathogenic microorganism using appropriate dose-response relationships (FAO/WHO, 2008). The level of pathogens that are ingested as estimated from the exposure assessment is used to establish the link between the exposure level (dose) and the rate of occurrence of illness or other adverse health effect (response). In QMRA, hazard characterization should provide a quantitative evaluation of the probability and severity resulting from exposure to pathogenic microorganisms or their toxins. At this step expert opinion can be very useful and according to WHO (1999), expert opinion can be used to determine the infectivity of microbial pathogens to ascertain host's response to a dose of pathogens exposed to. The hazard characterization step requires the development of appropriate dose-response relationships to assess the impact of exposure to a microbial hazard in food and several of these dose response relationships are available for various foodborne pathogens and/or microbial toxins (Teunis *et al.*, 1996).

2.5.1.1.4. Risk Characterization

At this step the results from the first three steps are put together to obtain a risk estimate or the probability of adverse health effect resulting from exposure to microorganism associated with consumption of food. For QMRA, this estimate is quantitative and must include a description of the uncertainties associated with the estimate (Dennis *et al.*, 2002). According to WHO (1999), the confidence limit of the overall risk estimation is affected by the variability, uncertainty, and assumptions made in all the previous steps. The amount of uncertainty associated with the estimate is therefore necessary for the communication of the risk. The introduction of Monte

Carlo analysis and other simulation modeling techniques have made it possible to factor the variability associated with the various steps in the food processing into the final risk estimate (Buchanan *et al*., 2000). In quantitative risk assessment, risk characterization involves the combination of exposure assessment and hazard characterization or dose-response assessment to mathematically express the probability of the effect on public health (Dennis *et al.*, 2002). This is the component of risk assessment which is of much interest to risk communicators and risk managers and must be well explained by risk assessors for onward communication or subsequent decision making (Dennis *et al.*, 2002).

2.5.2. Predictive models and Monte Carlo simulation

The exposure assessment component of the risk assessment process establishes the certainty that a given pathogen or microbial toxins will be present in food at the time of consumption or at a specified processing step. According to Lammerding and Fazil (2000), exposure assessment estimates the likelihood of individual or population exposure to a microbial hazard and the amount of microorganism that are likely to be ingested, however, pathogenic microorganisms in food are very dynamic and can increase or decrease at various stages from production to consumption (farm to fork). Exposure assessment should therefore take into consideration the dynamism of microorganism behavior from farm to fork. In quantitative exposure assessment, tools have been developed to facilitate the process. These tools include predictive microbiology (predictive models) and the Monte Carlo simulation (Collado *et al.*, 2011).

Predictive microbiology put together aspects of microbiology, mathematics and statistics to establish models that can predict the behavior of microorganisms under specified conditions

(Collado *et al*., 2011). The fundamental principle underlying the concept is that microorganisms are reproducible and can be described as a function of different variables in a model. In MRA, the transfer of pathogens through the various stages of food processes to the point of consumption is modeled. According to Nauta (2001), these kinds of models follow probability distributions of the amount of pathogens through the food pathway, considering the associated variability and uncertainty. Teunis *et al*., (1996) also stated that, various studies have produced quantitative descriptions of the dose-response relationships of microorganisms making it possible to establish the risk of becoming infected after the ingestion of some dose of pathogens. The models that are frequently used for most microbial pathogens are the exponential and beta-Poisson models, originally introduced by Haas (Haas, 1983; Haas *et al*., 1999).

According to Haas *et al*., (1999), the exponential model is based on three assumptions:

- microorganisms are distributed randomly and thus follow the Poisson distribution
- for infection to occur, at least one pathogen must survive within the host and
- the probability of infection per ingested or inhaled organism is constant

Mathematically, the probability of infection P (d) for the exponential model is given by the equation (Haas, 1983; Haas *et al*., 1999):

$$P(d) = 1 - e^{-rd} \qquad (1)$$

Where P (d) is the probability of infection at dose (d), d is the dose in Colony Forming Units (CFU) and r is a parameter of the dose-response function (model parameter specific for each pathogen) interpreted as the probability for one cell of microorganisms to surviving and reach a host site to successfully initiate a response (infection/illness). The 'r' is also related to the dose

required to cause infection in half the exposed population in the relation (Soller, 2006) represented as:

$$N_{50} = \text{In } (0.5)/r \qquad (2)$$

The dose-response relation for most protozoans and viruses follow the exponential model (Soller, 2006).

The beta-Poisson model also follows the first two assumptions of the exponential model. For the third assumption, the beta-Poisson mode requires that the probability of infection per ingested or inhaled organism be varied with the population. For this model "r" is not constant but is beta distributed, with two parameters (α and β) of the beta distribution. Mathematically the beta-Poisson model is given by the equation (Haas, 1983; Haas *et al*., 1999):

$$P(d) = 1 - (1 + d/\beta)^{-\alpha} \qquad (3)$$

Where, P (d) is the probability of infection at dose (d), d is the dose (CFU), β and α, are parameters of the beta distribution that describes the host pathogen interaction; α is an infectivity parameter and β, a shape parameter. At low doses the beta-Poisson is linear, however, as α increases, it approaches the exponential model (Haas *et al*., 1999). This model is mostly applicable to many bacteria and some viruses (Soller, 2006).

Replication of experiments or recreation of scenarios can be accomplished by means of computer simulations. Collado *et al*., (2011) reported that, Monte Carlo simulation can be used to assess many complex food safety related problems especially 'in systems with many degrees of freedom'. It can be applied in many fields and situations and is also not difficult to use. Exposure assessment and the mathematical models used for dose-response assessment explain the presence

of pathogens in food, their multiplication with time, their elimination by heating processes, ingestion of viable microbes in food, and consequent health effect on the consumer. Probability distributions can be used to characterize the variability and uncertainty associated with the input variables for the model. Monte Carlo simulation involves the use of probability distributions to produce estimates of the parameters involved in the model. According to Vose (2008), random sampling of each of the probability distributions in a model is used to estimate the likelihood of the model's potential outcomes. By iteration or re-calculation, simulation of the model can be accomplished. Monte Carlo simulation of the model can thus provide an estimate of the level of human illness and the uncertainty associated with that estimate. Computer Software are available for this process. According to Collado *et al*., (2011), available software include: Microsoft Excel @Risk, Cristal Ball and the numerical analysis software, Matlab.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Study Location

The study was conducted in KNUST and UEW-K, parts of Kumasi Metropolitan Area (KMA). Kumasi is located in the transitional forest zone and between latitude 6.35^{o} – 6.40^{o} and longitude 1.30^{o} – 1.35^{o}, an elevation of 250 – 300 meters above sea level with an area of about 254 square kilometers (KMA, 2006). The average temperature range is 21.5^{o}C - 30.7^{o}C (KMA, 2006). The population in Kumasi by the year 2000 was around 3.5 million (Osei and Duker, 2008). The Kumasi Metropolitan Assembly (KMA) estimates that approximately 10,000 registered food vendors operated within the Kumasi suburbs by the end of 2005 (Rheinländer, 2006). KNUST and UEW-K are located in the eastern and western sections of the Kumasi metropolis respectively (MLGRDE, 2006).

KNUST and UEW-K were chosen based on the findings of Ababio and Adi (2012). In their study, they identified five communities in Kumasi as highly populated because of the socioeconomic activities that take place in these areas. The identified areas were Kwame Nkrumah University of Science and Technology campus, Baba Yara Stadium and its surroundings, Adum in Kumasi and its surroundings, Komfo Anokye Teaching Hospital and its surrounding areas and the University of Education, Winneba, Kumasi campus and its surroundings.

3.1.2. Sample Collection

Mixed-vegetable salad samples were purchased from nine randomly selected canteens. Samples were taken on 3 separate occasions from each selected canteen from 8th to 15th November, 2013 between 10 am and 5 pm. In all instances, each sample was placed in labeled sterile polyethylene container, kept in icebox (containing ice-blocks) and transported to the laboratory. A total of 27 mixed vegetable salads were collected for microbial analysis.

A survey was also conducted alongside the sample collection using structured questionnaires that had both observational and responsive questions (Appendix A). General observations were carried out at the various canteens guided by the observational questions to evaluate the food handling practices employed by canteen workers during sales while consumers response to the responsive questions were also used to establish the consumption pattern of mixed vegetable salads. A total of 200 questionnaires were distributed to consumers, however, 156 were retrieved.

3.2. METHODS

3.2.1. Bacteria Culture and Enumeration

3.2.1.1. Media Preparation

Salmonella-Shigella agar (SSA) (from Liofilchem diagnostici, Italy), Mannitol-salt agar (MSA), Plate Count agar (PCA), Nutrient agar, Selinite Cystine broth (SCB) and Buffered Peptone water (BPW) all from Oxoid Ltd, England, were prepared according to Manufacturer's instruction, and sterilized by autoclaving at 121^{o}C for 15 min. Salmonella-Shigella agar and Selinite Cystine

broth, which did not require autoclaving, were sterilized by boiling for 15 min according to manufacturer's instruction.

3.2.1.2. Aerobic plate count (APC)

Aerobic plate count (APC) was performed by the pour plate method (American Public Health Association, 2001) using plate count agar (PCA). Stock solution of 10^{-1} dilution was prepared by homogenizing 1 g of the sample in 9 ml of physiological saline. Serial dilutions of 10^{-1} to 10^{-4} were prepared by tenfold dilution of each preceding dilution. A 1 ml aliquot from each dilution was poured onto the centre of Petri dishes and sterile molten PCA poured into the Petri dishes already containing the samples. The culture and the medium were mixed thoroughly by gently moving the plates and the plates allowed to solidify and incubated at 37^{o}C for 24 hours in an inverted position. After 24 hour incubation, colonies on PCA were counted and recorded in CFU/g of salad samples using the colony counter.

3.2.1.3. Enumeration and Isolation of *Staphylococcus aureus*

Staphylococcus aureus were enumerated and isolated by the pour plate method (American Public Health Association, 2001) using Mannitol-salt agar (MSA). The same serial dilutions and pour plate procedure used for APC were used for *Staphylococcus aureus* using MSA in place of PCA. After 24 hour incubation at 37^{o}C, golden yellow colonies were counted and recorded as presumptive *Staphylococcus* spp. in CFU/g using the colony counter. Presumptive *Staphylococcus* spp. colonies on MSA were sub cultured onto freshly prepared nutrient agar plates and confirmed by Grams staining and coagulase test using rabbit plasma (Murray *et al*.,

2003). Colonies on mannitol salt agar that were Gram positive and coagulase-positive were taken as *Staphylococcus aureus* (Appendix B).

3.2.1.4. Enumeration and isolation of *Salmonella* spp.

Salmonella spp. detection was carried out according to ISO-6579 method (International Standards Organization, 2002) using buffered peptone water as pre-enrichment medium, selenite cystine broth (SCB) as selective enrichment medium and Salmonella-Shigella agar (SSA) for selective plating. For enumeration, 1 g sample was thoroughly mixed in 9 ml buffered peptone water and serial dilutions of 10^{-1} to 10^{-4} prepared and spread on SSA followed by 24 hour incubation at 37^{o}C. For *Salmonella* spp. isolation, serial dilutions of 10^{-1} to 10^{-4} were incubated at 37^{o}C for 24 hours and 0.1 ml aliquot of serial dilutions inoculated onto 9 ml selenite cystine broth (SCB) followed by 48 hours incubation at 44^{o}C. After the 48 hours incubation, another 0.1 ml of the selective enrichment cultures were streaked on the surface of already prepared sterile Salmonella-Shigella agar (SSA) and incubated immediately. The plates were incubated at 37^{o}C for 48 hours for *Salmonella* spp. isolation. Black colonies on SSA are typical colonies of *Salmonella* spp.

3.2.2. Microbial Risk Assessment

For the microbial risk assessment, the focus of this study on the chain of vegetable salad production was the post salad preparation in various canteens to the point of consumption.

3.2.2.1. Hazard Identification

For the purpose of the risk assessment, *S. aureus* and *Salmonella* spp. were chosen as the model quantitative microbial risk assessment (QMRA) organisms. *S. aureus* is associated with staphylococcal food poisoning which causes self-limiting, acutely intense illness in most people while *Salmonella* serotypes *S. typhi* and *S. paratyphi* A are associated with typhoid fever (FDA, 2012). Their presence in food has been linked to cross-contamination and poor handling practices during food preparation (Gorman *et al.*, 2002, Dharod, 2007). Several studies have reported on outbreaks of foodborne illnesses related to vegetables and vegetable salads (Halablab *et al*., 2011, Meldrum *et al.*, 2009) as well as *S. aureus* and *Salmonella* spp. associated with these products (Ameko *et al*., 2012, Fung *et al.*, 2011, Poorna and Randhir, 2001). This research focused on the risk of infection associated with the consumption of already prepared mixed vegetable salads sold in the test canteens.

