EVALUATION OF BACTERIOLOGICAL QUALITY OF AIRCRAFT FOOD AT
THE JOMO KENYATTA INTERNATIONAL AIRPORT, NAIROBI, KENYA

BY

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I57/12686/05

A thesis submitted in partial fulfillment of the requirements for the award of the degree of

Master of Public Health of the School of Health Sciences, Kenyatta University.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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To Mum and Dad, for your support, patience, understanding, unconditional love and encouraging me to further my studies.
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DEFINITION OF OPERATIONAL TERMS

Batch: The quantity of product, which has been produced during a defined period of manufacture. A ‘batch’ may actually have been produced by a batch-wise process, or may correspond to a particular time duration during a run of a continuous process.

Blanch: Plunging of foods (particularly vegetables and fruits) firstly into boiling water for a brief period, and then into cold water to stop the cooking process.

Blast chiller: A cooling unit used for fast chilling of cooked food after cooking has been completed and before subsequent storage or handling. The cooling medium is usually very cold air, liquid nitrogen or liquid carbon dioxide.

Bulk meals: Refers to a ready-to-eat food batch not yet portioned out into smaller meals.

Critical control point: A step at which control can be applied and which is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Cold meal: Refers to a meal that will be eaten without heating or warming.

Colour code: Refers to the practice of affixing coloured stickers coded to the day of the week a product is produced or otherwise handled on all freshly prepared or purchased items. Colour coding may be done in accord with industry wide colour codes for the seven days of the week.

Compliance: Measures that satisfy the legal requirement.
Control: A measure, action or activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Cross contamination: The direct or indirect transfer of biological, chemical or physical contaminants from raw food or other sources to other food that may cause them to be unsafe for human consumption.

Documentation: All written production procedures, instructions and records, quality control procedures, and recorded test results involved in the manufacture of a product.

Dry foods: Food that has a low water activity, being less than the minimum growth water activity of micro-organisms of significance for the particular food.

Food safety: Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Food hygiene: Conditions and measures necessary for the production, processing, storage and distribution of food designed to ensure a safe, sound, wholesome product fit for human consumption.

Gastronorm: Food container

Hazard: Any unacceptable microbial, chemical, physical or allergenic contaminant

Hazard Analysis Critical Control Point (HACCP): A system which identifies, evaluates and controls hazards which are significant for food safety.
PRP: Prerequisite Programmes. Fundamental programmes critical to an operation that ensures the basic minimum requirements are in place to establish more elaborate processes such as HACCP. Examples include cleaning and sanitization and pest control.

Hot meal: Refers to a meal that will be heated or warmed before consumption.

Hygiene: All measures necessary to ensure the safety and quality of food at all stages in the food chain.

Risk: Likelihood of an event happening e.g. food contamination.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AEA</td>
<td>Association of European Airlines</td>
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<tr>
<td>AMP</td>
<td>Ampicilin</td>
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<td>APC</td>
<td>Aerobic Plate Count</td>
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<td>AUG</td>
<td>Augmentin</td>
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<td>AZT</td>
<td>Aztreonam</td>
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<tr>
<td>BA</td>
<td>British Airways</td>
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<td>C</td>
<td>Chloramphenical</td>
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<tr>
<td>CBD</td>
<td>Central Business District</td>
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<tr>
<td>CCP</td>
<td>Critical Control Point</td>
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<td>CEFI</td>
<td>Cefixime</td>
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<td>CEFO</td>
<td>Cefotaxime</td>
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<td>CEFT</td>
<td>Ceftazidime</td>
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<td>CN</td>
<td>Gentamicin</td>
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<tr>
<td>CXM</td>
<td>Cefuroxime</td>
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<tr>
<td>GHP</td>
<td>Good hygiene practices</td>
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<td>GMP</td>
<td>Good manufacturing practices</td>
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<td>HACCP</td>
<td>Hazard Analysis Critical Control Point</td>
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<td>HM</td>
<td>Hot meal</td>
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<td>ICMSF</td>
<td>International Commission on Microbiological Specifications for Foods</td>
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<td>IFAP</td>
<td>International Federation of Airline Pilots</td>
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<td>IFCA</td>
<td>International Flight Catering Association</td>
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<td>ISO</td>
<td>International Organization for Standardization</td>
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<td>JKIA</td>
<td>Jomo Kenyatta International Airport</td>
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<td>K</td>
<td>Kanamycin</td>
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<td>Abbreviation</td>
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<tr>
<td>KAA</td>
<td>Kenya Airport Authority</td>
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<td>KEBS</td>
<td>Kenya bureau of standards</td>
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<tr>
<td>KSMEF</td>
<td>Kenyan Standards for Microbiological Enumeration in Foods</td>
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<td>LSG</td>
<td>Lufthansa Service Gesellschaft</td>
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<td>PRP</td>
<td>Prerequisite programme</td>
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<td>RTE</td>
<td>Ready to Eat</td>
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<td>SPECS</td>
<td>Specifications</td>
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<td>SPSS</td>
<td>Statistical Package for Social Scientists</td>
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<td>SSOP</td>
<td>Sanitation standard operating procedures</td>
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<td>SXT</td>
<td>Sulphamethoxazole</td>
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<td>TE</td>
<td>Tetracycline</td>
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<td>WHO</td>
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ABSTRACT