3.2.2.2. Exposure assessment

Essential information from the survey data that were crucial to the microbiological quality of mixed vegetable salads and the levels of model organisms from the microbiological analysis were incorporated into the exposure assessment. Based on the frequency of consumption as indicated by the survey work, the following exposure scenarios were assessed:

- frequent consumption of vegetable salad by consumers (everyday consumption)
- average consumption of vegetable salad by consumers (once a week consumption)
- occasional consumption of vegetable salad by consumers (once a month consumption)

The assumption made for all the three scenarios was that there was no reduction in pathogen levels but a possible growth from post salad preparation to the point of consumption because the product was not heat treated prior to consumption and were kept at room temperature for a long time during sales. As a result, any increase in microbial numbers during sales will be passed on to the consumer. The exposure days per year were taken as 365, 52 and 12 days for frequent, average and occasional consumers respectively. The amount of mixed vegetable salad consumed per person per day that was used was 10 to 12 g, based on the research by Seidu *et al*., (2008). The dose of the model organism(s) used for the dose response assessment was obtained from the exposure assessment in the mathematical relation:

$$CFU_F = CFU_G \times W \qquad (4)$$

Where:

CFU_F = the final quantity of microorganisms consumed per day or per serving,

CFU_G = CFU/g (quantity of microorganisms per gram of salad)

W = the weight of salad consumed per serving

3.2.2.3. Hazard characterization (dose-response assessment)

Based on the outcome of the laboratory analysis of the model organisms, the exponential dose response model (Rose and Haas, 1999) was used for the dose response assessment to predict the probability of *Staphylococcus aureus* infection. Mathematically, the probability of infection for the exponential model used is given as:

$$P(d) = 1 - e^{-rd} \quad (1)$$

Where P (d) is the probability of infection, d is the dose (CFU) of microorganisms consumed per person per day and r is a dimensionless infectivity constant. The model parameter, "r" used was

7.64 x 10^{-8} (Rose and Haas, 1999). The final dose of *S. aureus* that are consumed (total exposure per day, CFU_F) were input into the mathematical model to obtain the probability of infection per day. This was subsequently run with Microsoft Excel @ risk software version 6.2.0.0 (Palisade Corporation) using 10,000 iterations.

3.2.2.4. Risk characterization

The annual probability of infection for each scenario was determined from the probability of infection and the number of days of exposure within the year in the relation:

$$P_{ann} = 1-(1-P(d))^{n} \quad (5)$$

Where n is the number of days of exposure within the year.

For each of the exposure scenarios Monte Carlo simulation was run using Microsoft Excel@ risk software version 6.2.0.0 (Palisade Corporation) sampling 10,000 iterations to determine the annual risk of infection for each exposure scenario. Mean risks of infections from the simulations were reported. The schematic of the inputs and output for the annual risk of infection (risk characterization) is presented in Figure 1 below.

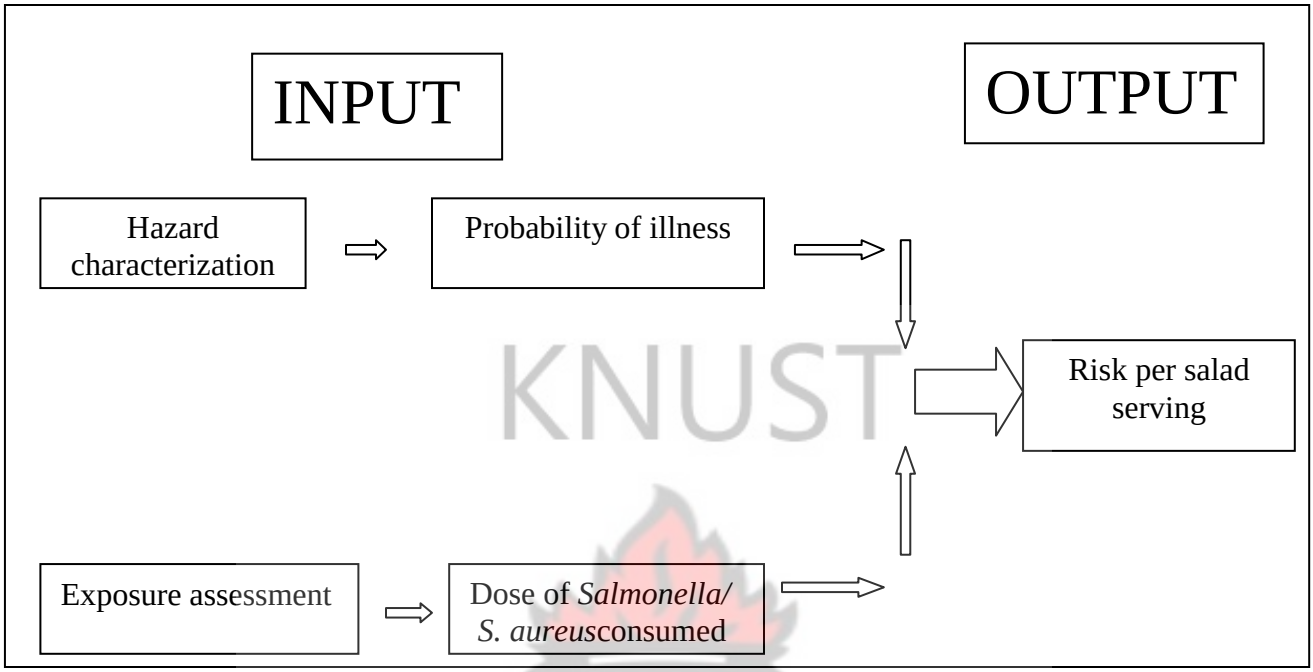

Fig. 1: The risk assessment process (Miller *et al.*, 2008)

3.3. Statistical Analysis

Data was analyzed by ANOVA appropriate to each experiment using Graph Pad Prism 5 software version 5.01 (2007) and any statistical significance of difference between means were tested at 95% confidence level ($P < 0.05$) by Tukey multiple test (Zar, 1996). Monte Carlo simulation of the models was carried out using Microsoft Excel @ risk software version 6.2.0.0 (Palisade Corporation).

CHAPTER FOUR

4.0 RESULTS

4.1. MICROBIAL LOAD OF THE MIXED VEGETABLE SALADS

The levels of microorganisms used for the microbiological risk assessment of mixed vegetable salads of all analyzed samples are presented in Table 1. Mean values of log CFU/g ± SD were used.

Table 1: Loads of total bacteria (APC), *S. aureus* and *Salmonella* spp. of the mixed vegetable salad samples from the selected canteens

Code of Canteen	Load (log CFU/g)		
	APC[a]	*S. aureus*[b]	*Salmonella* spp.[c]
AT	3.41 ± 0.68	4.12 ± 0.16	ND
H	4.48 ± 1.96	3.38 ± 0.74	ND
IFF	4.77 ± 1.19	4.96 ± 1.12	ND
AK	3.98 ± 0.49	4.92 ± 1.85	ND
GD	3.28 ± 0.85	2.97 ± 0.60	ND
THCS	3.10 ± 0.42	3.60 ± 1.01	ND
P1	4.83 ± 1.38	4.90 ± 2.11	ND
LRCS	4.81 ± 2.08	5.13 ± 0.26	ND
BC	4.09 ± 1.19	4.77 ± 0.25	ND

ND= not detected, [[a]acceptable: 10^6 to < 10^7 CFU/g (6 to <7 log CFU/g), [b]Unacceptable: ≥ 10^4 CFU/g (≥ 4 log CFU/g), [c] Unacceptable: Detected in 25 g (PHLS, 2000); [a]acceptable: < 10^5 CFU/g (< 5 log CFU/g), [b]acceptable: < 10^4 CFU/g (< 4 log CFU/g) (GSB, 2003)]

From Table1, *Salmonella* spp. was not detected in the mixed vegetable salads from any of the sources of risk (canteens). APC and *S. aureus* counts, however, varied among the risk sources. Mean APC and *S. aureus* counts ranged from 3.1 log CFU/g to 4.83 log CFU/g and 2.97 log CFU/g to 5.13 log CFU/g respectively. The highest mean APC of 4.83 log CFU/g was recorded

for canteen P1 and the lowest value of 3.1 log CFU/g for canteen THCS. The highest *S. aureus* count of 5.13 log CFU/g and lowest value of 2.97 log CFU/g were recorded for canteens LRCS and GD respectively. The differences in the mean total bacteria counts (APC) and *S. aureus* counts recorded for the mixed vegetable salads collected from the respective canteens were statistically insignificant ($P > 0.05$) (Appendix D1 and D2).

Figure 2, shows the relationship between APC and *S. aureus* count from the test canteens. From the Figure, apart from points H and GD, mean *S. aureus* count from all other canteens were slightly higher than APC.

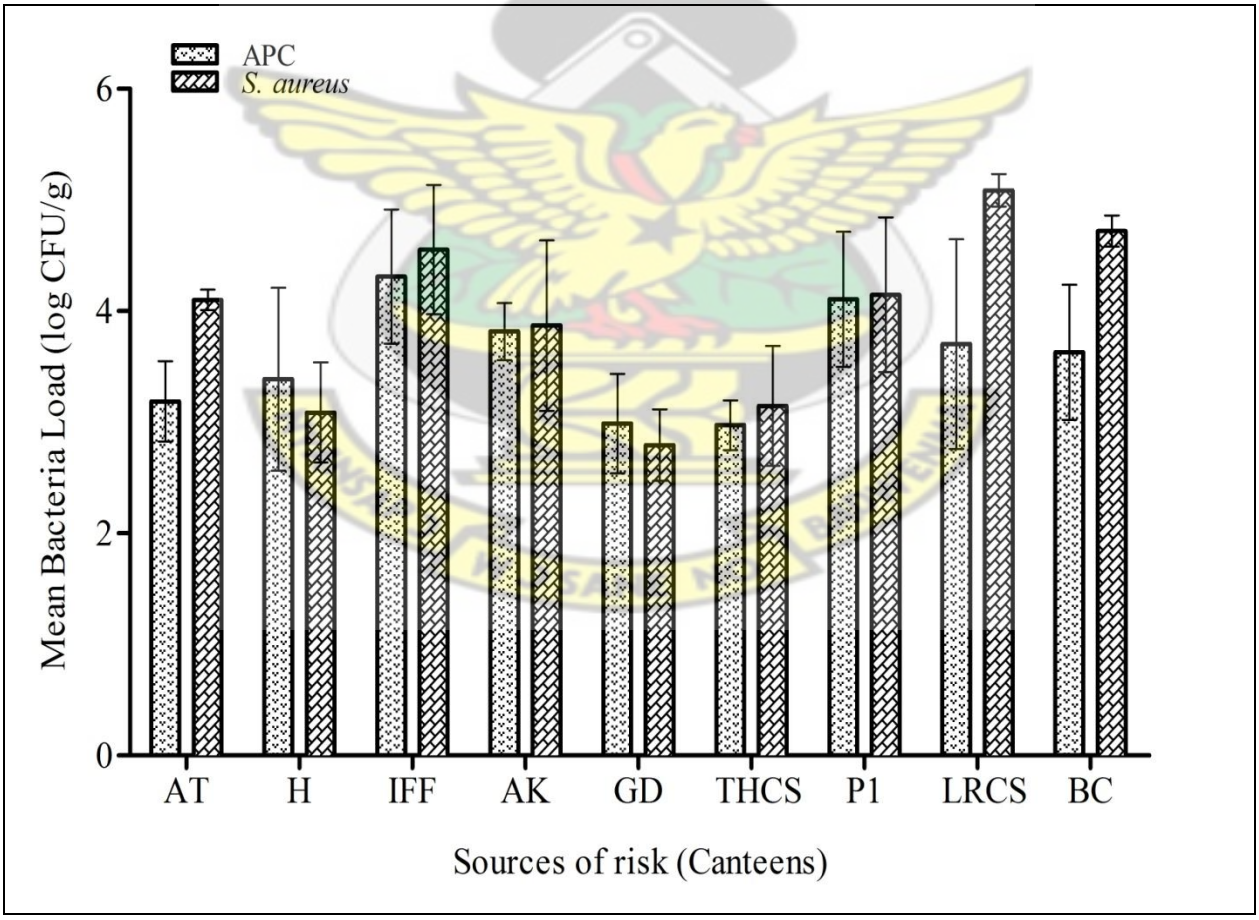

Fig. 2: APC and *S. aureus* loads in salad samples from the test canteens

4.2. POST PROCESSING HANDLING PRACTICES AND SALAD CONSUMPTION PROFILE

Post salad processing handling and salad consumption details were determined by structured questionnaire with both observational and responsive questions (Appendix A). The results are presented below.

4.2.1. Post Processing Handling Practices

Salad details and post preparation practices employed by canteen operators as determined by the questionnaire are presented in Table 2. The results show that all (100%) the canteens included in the study served mixed vegetable salad and majority of the canteens served salad with salad dressing (88.89%). All the samples did not have the same composition, but were predominantly made of a mixture of cabbage, (*Brasslike oleracea* L.) lettuce, (*Lactuca sativa* L.), cucumber (*Cucumis sativus* L.), carrots (*Daucus carota* L.), and spring onion (*Allium fistulosum* L.) in various proportions and combinations. Majority of the salads served in the various canteens were not heat treated (77.78%) and stored at room temperature (77.78%) when serving to consumers. Samples from only two locations (22.22%), LRCS and P1 (all in UEW-K), were partially cooked while all samples were dressed with salad cream or mayonnaise except one location (P1 in UEW-K). Most canteen operators also used containers with cover and serving utensil dedicated for that purpose (77.78% and 66.67% respectively).

Table 2: Post salad processing details

Parameter	Distribution of Respondents (Consumers)		Frequency (n=9)	Percentage (%)
	KNUST	UEW-K		
Type of Salad				
Lettuce only	0	0	0	**0**
Cabbage only	0	0	0	**0**
Cucumber only	0	0	0	**0**
Mixed vegetables	6	3	9	**100**
State of salad served				
Partially cooked	0	2	2	**22.22**
Not cooked	6	1	7	**77.78**
Storage condition for service				
Room temperature	5	2	7	**77.78**
Refrigerator	0	0	0	**0**
Not sure	1	1	2	**22.22**
State of storage container				
Covered	5	2	7	**77.78**
Not covered	0	0	0	**0**
Not sure	1	1	2	**22.22**
Salad dressing				
Served with dressing	6	2	8	**88.89**
Served without dressing	0	1	1	**11.11**
Utensil for serving				
Designated utensil	5	1	6	**66.67**
Shared utensil	0	0	0	**0**
Gloves protected hands	0	0	0	**0**
Bare hands	0	1	1	**11.11**
Not sure	1	1	2	**22.22**

4.2.2. Salad Consumption Profile

Salad consumption information obtained from the respondents is presented in Table 3.The result shows a total of 156 respondents (consumers) with 67.95% and 32.05% located in KNUST and UEW-K campuses respectively. From the table, almost all the respondents were between 18 and

35 years of age (96.8%). Percentage distribution of male and female respondents was 45.51% and 54.49% respectively.

The percentage score for source of vegetable salad consumption as shown in Table 3 indicates that 46.15% of the respondents consume salad from home, 29.49% from canteens, 16.67% from restaurants, 4.49% from street food joints and 3.21% from other sources. On the frequency of vegetable salad consumption, 37.82% of the respondents consume salad once a week, 37.18% consume it once a month and 13.46% consume it at least once a day while 11.54% consume it at unspecified occasions from all the sources. For respondents who consume salad from canteens, 45.65% consume it once a week, 34.78% consume it once a month, 15.21% consume it once a day and 4.35% consume it at unspecified occasions.

Table 3: Salad consumption details

Parameter	Distribution of Respondents (Consumers)		Frequency (N=156)	Percentage (%)
	KNUST	UEW-K		
Age				
18-35	101	50	151	**96.8**
> 35	3	2	5	**3.2**
Gender				
Male	47	24	71	**45.51**
Female	57	28	85	**54.49**
Source from which salad is consumed				
Home	49	23	72	**46.15**
Canteen	31	15	46	**29.49**
Restaurant	17	9	26	**16.67**
Street food joints	5	2	7	**4.49**
Others	2	3	5	**3.21**
Salad consumption frequency(total respondents)				
Once a day (frequent consumers)	13	8	21	**13.46**
Once a week (average consumer)	39	20	59	**37.82**
Once a month (occasional/rare consumer)	37	21	58	**37.18**
Others			18	**11.54**
Salad consumption frequency(canteens)			**n=(46)**	
Once a day(frequent consumers)	4	3	7	**15.21**
Once a week (average consumer)	16	7	21	**45.65**
Once a month (occasional/rare consumer)	11	5	16	**34.78**
Others	2	0	2	**4.35**

4.3. MICROBIAL RISK OF READY-TO-EAT MIXED VEGETABLE SALADS TO CONSUMERS

Based on the outcome of the microbial count and the estimated quantity of salad consumed per person per day, Monte Carlo simulation of *S. aureus* using the exponential model ($r = 7.64x\ 10^{-8}$) was run for 10,000 iterations. Risk assessment for three exposure scenarios (frequent consumers,

average consumers and occasional consumers) determined by the salad consumption profile were simulated.

4.3.1. Exposure Assessment and Hazard Characterization (Dose Response Assessment)

Figure 3shows the dose of *S. aureus* consumed per person per day after Monte Carlo simulation of 10,000 iterations. From the Figure, mean dose of 8.301 x 10^6 CFU (90% CI: 0.00 x 10^6 CFU – 1.20 x 10^7 CFU) was obtained after the simulation. Minimum and maximum doses of 8.54 x 10^2 CFU and 1.083 x 10^{10} CFU respectively were also recorded. The result indicates that an average dose of 8.301 x 10^6 CFU may be ingested from the consumption of mixed vegetable salad from the test canteens with a 95 % probability the dose of *S. aureus* that may be ingested will exceed 0.00 x 10^6but will however, not exceed 1.20 x 10^7 CFU.

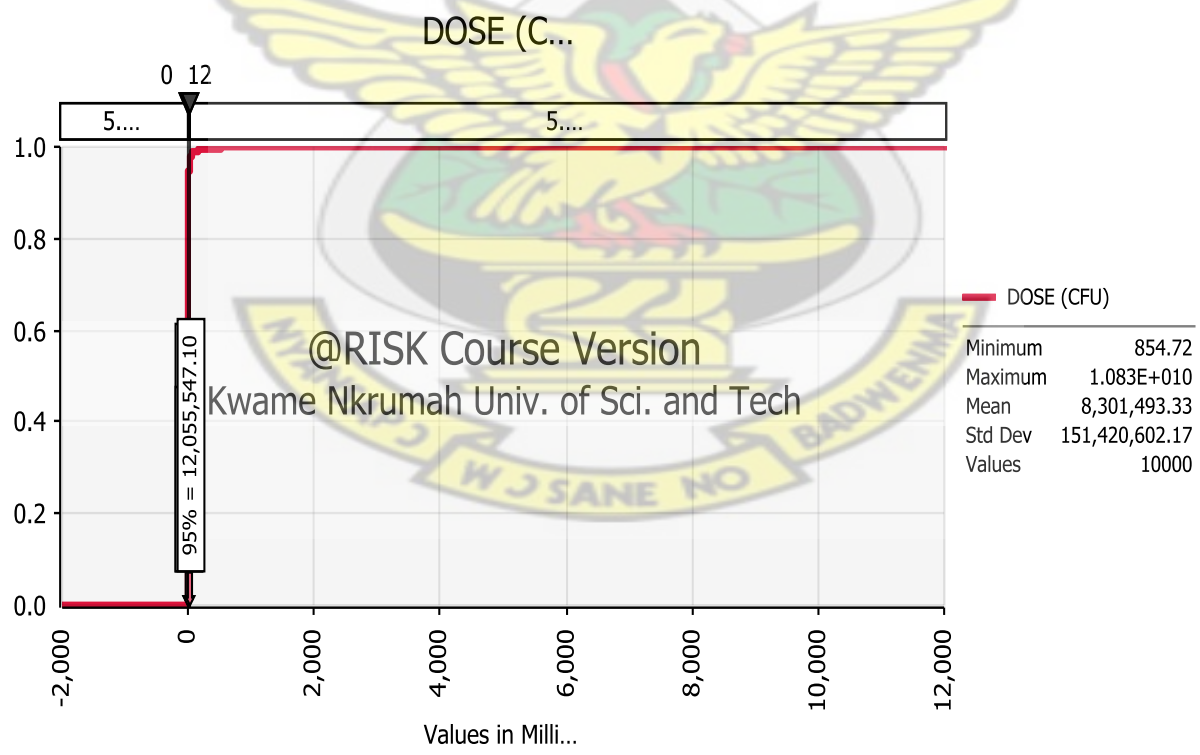

Fig. 3: Cumulative ascending distribution of the dose of S. aureus consumed per person per day after Monte Carlo simulation over 10,000 iterations

The dose response assessment (hazard characterization) resulting from ingestion of mixed vegetable salad from the test canteens after Monte Carlo simulation is shown in Figure 4. Figure 4 indicates a mean probability of infection of 1.84×10^{-1} (90% CI: $1.10 \times 10^{-2} - 7.10 \times 10^{-1}$) with minimum and maximum values being 1.50×10^{-4} and 16.3×10^{-1} respectively. This indicates that the average probability of *S. aureus* infection to consumers of mixed vegetable salad from the test canteens is most likely 18.4% with a 95 % probability that the probability of infection will exceed 1.1% but will however, not exceed 71.0%.

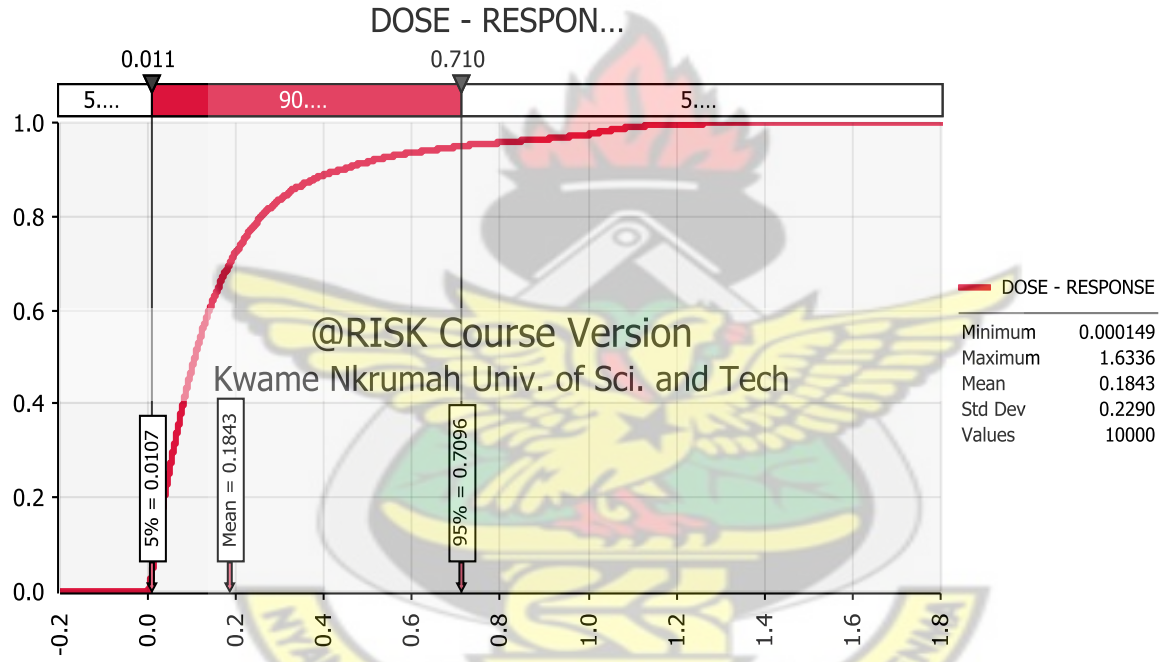

Fig. 4: Cumulative ascending distribution of the dose-response assessment of S. aureus associated with mixed vegetable salad after Monte Carlo simulation over 10,000 iterations

4.3.2. Annual Risk of *S. aureus* Infection associated with the Consumption of Mixed Vegetable Salad

The annual risk for the three exposure scenarios (frequent consumers, average consumers and occasional consumers) are presented in Figures 5 to 7.

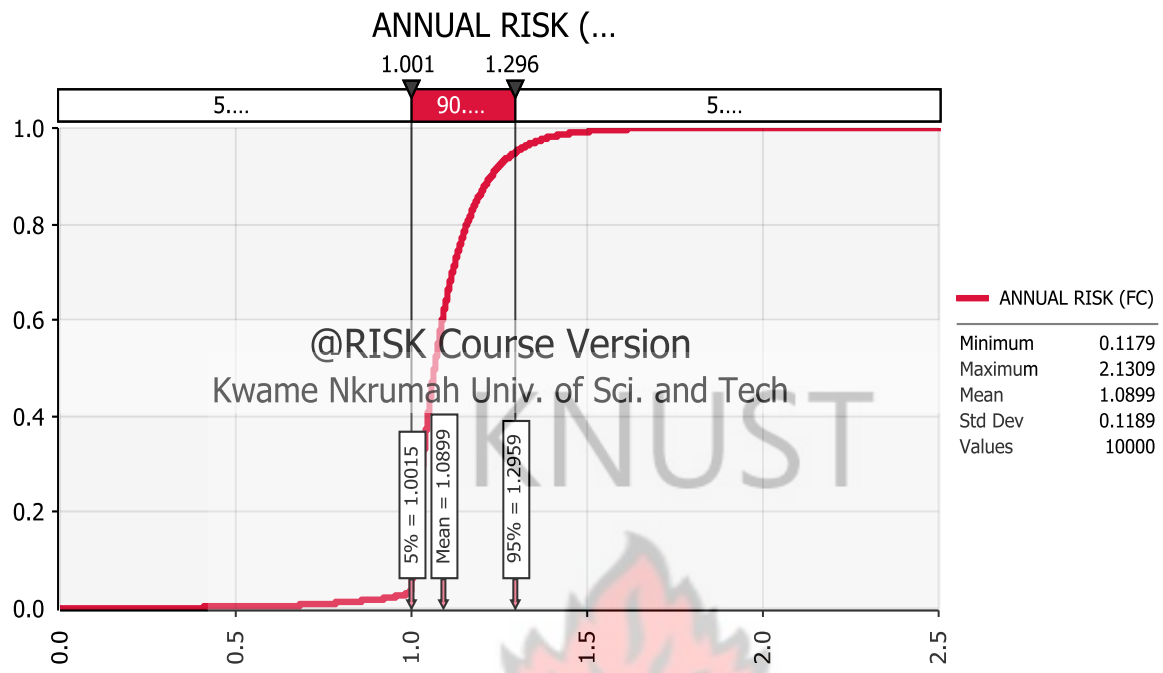

Fig. 5: Annual risk of *S. aureus* infection associated with the frequent (daily) consumption of the mixed vegetable salads from the test canteens after Monte Carlo simulation of 10,000 iterations