The production of aircraft meals can pose risks of global dimensions. Microbiological hazards are the most prominent risk factors associated with this kind of food production and arise owing to the complexity of the operation in the flight kitchen, long food production chains and onboard services with limited facilities. Food borne diseases constitute a significant cause of reduced economic activity in this sector and are also a growing public health problem worldwide. Regular microbiological testing of food as a part of the quality assurance system of flight kitchen is necessary to ensure the safety of meals. In order to lay a foundation for assessing these high-risk foods, this study sought to evaluate the microbiological quality of meals served on aircraft at the Jomo Kenyatta International Airport (JKIA), Nairobi. This was a descriptive cross sectional study and was expected to provide an insight on bacteria and their diversity occurring in airline food and thus its hygiene condition. Three hundred and sixty one meals were sampled purposively and conveniently and divided equally into four categories of (i) Starter dishes such as hors d’oeuvre, canapés and prawn cocktail-dishes that require a fair amount of handling during preparation and which are served without reheating, (ii) Main courses, mainly meals that are served hot, (iii) cold desserts; and (iv) Snack meals which include sandwiches, vol-au-vents and tartlets. Isolation of microorganisms was carried out in the laboratory, enumerated and data analysis was carried out using SPSS version 11.5. Frequencies and percentages of the variables were calculated and presented in graphs and tabular form. To examine the relationship among and between the variables, cross tabulations and the $\chi^2$ test, Pearson correlation coefficient were used. The antibiotic sensitivity profile of the microorganisms was evaluated against 12 antibiotics to shed light into difficulties that could be encountered if there is an infection by the isolated pathogens. In addition, a questionnaire was administered and structured to contain demographic characteristics, assess food safety knowledge, practices and attitude. Statistical significance was set at $p<0.05$. The results revealed contamination of aircraft food at 85% from all food sampled. Cold meals were more contaminated (68.7%) than hot meals (16.3%). The only pathogenic microorganism isolated was Staphylococcus aureus that accounted for 1.2% of the foods sampled. The bacteria isolated were most sensitive to Kanamycin and Aztreonam both at 81.8%, while they were resistant to Augmentin and Ampicillin at 81.2% and 84.1% respectively. Food handlers exhibited lapses in personal hygiene such as 87.8% of the food handlers admitting not to washing their hands upon entering the food production area. However with the appropriate controls, such contamination does not pose risk to the travelling consumers as they can be arrested before the meals are consumed. The results of the study will enable caterers to put effective quality control systems in place in order to prevent bacterial contamination of food. In addition, authorities such as Ministry of Public Health will find the study important in enforcing regulations such as compelling caterers of international magnitude to implement food safety systems such as Hazard Analysis Critical Control Point (HACCP). The information obtained in this study will also be used to streamline training modules to enhance food safety systems for in-flight food producers.
1.1 Background information

The first regular airline passenger service began in 1919 in Europe, between England and France, and food has been served on aircraft since the onset of this operation (Jones and Kipps, 1995). Initially the service included sandwiches, and beverages such as tea and coffee, but in the mid-1930s hot meals began to be served. Data on outbreaks of aircraft food poisoning is scanty with most incidents never brought out in public. However, few of such incidents have been published because of their magnitude and include 600 passengers falling sick in 1984 due to salmonella contaminated dishes, 176 passengers becoming sick onboard due to contaminated breakfast ham served and this was traced to a staff who had the same strain of staphylococcus in lesion on the finger and in 1992, 75 passengers flying from Peru to Los Angeles contracted cholera with epidemiological investigations linking the cases with the cold salad served on the flight (Erica, 2006).

Food borne diseases indicate that the majority of outbreaks result from faulty food handling practices (Clayton et al., 2002). In an era of frequent travel, safe food handling practices is imperative given the potential for widespread outbreaks of food-borne illnesses (Lynch et al., 2003). Lack of personal hygiene amongst food handlers is one of the most commonly reported practices contributing to food-borne illness while poor hand and surface hygiene is also a significant contributory factor (Cogan et al., 2002). The diseases occur as outbreak emergencies which often present a management dilemma because of the limited medical resources available on board (Godil and Godil, 1997). In addition, certain problems specific to air travel complicate the recognition and investigation of outbreaks caused by meals served on aircraft.
For example, if a causative agent has a longer incubation period than the flight takes, passengers become ill after disembarkation. This makes it difficult in such cases to recognize a cluster of food borne illness among travelers from many different countries and to trace the origin of the outbreak. However, very little data is available particularly in Kenya regarding the occurrence of pathogenic bacteria on food served in aircrafts.

It is important to identify the hazards associated with aircraft meals and to develop efficient control methods. Regular microbiological testing of food as a part of quality assurance system of flight kitchen is necessary to ensure the safety of meals. Hazard Analysis Critical Control Point (HACCP) is widely recognized in the food industry as a preventive system for managing food safety (Pierson and Corlett, 1992). The HACCP system identifies critical control points in food production process that are essential to monitor and control product’s safety. HACCPs preventive focus is more effective than testing a product and then destroying or reworking it (ICMSF, 1998). It is also an established safety management system in civil aviation (Hatakka, 2000) and many airline catering companies use the global quality policy described by LSG-Hygiene Institute, (1997). The overall cost of food borne illness include the cost of medical treatment, productivity loss, pain and suffering of affected people and losses such as expensive medical treatment, exacerbated conditions in patients seeking medical attention abroad within the public health sector (Harris, 1997).

1.2 Statement of the problem

In today’s world, airplane travelling has increased due to the nature of people’s lifestyle and work requirement. It is preferred due to safety and speed. The reason for travelling varies and in such aircrafts you will find variety of passengers some of whom are immune compromised such as the elderly, infants, and sick people travelling to seek medical treatment in other countries. The normal procedure with most air travel is that people are fed on board and this food has to be hygienically produced and be of high microbiological
standard. If sick travelers are fed with food that is contaminated, then their situation becomes exacerbated and the scenario could get worse if the affected personnel are crew members and worse still cockpit crew. Such emergencies present a dilemma because of the limited medical resources on board (Godil and Godil, 1997). Outbreaks resulting from exposures during air travel are particularly difficult to separate from illnesses attributable to preflight exposure (Al-Abri et al., 2005). Moreover, in most instances the incubation period after an in-flight exposure exceeds the flight time, so that illness occurs after passengers have dispersed. If their destinations are in different public health jurisdictions, identification of an epidemiological link between cases is especially challenging (Olsen et al., 2003). A study carried out by the International Federation of Airline Pilots Association (IFAPA) in 1991 showed that gastrointestinal distress accounts for 58.4% of serious incidents onboard while approximately 9000 air passengers and crew members are reported to suffer from aircraft food poisoning annually with an average of 11 reported deaths (Hatakka, 2000). When a food poisoning incident is brought to the caterers’ attention, most caterers normally shift blame to what the passenger could have eaten before absolving themselves from any blame (Hatakka, 2000). The problem therefore remains unsolved and recurrence is a common feature. This study, investigated the microbiological quality of aircraft bound meals and the possible points of contamination along the production chain. The data gives insight on the possible contamination areas that could be investigated in case of food poisoning.