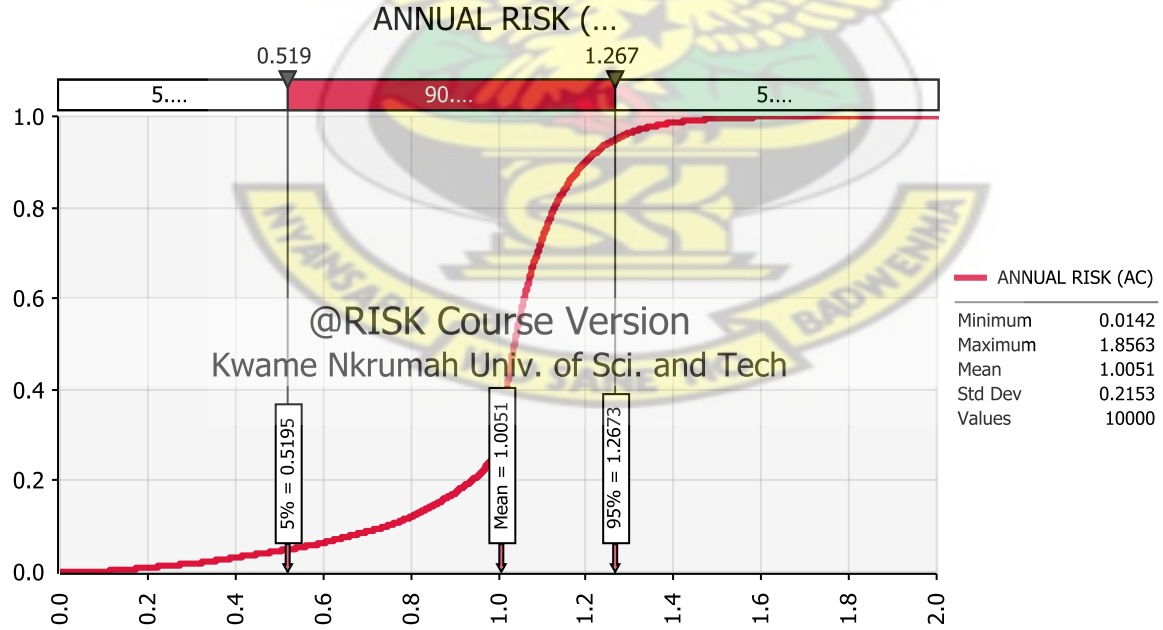

Fig. 6: Annual risk of *S. aureus* infection associated with the average (weekly) consumption of mixed vegetable salads from the test canteens after Monte Carlo simulation over 10,000 iterations

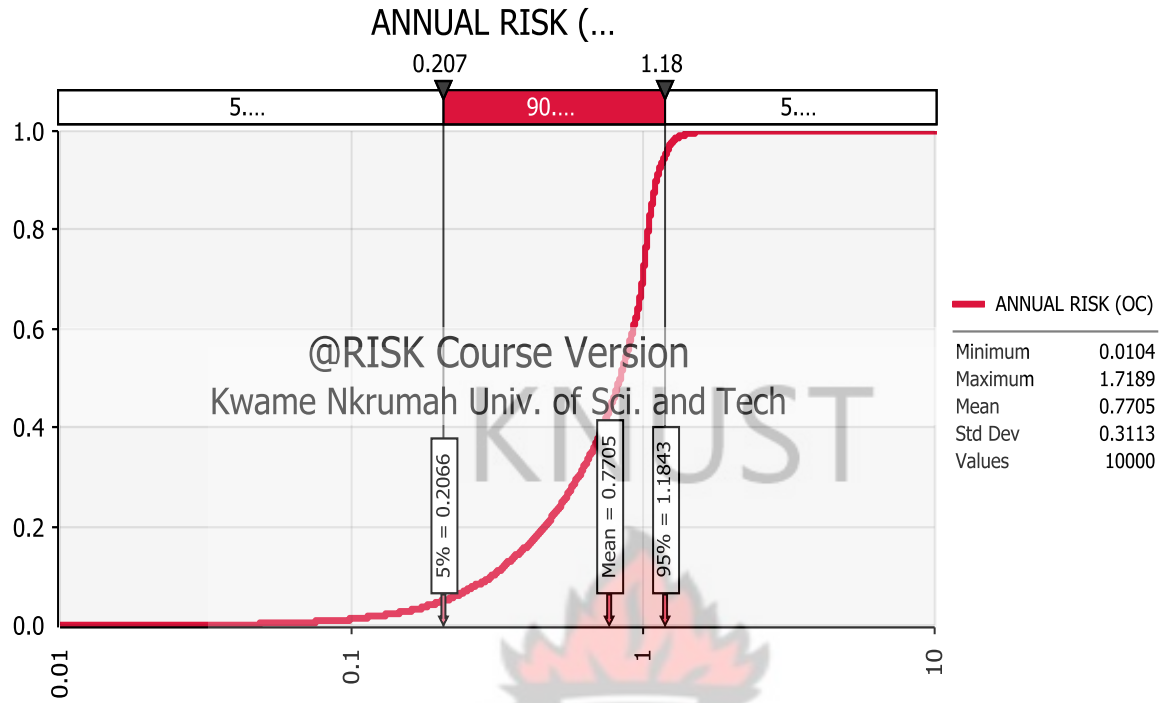

Fig. 7: Annual risk of *S. aureus* infection associated with the occasional (monthly) consumption of mixed vegetable salads from the test canteens after Monte Carlo simulation over 10,000 iterations

From the results of the risk assessment, the mean annual risk associated with the consumption of mixed vegetable salads from the test canteens for frequent consumers (everyday) was 10.90×10^{-1} (90% CI: 10.01×10^{-1} – 12.96×10^{-1}), with minimum and maximum risk being 1.18×10^{-1} and 21.31×10^{-1} (Figure 5). With this mean annual risk, daily consumption of mixed vegetable salad from the test canteens may likely result in approximately 11 out of 10 consumers being infected with *S. aureus* with 95 % probability that the risk of infection will exceed approximately 10 out of 10 consumers being infected. From Figure 6, the mean annual risk for average consumers (weekly) was 10.05×10^{-1} (90% CI: 5.19×10^{-1} – 12.67×10^{-1}). The maximum and minimum risk for this category of salad consumers were 18.56×10^{-1} and 0.14×10^{-1} respectively. Thus, for weekly consumption of mixed vegetable salad from the test canteens, approximately 10 out of 10

consumers may likely be infected with *S. aureus* with 95 % probability that the risk of infection will exceed approximately 5 out of 10 consumers being infected. Figure 7, also indicates mean annual risk of 7.71×10^{-1} (90% CI: 2.07×10^{-1} – 11.8×10^{-1}) as well as minimum and maximum risk of 0.10×10^{-1} and 17.19×10^{-1} respectively. The maximum and minimum risk for this category of salad consumers were 18.56×10^{-1} and 0.14×10^{-1} respectively. This indicates that monthly consumption of mixed vegetable salad from the test canteens may result in approximately 8 out of 10 consumers being infected with *S. aureus* with 95 % probability that the risk of infection will exceed approximately 2 out of 10 consumers being infected. The risk of consuming mixed vegetable salad, therefore, differs for the three categories of exposures.

CHAPTER FIVE

4.0. DISCUSSION

Consumption of ready to eat mixed vegetable salads is greatly patronized, especially, by majority of city dwellers in Ghana. One of the main reasons for such a high patronage of the product is the well reported nutritional and health benefits, however, it is also well reported that fresh cut vegetables, the main ingredients of mixed vegetable salads, are potential vehicles for the transmission of pathogenic foodborne microbes (Nguyen-the and Carlin, 1994; Beuchat, 1996). From this study all the samples examined from the various canteens were contaminated with total aerobic mesophiles (APC) and *S. aureus*.

From the results, APC of 3.1 log CFU/g to 4.83 log CFU/g were recorded. APC of samples from all the test canteens were within the standard requirements of both the Ghana Standards Board (< 5 log CFU/g) (GSB, 2003) and the UK Public Health Laboratory Services (6 to < 7 log CFU/g) (PHLS, 2000). APC for various salad ingredients have been reported by some researchers. Abdullahi and Abdulkareem (2010), observed average APC of 2.3×10^8 CFU/g (8.36 log CFU/g) for cabbage, 2.5×10^8 CFU/g (8.40 log CFU/g) for lettuce and 1.1×10^6 CFU/g (6.04 log CFU/g) for cucumber. Aboh *et al*., (2011), also reported of APC ranging from 1.6×10^6 CFU/g (6.2 log CFU/g) to 2.9×10^8 CFU/g (8.46 log CFU/g) for salad vegetables. In a related study in Accra (Mensah *et al*., 2002) and Kumasi (Feglo and Sakyi, 2012), mean APC of 6.3 log CFU/g and 5.13 log CFU/g respectively were recorded in salad samples. The APC results of this work generally appear relatively lower than most of the research findings as well as the standard values of < 5 log CFU/g (GSB, 2003) and 6 to < 7 log CFU/g (PHLS, 2000). High APC may indicate poor handling, inappropriate processing or a general lack of hygiene, indicating that the

canteen operators involved in this study probably employed some level of proper handling and hygienic practices. APC determination may, however, include species that inhibit the growth of other pathogenic bacteria strains in a mixed population such as *S. aureus* (Medveďová and Valík, 2012) and may even include strains like *Salmonella* and *Listeria* which are considered unacceptable when detected in food samples. Moreover, bacteria have the potential to grow when provided the right conditions with time, therefore, although the levels of APC as determined by this research are within acceptable limit and are far lower than the results of similar researches, canteen operators should still be encouraged to employ proper handling and hygienic practices during vegetable salad processing. This is especially necessary considering the fact that the salads were kept at room temperature for extended period of time during sales.

S. aureus is a pathogen known to be carried by food handlers (Beuchat, 1998). The presence of *S. aureus* in mixed fresh-cut vegetables indicates poor hygienic practices and levels higher than 4 log CFU/g are potentially hazardous. From this study, *S. aureus* count ranged from 2.97 log CFU/g to 5.13 log CFU/g. Samples from canteens, H, GD and THCS were within the standard values of $<10^4$ CFU/g (< 4 log CFU/g) of both the Ghana Standards Board (GSB, 2003) and the United Kingdom Public Health Laboratory Services (PHLS, 2000) while samples from the remaining six points (AT, IFF, AK, LRCS, P1 and BC) were all above the standard values (Table 1). Various researchers have reported of isolation of *S. aureus* from vegetables and vegetable salads. Myhara *et al*., (2003), reported of mean *S. aureus* count of 5.4 x 10^2 CFU/g (2.7 log CFU/g) in salad from parts of Accra. Gitahi *et al*., (2012), also recorded *S. aureus* counts of 3.13 log CFU/g to 4.69 log CFU/g in street food vegetables from five locations in industrial area of Nairobi city. In a similar work in India, Sabbithi *et al*., (2014), reported of *S. aureus* count of 2.0

log CFU/g – 5.2 log CFU/g and 2.0 log CFU/g – 5.0 log CFU/g in carrot and onion, respectively. The *S. aureus* counts recorded in the current study is contrary to the findings of Myhara *et al*., (2003), which is within the acceptable standard of <4 log CFU/g but is close to what was observed by Gitahi *et al*., (2012) and Sabbithi *et al*., (2014), where some of the findings are beyond the acceptable standard.

The high levels of *S. aureus* in most canteens (66.67%) indicate poor handling practices during and/or after salad preparation. The common sources of *S. aureus* food contamination are the nose, throat, skin, and hair of healthy humans and animals as well as feathers of birds where they occur naturally (Tatini, 1973; Smith *et al.*, 1983; Garvani, 1987). Food handlers are the main agents of transmission of *S. aureus* into food. According to Loir *et al*., (2003), improper handling of food by contaminated hands or other improper food handling practices such as coughing or sneezing during food preparation usually after heat treatment of the food contribute significantly to *S. aureus* contamination of food. FDA (2012) also indicated that, unless heat processes are applied, staphylococci are expected to exist in any as well as all foods that are handled directly by humans or are of animal origin. Therefore, the high levels of *S. aureus* identified in samples from most of the test canteens (66.67%) suggest time and temperature abuse of the product by most canteen operators and lack of proper handling practices during sales.

S. aureus species are mainly involved in staphylococcal food intoxication cases (Khambaty *et al.*, 1994). According to FDA (2012), the intoxication dose of staphylococcal enterotoxins (SE) is less than 1.0µg which can be produced when *S. aureus* populations exceed 100, 000 organisms/g in food. From the results, mean *S. aureus* count of 1.35×10^5 CFU/g (5.13 log

CFU/g) was recorded in the samples from canteen LRCS which exceeds the minimum population of 100,000 CFU/g required to cause staphylococcal intoxication. The mean *S. aureus* count for canteens AT, IFF, AK, P1 and BC were: 1.31 x 10^4 CFU/g (4.12 log CFU/g), 9.17x 10^4 CFU/g (4.96 log CFU/g), 8.23 x 10^4 CFU/g (4.92 log CFU/g), 7.87 x 10^4 CFU/g (4.90 log CFU/g) and 5.85 x 10^4 CFU/g (4.77 log CFU/g) respectively. All these values exceed 10,000 CFU/g, minimum bacteria population (10,000 - 20,000 CFU/g) required to produce 100 to 200 ng of enterotoxin which can cause illness in delicate individuals (FDA, 2012). FDA (2012) also pointed out that the population of *S. aureus* at the time of analysis may be significantly different, and not representative of the highest population that occurred in the product, which in fact should be considered when examining foods. Thus, while consumption of mixed vegetable salads from canteen LRCS might present serious microbiological risk that needs urgent intervention to avoid any outbreak with respect to *S. aureus*, canteens AT, IFF, AK, P1 and BC equally have the potential to cause outbreak and require similar attention as point LRCS.