1.3 Justification

Travelling by air has increased in today’s world and such travel comes with its own challenges. Among these challenges is the food that is served on board being loaded long before the departure time. This challenge is further exacerbated by the close food packing and stacking arrangement in the airplane kitchen combined with the low humidity in the pressurized cabin that could aid the multiplication of bacteria (Wilson, 2003).
Microbiological hazards are the most prominent risk factors associated with airline food production (Hatakka, 2000). Furthermore, due to limited food borne disease investigations and surveillance in most countries including Kenya, most outbreaks go undetected (Ombui et al., 2001; Kelly et al., 2001). JKIA is an international airport serving over 20 international airlines that source their food from one point. JKIA is therefore a good representative sample for conducting such a study. The source of food is also representative enough to enable this research address the problem being investigated which was to establish the bacteriological quality of this food right from delivery to the final dispatch and enumerate the necessary controls that fail along the food chain. Appendix 2 is a flow diagram showing various stages of the food production chain at JKIA.

1.4 Significance of results

Data from this study will be of use to hygiene officers, food handlers and airport health officers in improving and strengthening hygienic production of aircraft meals to avoid bacterial food contamination. The study will also provide basis for knowledge on food poisoning bacteria valuable to tourist hotels and other food establishments that are involved in mass food production. In addition, the results will enable authorities in the Ministry of Public Health facilitate the implementation of sanitation programmes that will be beneficial to caterers and reduce possibilities of food contamination.

1.5 Research questions

i. What are the bacterial species occurring in aircraft food at JKIA?

ii. What is the bacteriological quality of hot and cold meals?

iii. What are the food safety controls that fail in the food chain?

iv. What is the antibiotic sensitivity profile of bacterial pathogens occurring in food served in aircrafts at JKIA?
1.6 Objectives

1.6.1 General objective

To evaluate the microbiological quality of meals served on aircraft at the Jomo Kenyatta International Airport (JKIA), Nairobi.

1.6.2 Specific objectives

i. To identify the bacterial species occurring in aircraft food at JKIA.

ii. To examine the bacteriological quality of hot and cold meals.

iii. To identify the food safety controls that fail in the food chain.

iv. To determine the antibiotic sensitivity profiles of the isolated bacterial pathogens in aircraft meals served at JKIA.

1.7 Null hypothesis

H₀: Meals served in aircrafts operating at the Jomo Kenyatta International Airport, Nairobi have no bacterial contamination.

1.8 Limitations and delimitations

Limitation: Food samples were picked at different stages from receiving through production process to the dispatch. Food was not sampled in the aircraft because of the logistics involved such as lengthy clearance from the airline management, various security regulators, and also the fact that such food may have become contaminated after delivery to the aircraft.

Delimitations: The food was sampled in all potential areas of contamination along the production line. In addition the food handlers were assessed in their practices, attitude and food safety knowledge as this has an effect on food contamination.
1.9 Conceptual framework

A framework to find out the key factors that affect the food quality is explained as follows: Raw materials would affect the quality of final meal if it has an excess of microbial load. In addition if the food safety systems have pitfalls, then microbes will be able to find their way into the final meal.

The effectiveness of the system is ensured through constant monitoring while detection of the microbes either in the final meal or the raw materials can only be determined through elaborate microbiological analysis. The type of laboratory analysis whether convectional or molecular also has an effect in establishing the quality of the final meal. A framework showing the above interactions is shown in Figure 1.

![Conceptual model](image)

Figure 1. Conceptual model. Modified from (Luning et al., 2002)
CHAPTER TWO: LITERATURE REVIEW

2.1 Air travel and In-flight catering

The advent of the jet aircraft in passenger services in the mid-1960s contributed to the growth of mass tourism. In 1950, there were 25 million international tourist arrivals, in 1960, 69 million, in 1970, 160 million and in the 1990s, 400 to 600 million tourist arrivals recorded worldwide yearly (Jones and Kipps 1995, World Tourism Organization 2000). This huge increase in air traffic has created a need for a certain type of mass catering. The scope can vary from a small kitchen to a large catering establishment producing up to 40,000 meals per day (Kirk, 1995), including provisions for long-haul flights and handling the detailed specifications for many different airlines. A large flight kitchen may have contracts with tens of airlines. The way food is prepared today in large units resembles processing in a food manufacturing plant rather than a catering kitchen.