The results from this study also reveal that mean *S. aureus* count from majority of the canteens (H and GD excluded) were slightly higher than APC. For the canteens with *S. aureus* count slightly higher than APC, apart from point THCS, all the other points had *S. aureus* population highly likely to cause infection. This confirms that poor handling practices were employed during and/or after salad preparation in majority of the canteens. In addition, according to Medveďová and Valík (2012), staphylococci compete poorly with indigenous bacteria and are inhibited by the antagonistic activities of other organisms. They, therefore, recommended that the presence of *S. aureus* in foods must be considered in relation to the amount and types of the accompanying flora. This suggests that APC of salad samples from majority of the canteens possibly included species that interfered with *S. aureus* growth along other accompanying flora,

however, on a selective media all other microbes were excluded making it possible for all viable organisms to grow. Moreover, correlation analysis of the data suggests a positive linear correlation between APC and *S. aureus* count ($r_s = 0.6500$) but there was not enough evidence at 5% level of significance to conclude that there was a significant linear correlation between them (Appendix C). This suggests that all the quantity of *S. aureus* that were detected on the selective plates in the samples were not necessarily viable on the PCA to add up to the values of the APC.

Salmonella spp. is widely dispersed in nature. Poultry and other meat products, eggs and dairy products, are the most commonly implicated sources of outbreaks involving *Salmonella* (D'Aoust 2000; Olsen *et al.,* 2000), however, fresh produce has also been implicated as the source of major outbreaks, particularly in recent times (Mensah *et al.,* 2001; Fung *et al.*, 2011). From the results, *Salmonella* spp. was not detected in the samples from any of the canteens. According to PHLS (2000), the presence of *Salmonella* spp. in any quantity represents high risk. In related studies in Accra and Lomé, Myhara *et al.,* (2003) and Adjrah *et al.,* (2013), respectively did not detect *Salmonella* spp. in any of the salad samples evaluated, similar to the findings of this study. Other researches carried out on salad and salad vegetables in Uganda (Mugampoza *et al.,* 2013) and Iran (Moayed *et al.,* 2013) also recorded no *Salmonella* spp. in any of the samples analyzed. In this study, the salad samples from all the canteens met the standard requirement of no *Salmonella* in 25 g of food samples and comparable to the findings of some previous works. *Salmonella* originate from the gastrointestinal tract of man and animals and their presence in food products, therefore, indicate faecal contamination and cross contamination during preparation. Their absence as determined by this research suggests that the canteen operators used practices that eliminated or minimized cross contamination of salad

ingredients and finished product with meat and other products likely to be infested with *Salmonella* spp. It also suggests the sources of salad ingredients were free of faecal contamination.

The results from the survey conducted for the test canteens show that mixed vegetable salads were served in all the canteens included in the study, while majority (88.89%) served salad with salad dressing. This finding agrees with the works of Ameko *et al*., (2012) and Rheinländer (2006), among street food vendors in Accra and Kumasi respectively. Both studies identified the make-up of the salad served as composed of various kinds of vegetables including lettuce, cabbage, tomato, onion and carrot. While consumers may patronize mixed vegetable salads due to the perceived nutritional benefits (James and Ngarmsak, 2011; Kalia and Gupta, 2006), canteen operators are encouraged to serve the product to attract more customers and thus increase profit.

From the study, most canteen operators used containers with cover (77.78%) and serving utensils dedicated for serving only salad (66.67%), however, majority of the salad served in the various canteens were not heat treated and stored at room temperature when serving to consumers (indicated by 77.78% in each case). This suggests that while most canteen operators may be well intended and more concerned about the safety and well being of consumers, they knowingly or unknowingly ignore measures that can prevent microbial multiplication in food products during sales. This finding agrees with the results of Akonor and Akonor (2013), who indicated that most domestic food handlers were more knowledgeable in the areas of food safety concerns, general and personal hygiene and handling leftover food, than they were in cross-contamination and the

dynamics of pathogens in causing food borne diseases. The findings of Ababio and Adi (2012) also indicated that most food handlers in the Kumasi Metropolis lacked adequate knowledge about foodborne diseases and failed to apply control measures. Perishable foods like vegetable salads can promote microbial growth when provided the right temperature and other conditions. It is very important, therefore, that regulatory agencies such as Food and drug authority (FDA) and metropolitan assemblies pay close attention to microbiological food safety control systems employed by canteen operators and other food vendors to ensure adequate food safety to consumers.

In the case of the salad consumption profile, the study revealed that 46.15% of the respondents consumed salad from home, 29.49% from canteens, 16.67% from restaurants, 4.49% from street food joints and 3.21% from other sources. Thus, a total of 53.85% of the respondents patronized salad sold outside the home. This compares with other works which suggested high interest in the food vending business. Nyarango *et al.*, (2003), reported that over 2.5 billion people regularly support food sold outside the home, worldwide. Moreover females make up 54.49% of the respondents and most females generally prefer homemade foods which may account for a significant number of the respondents (46.15%) interested in homemade salads. Out of the total of 53.85% who consume salad outside the home, majority (29.49% of 53.85%) consume it from canteens. This could be due to the moderate cost of food sold at canteens. The cost of a plate of rice from the canteens included in the research ranged between three Ghana Cedis (Gh₵ 3.00) and eight Ghana Cedis (Gh₵ 8.00), while the same meal may cost Fifteen Ghana Cedis (Gh₵ 15.00) to thirty Ghana Cedis (Gh₵ 30.00) in restaurants. This is in agreement with the works of

Maxwell (1998) and Rheinländer (2006), who suggested that reduced prices of food is one of the major reasons why people patronize food vending business in Ghana.

From this study, 37.82% of the respondents consumed salad at least once a week, 37.18% consumed it at least once a month while 13.46% consumed it every day. For those who consumed salad from canteens, 45.65% and 15.21% respectively consumed it at least once a week or once a day making a total of 60.86% of the respondents who consumed it quite frequently from canteens. Such a high patronage of mixed vegetable salads may be attributed to the widely publicized health and nutritional benefits associated with the consumption of vegetables and vegetable salads (Kalia and Gupta, 2006; James and Ngarmsak, 2011). According to Amoah *et al.* (2007), westernization of the Ghanaian culture has made salad consumption essential component of our diet. The work of Rhelander (2006), in Kumasi, also revealed that, food hygiene is not the decisive factor for customers but personal trust for vendors rather seems to overrule and replace concerns and criteria of proper food handling and food hygiene. It is, therefore, necessary that consumers of vegetable salad be properly sensitized to be conscious of the safety of the product.

The microbiological risk of ready to eat mixed vegetable salads to consumers was evaluated using *S. aureus* and *Salmonella* spp. From the bacteriological analysis, *S. aureus* was determined in salad samples from all the canteens at levels likely to cause infection from majority (66.67%) of the canteens while *Salmonella* spp. was not detected in any of the samples.

The mean dose of *S. aureus* consumed per person per day from consumption of mixed vegetable salad was above the minimum population of 100, 000 CFU required to cause infection (FDA, 2012). The exposure assessment indicated a mean dose of 8.301 x 10^6 CFU per person per day for the three exposure scenarios. With 90% of the doses between 0.00 x 10^6 CFU and 1.20 x 10^7 CFU, there is the possibility of high risk of *S. aureus* infection from consumption of mixed vegetable salads from the canteens. The mean probability of consumers being infected with *S. aureus* was1.84 x 10^{-1} and this represents 18.4% chances of an individual being infected with *S. aureus* from the consumption of vegetable salads. In 90% of the cases, the chances of consumers being infected with *S. aureus* from the consumption of vegetable salads were between 1.1% and 71.0% (90% CI: 1.10 x 10^{-2} – 7.10 x 10^{-1}). It may be argued that, the mean probability of infection of 1.84 x 10^{-1} appears quite low (< 20%), however, according to Haas *et al*., (1993), a single pathogen ingested can multiply to cause infection, therefore, the seemingly low probability of infection does not necessarily indicate low risk.

The mean annual risks of *S. aureus* infection in the three scenarios were 10.90 x 10^{-1}, 10.05 x 10^{-1} and 7.71 x 10^{-1} for frequent, average and occasional consumers respectively. The annual risk of *S. aureus* infection for all the scenarios exceeded the WHO tolerable risk of 10^{-4} per person per year (WHO, 2006). Annual risk of 10^{-1}, suggests a risk of one infection in 10 consumers. Thus, for frequent and average consumers, approximately 11 and 10 out of 10 mixed vegetable salad consumers respectively stand a chance of *S. aureus* infection while approximately 8 out of 10 occasional consumers stand a risk of *S. aureus* infection. These risks are very likely high and above the recommended tolerable level by a magnitude order of 3 (10^{-3}). The risk of *S. aureus* infection for frequent consumers of approximately 11 out of 10 indicates over 100% certainty

(110%) that daily consumption of mixed vegetable salads from the test canteens will most likely result in infection. The risk of *S. aureus* infection as determined by this study is also higher than the annual risk of *E. coli* infection of 3.63×10^{-1} in lettuce consumption reported by Ackerson and Awuah (2012). However, although all the consumers of mixed vegetable salads from the canteens were at high risk of *S. aureus* infection, more frequent consumers stand greater risk than occasional consumers.

The very high likelihood of the risk of *S. aureus* infection can be attributed to the relatively high levels of *S. aureus* enumerated and the frequency of consumption. Since greater number of mixed vegetable salad consumers from canteens consumed it quite frequently (60.86%) based on the survey carried out in this work, there is an indication of greater public health risk which could possibly result in an outbreak.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The aerobic plate count (APC) of mixed vegetable salads analyzed from the sources of risk (test canteens) ranged between 3.1 log CFU/g to 4.83 log CFU/g, which were all within both the Ghana Standard Board (GSB) and the UK Public Health Laboratory Services (PHLS) acceptable references of < 5.0 log CFU/g and 6 to < 7 log CFU/g, respectively. *Salmonella* spp. was not detected in salad samples from any of the test canteens in compliance with both GSB and PHLS standard requirements, however, *S. aureus* were isolated at levels ranging between 2.97 log CFU/g to 5.13 log CFU/g. The levels of *S. aureus* are higher than both the GSB and PHLS acceptable standards of < 4 log CFU/g in majority (66.67%) of the test canteens, making the mixed vegetable salads sold in majority of the canteens in KNUST and UEW-K and their environs a potential source of food poisoning to consumers. From the study, majority (77.78%) of canteen operators stored salad at room temperature during sales while a total of 60.86% of patrons of mixed vegetable salad sold from canteens consume it quite frequently. Storage conditions of mixed vegetable salads during sales and the frequency of consumption, respectively represent the post processing handling practices and consumption patterns that contribute significantly to the microbiological quality of mixed vegetable salads from canteens in and around KNUST and UEW-K and the risk of *S. aureus* infection. The annual risk of *S. aureus* infection analyzed for the three exposure scenarios, frequent, average and occasional consumers were 10.90×10^{-1}, 10.05×10^{-1} and 7.71×10^{-1}, respectively. This represents a potentially high risk of *S. aureus* infection from the consumption of mixed vegetable salads sold in canteens

located in and around the campuses of KNUST and UEW-K with most frequent consumers being at higher risk than occasional consumers.

6.2. RECOMMENDATION

It is recommended that further research be conducted in other parts of Ghana using other pathogens, other types of foods and other food service establishments to establish a comprehensive profile of microbial risk and/or safety of various food products.

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APPENDICES

APPENDIX A

KWAME NRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

INSTITUTE OF DISTANCE LEARNING

MICROBIAL RISK ASSESSMENT OF MIXED VEGETABLE SALADS FROM SELECTED CANTEENS

QUESTIONNAIRE FOR SALAD PREPARATION AND COSUMPTION DETAILS

The information provided is for academic purposes only and will be treated confidentially. Please tick the appropriate option.

A. PREMISES DETAILS

Name……

Location……

Comment on appearance of preparation/storage area…….....

……

……..

B. SALAD DETAILS

Type of salad: [] lettuce [] cucumber [] cabbage [] mixed [] other/ specify…..

How is the salad stored/kept for service: [] at room temperature [] in a refrigerator [] others……..

Is salad covered: [] Yes [] No

Is salad handled/served using: [] designated serving utensil [] shared utensil [] gloves protected hand [] bare hand [] other/specify…….

C. SALAD CONSUMPTION DETAILS

Name (optional)…….

Age: [] below18 [] 18-35 []above35 Sex: M/F Occupation……

How many times in a year do you eat salad: [] every day [] once a week [] once a month [] others…….

Where do you eat salad from: home [] restaurant [] canteen [] on the street [] others ……

Why do you eat salad: [] for health benefits [] for taste [] for its aesthetic appeal [] social interaction [] others/specify……

Have you ever experienced any discomfort/sickness attributed to consumption of salad: [] Yes [] No

If yes, specify the kind of discomfort/sickness…….