It is important to identify the hazards associated with aircraft meals and to develop efficient control methods. Regular microbiological testing of food as a part of the quality assurance system of flight kitchen is necessary to ensure the safety of meals. Controlling the health status of food handling staff and training in food hygiene field is of great importance.

2.2 Flight kitchen operations

Flight kitchen production is a typical form of mass catering, but has some unique features distinct from food preparation in restaurants and hotels. The time difference between food production in the flight kitchen and the final serving on board an aircraft with limited kitchen facilities makes flight catering a high-risk food preparation operation. The complexity of the production procedures in the flight kitchen also increases the microbiological hazards associated with this type of food preparation (Hatakka, 2000).
Major factors affecting the hygienic quality of the food are the size of the operation, the complexity of the in-flight service, the number of airlines catered for, the number of flights serviced during the day and the duration of the flights to be serviced (Hatakka, 2000).

2.3 Food handling on aircraft

Food storage and preparation for serving takes place in aircraft galleys, which mostly have limited space and equipment for this purpose. In common with any kitchen, a galley has to provide the following: cold storage areas, regeneration ovens, water boilers and beverage machines and the stowage of waste products. Chilled and frozen meals served hot must be re-heated, so that a core temperature of at least 72°C is reached to destroy any surviving pathogenic micro-organisms (LSG-Hygiene Institute, 1997). In the 1970s, hot meal trays were transported to aircraft in hot ovens for short-haul flights and kept there until serving, the temperature of food being over 63°C (Bailey, 1977). Currently, a cook-chill system is mostly used, although foods to be served hot can still be transported hot to small aircraft if they are not equipped with ovens (LSG-Hygiene Institute, 1997).

2.4 Epidemiology of food borne diseases

Food borne outbreaks traced to meals served on aircraft are most probably under-reported for several reasons. The incubation period is often longer than the flight time, and passengers are unaware of each other’s illness. Therefore recognizing a cluster of food borne illness becomes difficult. When an outbreak is identified, it portrays a negative picture with great financial losses both to the airline and the catering unit (Pakkala, 1989). Therefore airline companies, just as any other companies providing food service, do not like publishing any data on food borne outbreaks. These authorities need to recognize outbreaks associated with aircraft meals. In order to prevent dissemination or recurrence of outbreaks and of health hazards, rapid international exchange of information is also needed (Hatakka, 2000).
2.5 Causes and incidents associated with food poisoning on aircrafts

A Salmonella outbreak was reported in 1986 affecting a total of 226 people, charter-flight passengers from Helsinki to Rhodes, Greece. The outbreak was caused by *Salmonella enterica*, via egg sandwiches and meals served on the flight and also cold cuts eaten by the flight kitchen catering staff (LSG Hygiene Institute, 1997). In 1988 a food borne outbreak by *Shigellosis* occurred in a commercial airline in the US (Hedberg *et al.*, 1989). Confirmed or probable *Shigellosis* was identified among 240 passengers on 219 flights to 24 states, the District of Columbia, and four countries in September and October of 1996. The outbreak was associated with the strain *Shigella sonnei*, which was isolated from airline passengers, and flight attendants.

According to Association of European Airlines (2006), traces of fatal *Escherichia coli* bacteria were found in meals intended for British Airways. Gate gourmet the airline food company cited 8 meals between August 2003 and August 2004 that were infected with food-poisoning bacteria. *E. coli* was reported in a lemon-chicken salad and a prawn with lemon herbs meal in August 2003, in honey-glazed chicken and mustard mayonnaise bloomer in October 2003. The bacterium was also reported in four other unnamed sandwiches in November 2003 and in a pesto butter steak fillet in March 2004 (Denise *et al.*, 2006). In 1975, 196 (57%) of 344 passengers and 1 steward aboard a commercial aircraft Boeing 747 from Tokyo to Paris contracted a gastrointestinal illness characterized by nausea, vomiting, abdominal cramps, and diarrhoea; 142 passengers and the steward were admitted in hospital (Barbara, 1989). Symptoms developed shortly after a ham and omelette breakfast had been served. An investigation strongly incriminated ham as the vehicle of the outbreak, and the source seemed to have been a cook who had lesions on his fingers. The attack-rate was 86% for passengers who ate the ham handled by this cook and 0% for passengers who ate ham handled exclusively by other food preparer.
Before being served, the ham and omelette had been held at room temperature for 14 h and at 10 degrees C (50 degrees F) for 14 ½ hours. Specimens of stool and vomitus from ill passengers, left-over food, and the finger lesions of the cook were positive for \textit{Staphylococcus aureus} of identical phage types and antibiotic sensitivities. Preformed enterotoxin was detected in the left-over ham and omelette. In the spring of 1984, British Airways was involved in a major food poisoning outbreak which affected nearly 1000 passengers, aircrew and ground personnel. The operational impact was worldwide and could have resulted in the cessation of the airline's day-to-day operations (Carole et al., 1990). In another outbreak 47 airline passengers suffered from illness associated with eating garden salad made from iceberg lettuce and shredded carrots (Beuchat, 1996).