What do you know about microbial contamination of vegetable salad……

……

APPENDIX B

Table B: *S. aureus* confirmatory test results

CODE	GRAM STAINING	COAGULASE TEST	CONCLUSION
AT	+	+	*S. aureus*
H	+	+	*S. aureus*
IFF	+	+	*S. aureus*
AK	+	+	*S. aureus*
GD	+	+	*S. aureus*
THCS	+	+	*S. aureus*
P1	+	+	*S. aureus*
LRCS	+	+	*S. aureus*
BC	+	+	*S. aureus*

APPENDIX C: CORRELATION ANALYSIS BETWEEN APC AND *S. aureus*

	S. aureus
Number of XY Pairs	9
Spearman r	0.6500
P value (two-tailed)	0.0666
P value summary	Ns
Exact or approximate P value?	Exact
Is the correlation significant? (alpha=0.05)	No

APPENDIX D: ANALYSIS OF VARIANCE (ANNOVA) AND TURKEYS MULTIPLE COMPARISON TEST

Appendix D1: Analysis of Variance and Turkey's multiple comparison test for *S. aureus*

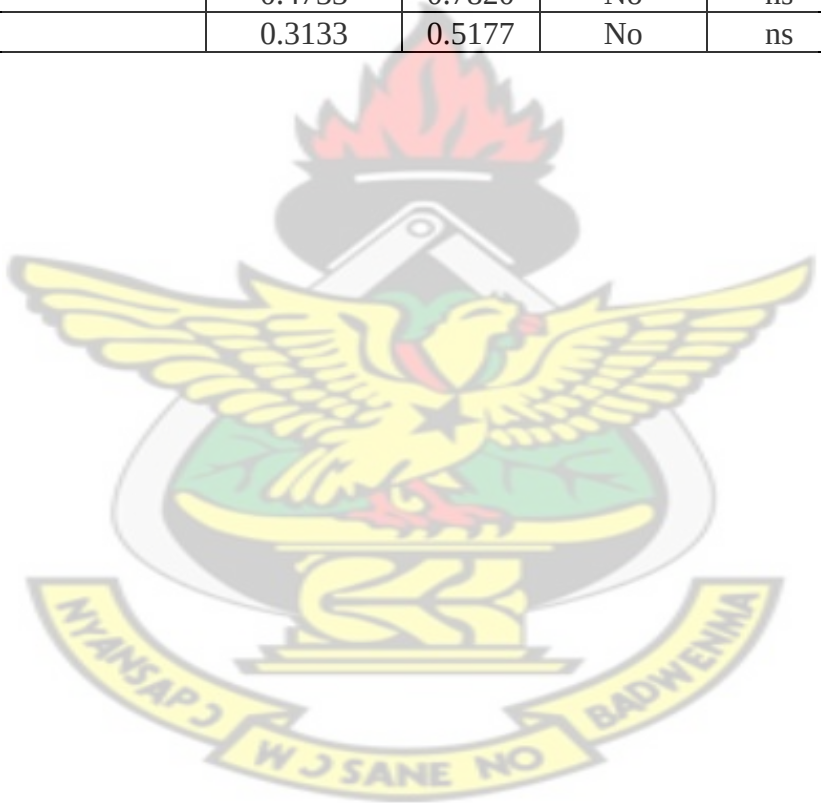
Table Analyzed	***S. aureus***				
One-way analysis of variance					
P value	0.0801				
P value summary	Ns				
Are means significantly different? (P < 0.05)	No				
Number of groups	9				
F	2.187				
R squared	0.4929				
ANOVA Table	**SS**	**Df**	**MS**		
Treatment (between columns)	15.03	8	1.878		
Residual (within columns)	15.46	18	0.8588		
Total	30.49	26			
Tukey's Multiple Comparison Test	**Mean Diff.**	**Q**	**Significant? P < 0.05?**	**Summary**	**95% CI of diff**
AT vs H	1.013	1.894	No	Ns	-1.638 to 3.665
AT vs IFF	-0.4533	0.8473	No	Ns	-3.105 to 2.198
AT vs AK	0.2300	0.4299	No	Ns	-2.422 to 2.882
AT vs GD	1.307	2.442	No	Ns	-1.345 to 3.958
AT vs THCS	0.9533	1.782	No	Ns	-1.698 to 3.605
AT vs P1	0.2867	0.5358	No	Ns	-2.365 to 2.938
AT vs LRCS	-0.9867	1.844	No	Ns	-3.638 to 1.665
AT vs BC	-0.6200	1.159	No	Ns	-3.272 to 2.032
H vs IFF	-1.467	2.741	No	Ns	-4.118 to 1.185
H vs AK	-0.7833	1.464	No	Ns	-3.435 to 1.868
H vs GD	0.2933	0.5482	No	Ns	-2.358 to 2.945
H vs THCS	-0.06000	0.1121	No	Ns	-2.712 to 2.592
H vs P1	-0.7267	1.358	No	Ns	-3.378 to 1.925
H vs LRCS	-2.000	3.738	No	Ns	-4.652 to 0.6517
H vs BC	-1.633	3.053	No	Ns	-4.285 to 1.018
IFF vs AK	0.6833	1.277	No	Ns	-1.968 to 3.335
IFF vs GD	1.760	3.289	No	Ns	-0.8917 to 4.412
IFF vs THCS	1.407	2.629	No	Ns	-1.245 to 4.058

IFF vs P1	0.7400	1.383	No	Ns	-1.912 to 3.392
IFF vs LRCS	-0.5333	0.9968	No	Ns	-3.185 to 2.118
IFF vs BC	-0.1667	0.3115	No	Ns	-2.818 to 2.485
AK vs GD	1.077	2.012	No	Ns	-1.575 to 3.728
AK vs THCS	0.7233	1.352	No	Ns	-1.928 to 3.375
AK vs P1	0.05667	0.1059	No	Ns	-2.595 to 2.708
AK vs LRCS	-1.217	2.274	No	Ns	-3.868 to 1.435
AK vs BC	-0.8500	1.589	No	Ns	-3.502 to 1.802
GD vs THCS	-0.3533	0.6604	No	Ns	-3.005 to 2.298
GD vs P1	-1.020	1.906	No	Ns	-3.672 to 1.632
GD vs LRCS	-2.293	4.286	No	Ns	-4.945 to 0.3584
GD vs BC	-1.927	3.601	No	Ns	-4.578 to 0.7250
THCS vs P1	-0.6667	1.246	No	Ns	-3.318 to 1.985
THCS vs LRCS	-1.940	3.626	No	Ns	-4.592 to 0.7117
THCS vs BC	-1.573	2.941	No	Ns	-4.225 to 1.078
P1 vs LRCS	-1.273	2.380	No	Ns	-3.925 to 1.378
P1 vs BC	-0.9067	1.695	No	Ns	-3.558 to 1.745
LRCS vs BC	0.3667	0.6853	No	ns	-2.285 to 3.018

Appendix D2: Analysis of Variance and Turkey's multiple comparison test for APC

Table Analyzed	**APC**				
One-way analysis of variance					
P value	0.7262				
P value summary	Ns				
Are means significantly different? (P < 0.05)	No				
Number of groups	9				
F	0.6510				
R squared	0.2244				
ANOVA Table	**SS**	**Df**	**MS**		
Treatment (between columns)	5.724	8	0.7155		
Residual (within columns)	19.78	18	1.099		
Total	25.51	26			
Tukey's Multiple Comparison Test	**Mean Diff.**	**Q**	**Significant? P < 0.05?**	**Summary**	**95% CI of diff**
AT vs H	-0.2000	0.3304	No	ns	-3.200 to 2.800
AT vs IFF	-1.123	1.856	No	ns	-4.123 to 1.876
AT vs AK	-0.6300	1.041	No	ns	-3.630 to 2.370
AT vs GD	0.2000	0.3304	No	ns	-2.800 to 3.200
AT vs THCS	0.2133	0.3525	No	ns	-2.786 to 3.213
AT vs P1	-0.9167	1.514	No	ns	-3.916 to 2.083
AT vs LRCS	-0.7567	1.250	No	ns	-3.756 to 2.243
AT vs BC	-0.4433	0.7325	No	ns	-3.443 to 2.556
H vs IFF	-0.9233	1.525	No	ns	-3.923 to 2.076
H vs AK	-0.4300	0.7104	No	ns	-3.430 to 2.570
H vs GD	0.4000	0.6609	No	ns	-2.600 to 3.400
H vs THCS	0.4133	0.6829	No	ns	-2.586 to 3.413
H vs P1	-0.7167	1.184	No	ns	-3.716 to 2.283
H vs LRCS	-0.5567	0.9197	No	ns	-3.556 to 2.443
H vs BC	-0.2433	0.4020	No	ns	-3.243 to 2.756
IFF vs AK	0.4933	0.8151	No	ns	-2.506 to 3.493
IFF vs GD	1.323	2.186	No	ns	-1.676 to 4.323
IFF vs THCS	1.337	2.208	No	ns	-1.663 to 4.336
IFF vs P1	0.2067	0.3414	No	ns	-2.793 to 3.206
IFF vs LRCS	0.3667	0.6058	No	ns	-2.633 to 3.366
IFF vs BC	0.6800	1.123	No	ns	-2.320 to 3.680
AK vs GD	0.8300	1.371	No	ns	-2.170 to 3.830

AK vs THCS	0.8433	1.393	No	ns	-2.156 to 3.843
AK vs P1	-0.2867	0.4736	No	ns	-3.286 to 2.713
AK vs LRCS	-0.1267	0.2093	No	ns	-3.126 to 2.873
AK vs BC	0.1867	0.3084	No	ns	-2.813 to 3.186
GD vs THCS	0.01333	0.02203	No	ns	-2.986 to 3.013
GD vs P1	-1.117	1.845	No	ns	-4.116 to 1.883
GD vs LRCS	-0.9567	1.581	No	ns	-3.956 to 2.043
GD vs BC	-0.6433	1.063	No	ns	-3.643 to 2.356
THCS vs P1	-1.130	1.867	No	ns	-4.130 to 1.870
THCS vs LRCS	-0.9700	1.603	No	ns	-3.970 to 2.030
THCS vs BC	-0.6567	1.085	No	ns	-3.656 to 2.343
P1 vs LRCS	0.1600	0.2643	No	ns	-2.840 to 3.160
P1 vs BC	0.4733	0.7820	No	ns	-2.526 to 3.473
LRCS vs BC	0.3133	0.5177	No	ns	-2.686 to 3.313



APPENDIX E: MICROBIAL COUNT

Table E1: *S. aureus* count

Code	R1 (CFU/g)	R1 (log CFU/g)	R2 (CFU/g)	R2 (log CFU/g)	R3 (CFU/g)	R3 (log CFU/g)	Avg. (CFU/g)	Avg. (log CFU/g)	Avg. (log CFU/g ± SD)
AT	$8.91x10^3$	3.95	$1.20x10^4$	4.08	$1.85x10^4$	4.27	$1.31x10^4$	4.12	**4.12±0.16**
H	$1.55x10^2$	2.19	$4.31x10^3$	3.64	$2.68x10^3$	3.43	$2.38x10^3$	3.38	**3.38±0.74**
IFF	$1.35x10^5$	5.13	$2.44x10^3$	3.39	$1.38x10^5$	5.14	$9.17x10^4$	4.96	**4.96±1.12**
AK	$2.44x10^5$	5.39	$8.70x10^2$	2.94	$1.90x10^3$	3.28	$8.23x10^4$	4.92	**4.92±1.85**
GD	$1.02x10^3$	3.01	$1.62x10^3$	3.21	$1.45x10^2$	2.16	$9.25x10^2$	2.97	**2.97±0.60**
THCS	$1.37x10^2$	2.14	$2.02x10^3$	3.31	$9.81x10^3$	3.99	$3.99x10^3$	3.60	**3.60±1.01**
P1	$8.50x10^1$	1.93	$1.49x10^4$	4.17	$2.21x10^5$	5.34	$7.87x10^4$	4.90	**4.90±2.11**
LRCS	$1.88x10^5$	5.27	$6.24x10^4$	4.8	$1.54x10^5$	5.19	$1.35x10^5$	5.13	**5.13±0.26**
BC	$4.90x10^4$	4.69	$9.55x10^4$	4.98	$3.09x10^4$	4.49	$5.85x10^4$	4.77	**4.77±0.25**

Table E2: Aerobic Plate Count (APC)

Code	R1 (CFU/g)	R1 (log CFU/g)	R2 (CFU/g)	R2 (log CFU/g)	R3 (CFU/g)	R3 (log CFU/g)	Avg. (CFU/g)	Avg. (log CFU/g)	Avg. (log CFU/g ± SD)
AT	$5.04x10^3$	3.70	$2.36x10^3$	3.37	$3.10x10^2$	2.49	2.67x103	3.41	**3.41±0.68**
H	$8.86x10^4$	4.95	$1.40x10^2$	2.15	$1.15x10^3$	3.06	$3.00x10^4$	4.48	**4.48±1.96**
IFF	$5.30x10^4$	4.72	$1.32x10^3$	3.12	$1.24x10^5$	5.09	$5.93x10^4$	4.77	**4.77±1.19**
AK	$2.12x10^4$	4.33	$3.77x10^3$	3.58	$3.45x10^3$	3.54	$9.47x10^3$	3.98	**3.98±0.49**
GD	$1.30x10^2$	2.11	$1.87x10^3$	3.27	$3.77x10^3$	3.58	$1.92x10^3$	3.28	**3.28±0.85**
THCS	$4.70x10^2$	2.67	$6.87x10^3$	2.84	$2.58x10^3$	3.41	$1.25x10^3$	3.10	**3.10±0.42**
P1	$5.54x10^3$	3.74	$1.92x10^3$	3.28	$1.95x10^5$	5.29	$6.74x10^4$	4.83	**4.83±1.38**
LRCS	$8.18x10^4$	4.91	$7.50x10^1$	1.88	$1.09x10^5$	5.04	$6.38x10^4$	4.81	**4.81±2.08**
BC	$2.54x10^4$	4.40	$2.70x10^2$	2.43	$1.15x10^4$	4.06	$1.24x10^4$	4.09	**4.09±1.19**

Table E3: *Salmonella* spp.

Code	R1 (CFU/g)	R1 (CFU/g)	R2 (CFU/g)
AT	ND	ND	ND
H	ND	ND	ND
IFF	ND	ND	ND
AK	ND	ND	ND
GD	ND	ND	ND
THCS	ND	ND	ND
P1	ND	ND	ND
LRCS	ND	ND	ND
BC	ND	ND	ND

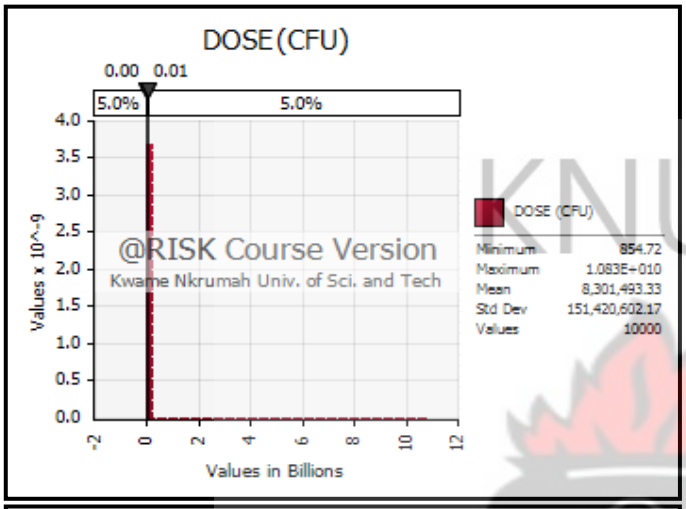
ND = not detected

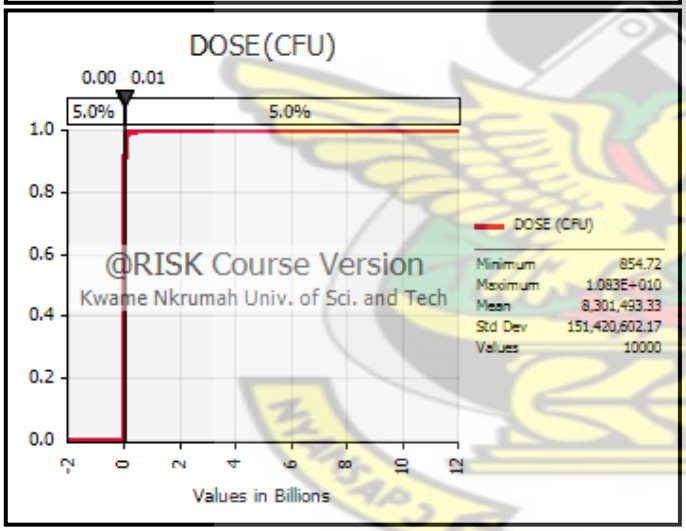
APPENDIXAPPENDIX F: @RISK OUTPUTS

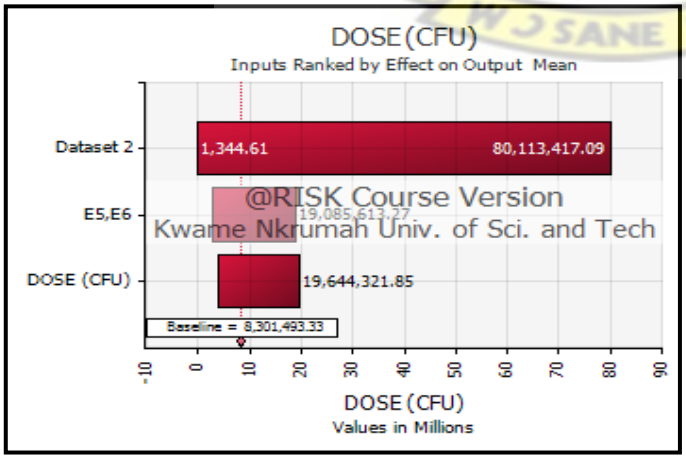
Appendix F1: Dose of *S. aureus*

@RISK Output Report for DOSE (CFU)

Performed By: DOUGBELLA
Date: Tuesday, October 28, 2014 1:07:38 PM







Simulation Summary Information	
Workbook Name	D. Amoah data.xlsx, D. Amoah data 1.xlsx
Number of Simulations	1
Number of Iterations	10000
Number of Inputs	58
Number of Outputs	51
Sampling Type	Latin Hypercube
Simulation Start Time	10/28/2014 12:54
Simulation Duration	00:00:44
Random # Generator	Mersenne Twister
Random Seed	209651587

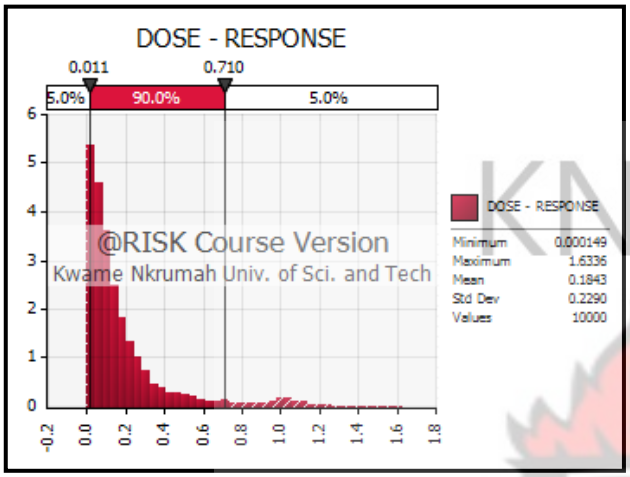
Summary Statistics for DOSE (CFU)			
Statistics		**Percentile**	
Minimum	854.7212691	**5%**	1286.6338
Maximum	10831087369	**10%**	2013.7327
Mean	8301493.334	**15%**	3224.3617
Std Dev	151420602.2	**20%**	5203.9183
Variance	2.29282E+16	**25%**	8292.7925
Skewness	52.01078973	**30%**	12732.251
Kurtosis	3260.983498	**35%**	19284.462
Median	63669.78434	**40%**	28915.711
Mode	1004.966954	**45%**	43613.537
Left X	1286.633792	**50%**	63669.784
Left P	5%	**55%**	95568.977
Right X	12055547.1	**60%**	143392.25
Right P	95%	**65%**	216075.86
Diff X	12054260.47	**70%**	338040.11
Diff P	90%	**75%**	545484.66
#Errors	0	**80%**	928415.98
Filter Min	Off	**85%**	1691823.8
Filter Max	Off	**90%**	3802823.8
#Filtered	0	**95%**	12055547

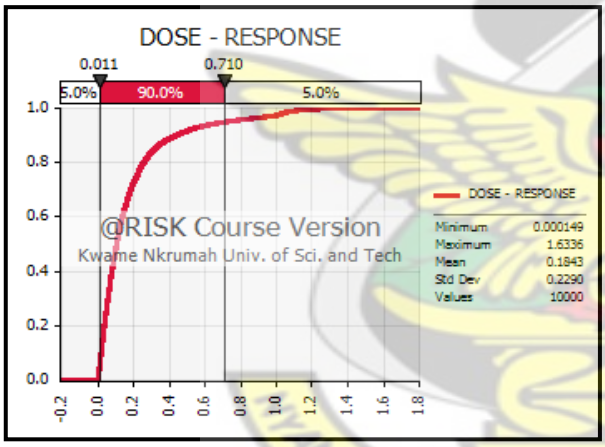
Change in Output Statistic for DOSE (CFU)			
Rank	**Name**	**Lower**	**Upper**
1	Dataset 2	1344.6072	80113417
2	E5,E6	2903051.9	19085613
3	DOSE (CFU)	4059007	19644322

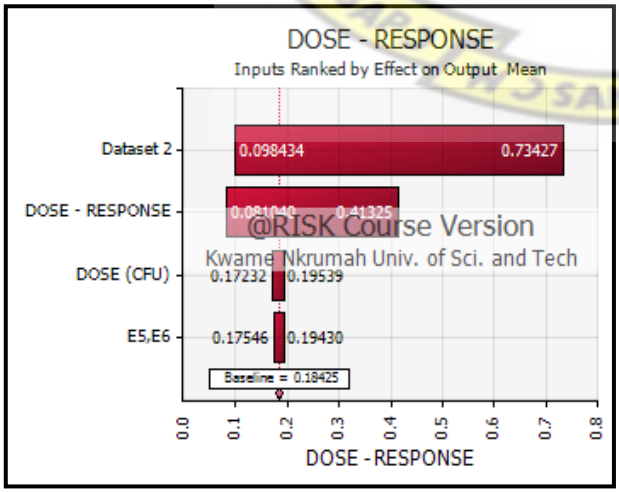
Appendix F2: Probability of infection for *S. aureus*

@RISK Output Report for DOSE - RESPONSE

Performed By: DOUGBELLA
Date: Tuesday, October 28, 2014 1:07:39 PM







Simulation Summary Information	
Workbook Name	D. Amoah data.xlsx, D. Amoah data 1.xlsx
Number of Simulations	1
Number of Iterations	10000
Number of Inputs	58
Number of Outputs	51
Sampling Type	Latin Hypercube
Simulation Start Time	10/28/2014 12:54
Simulation Duration	00:00:44
Random # Generator	Mersenne Twister
Random Seed	209651587

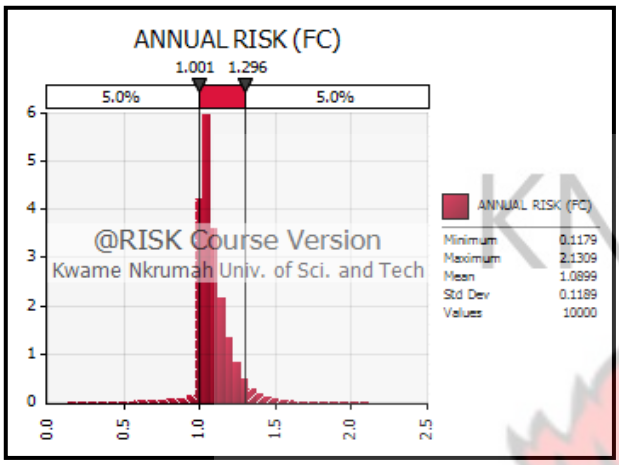
Summary Statistics for DOSE – RESPONSE			
Statistics		**Percentile**	
Minimum	0.00014886	**5%**	0.010686
Maximum	1.633610253	**10%**	0.0198596
Mean	0.184252149	**15%**	0.0281934
Std Dev	0.228965364	**20%**	0.0369226
Variance	0.052425138	**25%**	0.0467786
Skewness	2.495787801	**30%**	0.0574335
Kurtosis	9.582754192	**35%**	0.0684008
Median	0.106275772	**40%**	0.0799371
Mode	0.02590282	**45%**	0.0923424
Left X	0.010685981	**50%**	0.1062758
Left P	5%	**55%**	0.1212152
Right X	0.709618975	**60%**	0.1384862
Right P	95%	**65%**	0.160775
Diff X	0.698932994	**70%**	0.1856147
Diff P	90%	**75%**	0.2172205
#Errors	0	**80%**	0.2585545
Filter Min	Off	**85%**	0.318469
Filter Max	Off	**90%**	0.4379322
#Filtered	0	**95%**	0.709619

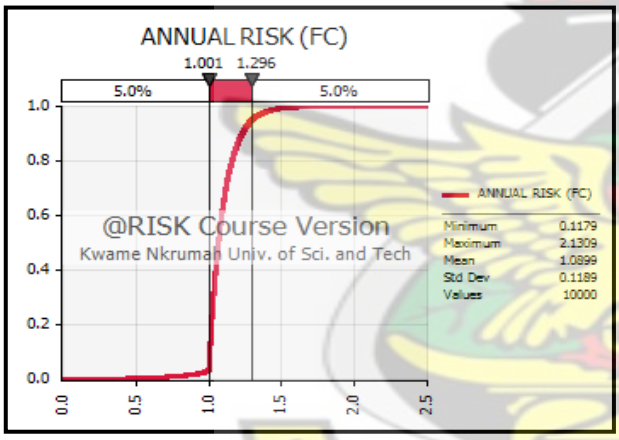
Change in Output Statistic for DOSE – RESPONSE			
Rank	**Name**	**Lower**	**Upper**
1	Dataset 2	0.0984335	0.7342733
2	DOSE - RESPONSE	0.0810403	0.4132475
3	DOSE (CFU)	0.1723218	0.1953886
4	E5,E6	0.1754585	0.1943035

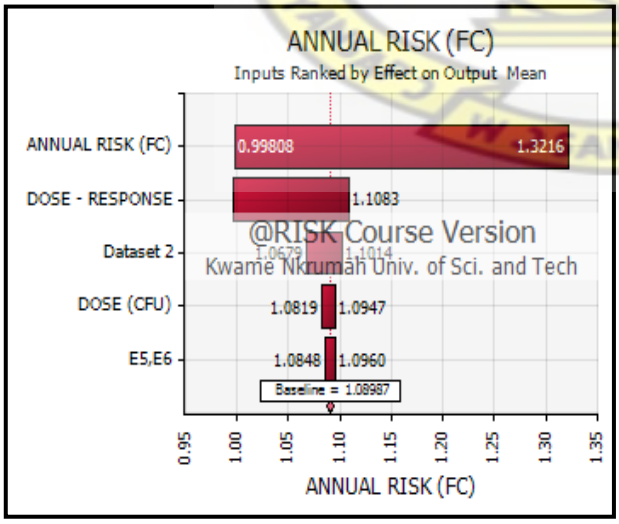
Appendix F3: Annual risk of *S. aureus* infection (frequent consumers)

@RISK Output Report for ANNUAL RISK (FC)

Performed By: DOUGBELLA
Date: Tuesday, October 28, 2014
1:07:40 PM







Simulation Summary Information	
Workbook Name	D. Amoah data.xlsx, D. Amoah data 1.xlsx
Number of Simulations	1
Number of Iterations	10000
Number of Inputs	58
Number of Outputs	51
Sampling Type	Latin Hypercube
Simulation Start Time	10/28/2014 12:54
Simulation Duration	00:00:44
Random # Generator	Mersenne Twister
Random Seed	209651587

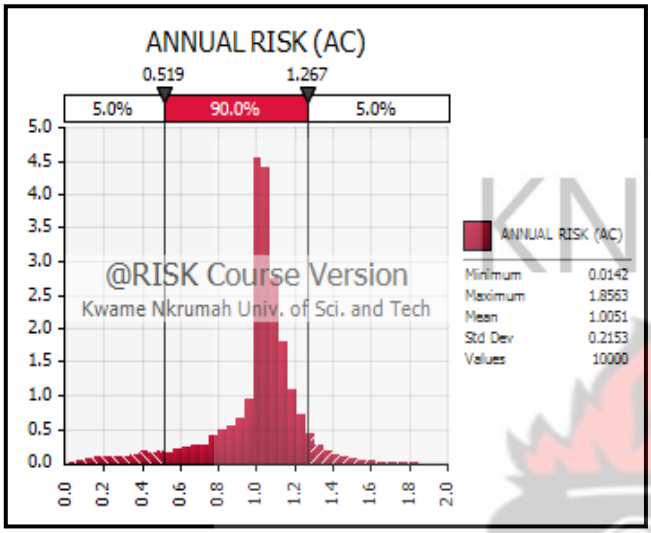
Summary Statistics for ANNUAL RISK (FC)			
Statistics		**Percentile**	
Minimum	0.117939355	**5%**	1.0014811
Maximum	2.130883433	**10%**	1.0068212
Mean	1.089865154	**15%**	1.0125219
Std Dev	0.118917215	**20%**	1.018696
Variance	0.014141304	**25%**	1.0251556
Skewness	-0.169707865	**30%**	1.0318849
Kurtosis	13.83218212	**35%**	1.0393678
Median	1.06569285	**40%**	1.0472874
Mode	1.001977534	**45%**	1.0559425
Left X	1.001481061	**50%**	1.0656929
Left P	5%	**55%**	1.0760826
Right X	1.295941666	**60%**	1.0880649
Right P	95%	**65%**	1.1015538
Diff X	0.294460604	**70%**	1.1171081
Diff P	90%	**75%**	1.1356941
#Errors	0	**80%**	1.1579897
Filter Min	Off	**85%**	1.1866569
Filter Max	Off	**90%**	1.2269459
#Filtered	0	**95%**	1.2959417

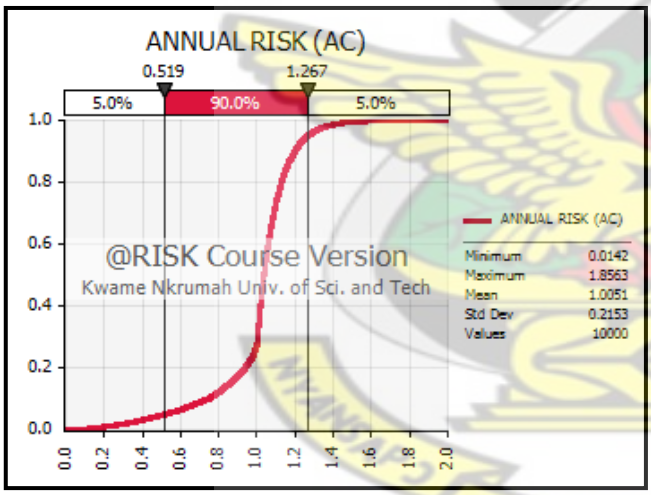
Change in Output Statistic for ANNUAL RISK (FC)			
Rank	**Name**	**Lower**	**Upper**
1	ANNUAL RISK (FC)	0.9980795	1.3216118
2	DOSE - RESPONSE	0.9970662	1.1083024
3	Dataset 2	1.0679374	1.1013517
4	DOSE (CFU)	1.0819023	1.0947384
5	E5,E6	1.0848425	1.0960177

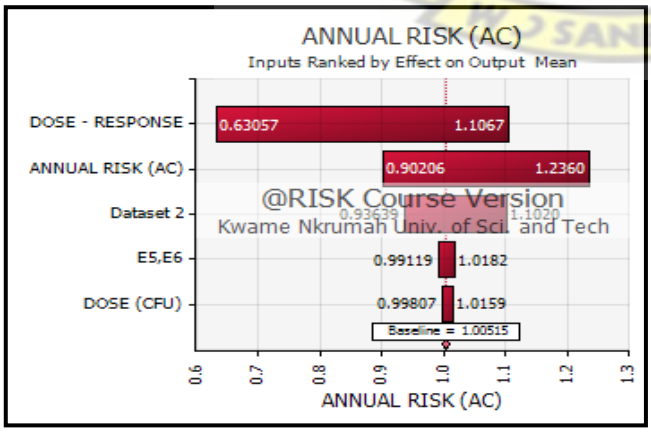
Appendix F4: Annual risk of *S. aureus* infection (average consumers)

@RISK Output Report for ANNUAL RISK (AC)

Performed By: DOUGBELLA
Date: Tuesday, October 28, 2014 1:07:42 PM







Simulation Summary Information	
Workbook Name	D. Amoah data.xlsx, D. Amoah data 1.xlsx
Number of Simulations	1
Number of Iterations	10000
Number of Inputs	58
Number of Outputs	51
Sampling Type	Latin Hypercube
Simulation Start Time	10/28/2014 12:54
Simulation Duration	00:00:44
Random # Generator	Mersenne Twister
Random Seed	209651587

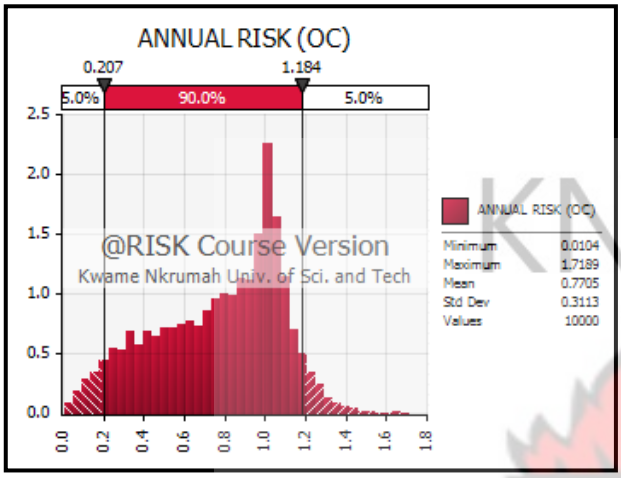
Summary Statistics for ANNUAL RISK (AC)			
Statistics		**Percentile**	
Minimum	0.014191225	**5%**	0.5194883
Maximum	1.856335385	**10%**	0.7459226
Mean	1.005146234	**15%**	0.8613256
Std Dev	0.215273826	**20%**	0.9379641
Variance	0.04634282	**25%**	0.9877813
Skewness	-1.556887781	**30%**	1.0035122
Kurtosis	6.955788972	**35%**	1.0108816
Median	1.037019166	**40%**	1.0192409
Mode	1.010284354	**45%**	1.0274424
Left X	0.519488332	**50%**	1.0370192
Left P	5%	**55%**	1.0472539
Right X	1.267304965	**60%**	1.059289
Right P	95%	**65%**	1.072961
Diff X	0.747816633	**70%**	1.0891461
Diff P	90%	**75%**	1.1071968
#Errors	0	**80%**	1.1290441
Filter Min	Off	**85%**	1.1577862
Filter Max	Off	**90%**	1.1987218
#Filtered	0	**95%**	1.267305

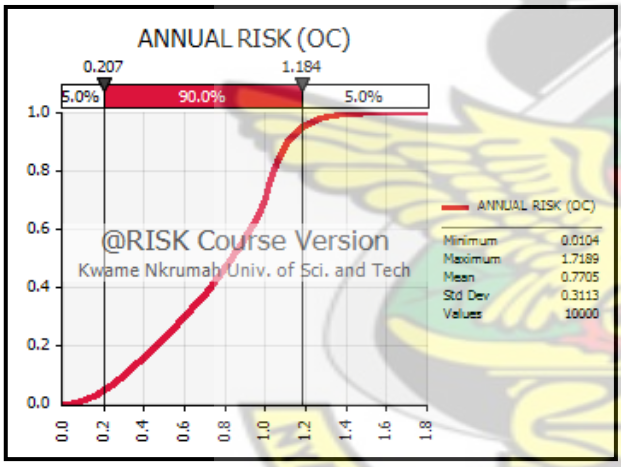
Change in Output Statistic for ANNUAL RISK (AC)			
Rank	**Name**	**Lower**	**Upper**
1	DOSE – RESPONSE	0.6305655	1.1066789
2	ANNUAL RISK (AC)	0.9020572	1.2359767
3	Dataset 2	0.9363939	1.1019813
4	E5,E6	0.9911874	1.0182259
5	DOSE (CFU)	0.9980738	1.0158568

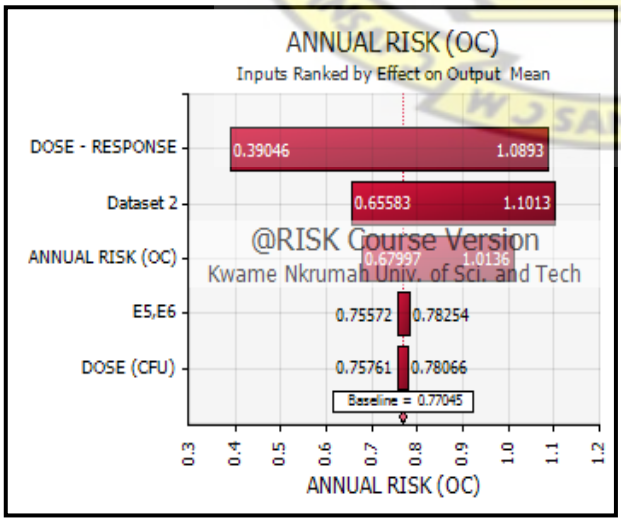
Appendix F5: Annual risk of *S. aureus* infection (occasional consumers)

@RISK Output Report for ANNUAL RISK (OC)

Performed By: DOUGBELLA
Date: Tuesday, October 28, 2014 1:07:43 PM







Simulation Summary Information	
Workbook Name	D. Amoah data.xlsx, D. Amoah data 1.xlsx
Number of Simulations	1
Number of Iterations	10000
Number of Inputs	58
Number of Outputs	51
Sampling Type	Latin Hypercube
Simulation Start Time	10/28/2014 12:54
Simulation Duration	00:00:44
Random # Generator	Mersenne Twister
Random Seed	209651587

Summary Statistics for ANNUAL RISK (OC)			
Statistics		**Percentile**	
Minimum	0.010415548	**5%**	0.2065856
Maximum	1.718895905	**10%**	0.3038079
Mean	0.770453346	**15%**	0.3833364
Std Dev	0.311338775	**20%**	0.4587744
Variance	0.096931833	**25%**	0.5308563
Skewness	-0.404548628	**30%**	0.6002157
Kurtosis	2.291840013	**35%**	0.6655192
Median	0.834098543	**40%**	0.7296499
Mode	1.003330137	**45%**	0.7823673
Left X	0.206585632	**50%**	0.8340985
Left P	5%	**55%**	0.8814439
Right X	1.184335422	**60%**	0.9251467
Right P	95%	**65%**	0.9667225
Diff X	0.97774979	**70%**	0.9975829
Diff P	90%	**75%**	1.0165264
#Errors	0	**80%**	1.0414559
Filter Min	Off	**85%**	1.070255
Filter Max	Off	**90%**	1.1105326
#Filtered	0	**95%**	1.1843354

Change in Output Statistic for ANNUAL RISK (OC)			
Rank	**Name**	**Lower**	**Upper**
1	DOSE - RESPONSE	0.3904599	1.0892908
2	Dataset 2	0.6558312	1.1013388
3	ANNUAL RISK (OC)	0.6799676	1.0135821
4	E5,E6	0.7557238	0.7825427
5	DOSE (CFU)	0.7576138	0.7806565