On a flight from Lima, Peru to Los Angeles in 1992, 75 passengers had a staphylococcus food poisoning and an emergency landing had to be done (Eberhart-phillips et al., 1996). In another case a flight to Canary Island had food poisoning that affected 455 passengers on different aircraft but the source was from one caterer (Hatakka, 2000). Yet in another incidence, British Airways had a food poisoning case that affected 1000 passengers, crew and ground personnel (Carole et al, 1990). The food poisoning was linked to \textit{Salmonella enteriditis}.

In August 2004, an outbreak of \textit{Shigella sonnei} infection affected air travellers who departed from Hawaii. Forty-seven passengers with culture-confirmed \textit{Shigellosis} and 116 probable cases who travelled on 12 flights dispersed to Japan, Australia, 22 US states, and American Samoa. All flights were served by one caterer. Food histories and menu reviews identified raw carrot served onboard as the likely vehicle of infection. Attack rates for diarrhoea on three surveyed flights with confirmed cases were 54% (110/204), 32% (20/63), and 12% (8/67). A total of 2700 meals were served on flights with confirmed cases; using attack rates observed on surveyed flights, we estimated that 300–1500 passengers were infected (Gaynor et al, 2007).
2.6 Food poisoning

Food poisoning is an acute illness which usually occurs within 1 to 36 hours of eating contaminated or poisonous food. Symptoms normally last from one to seven days and often include diarrhea, vomiting, abdominal pain, nausea, fever and prostration. Bacteria related food poisoning is the most common, but fewer than 20 of the many thousands of different bacteria are concerned. More than 90 percent of cases of food poisoning each year are caused by the bacteria; *Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus*, and entero-pathogenic *Escherichia coli*, (ICMSF, 1998). These bacteria are commonly found on many raw foods. Therefore, illness can be prevented by (1) controlling the initial number of bacteria present, (2) preventing the small number from multiplying, (3) destroying the bacteria by proper cooking and (4) avoiding re-contamination (ICMSF, 1998).

Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods (Hatakka, 2000). Mishandling of raw and cooked foods allows bacteria to grow. The temperature range in which most bacteria grow is between 5°C and 60°C. It is recommended that raw and cooked foods should not be kept at this danger temperature zone any longer than is necessary. Undercooking or improper processing of home-canned foods also cause very serious food poisoning (ICMSF, 1998).

2.7 Bacterial pathogens associated with food poisoning

2.7.1 *Staphylococcus aureus*

Man's respiratory passages, skin and superficial wounds are common sources of *Staphylococcus aureus*. When *Staphylococcus aureus* is allowed to grow in foods, it can produce a toxin that causes illness. The toxin produced is heat stable and is not destroyed
during cooking although cooking destroys the bacteria. *Staphylococcal* food poisoning occurs most often in foods that require hand preparation, such as potato salad, ham salad and sandwich spreads. When these types of foods are left at room temperature for prolonged periods of time, bacteria grow and produce toxin. Good personal hygiene while handling foods helps to keep *Staphylococcus aureus* out of foods, and refrigeration of raw and cooked foods prevents the growth of these bacteria if any are present (Hatakka, 2000). Symptoms include Nausea, vomiting, abdominal pain, which appear 1-6 hours after ingestion (Dewell *et al.*, 1999).

### 2.7.2 *Salmonella typhimurium*

The gastrointestinal tracts of animals and human are common sources of *Salmonella typhimurium*. High protein foods such as meat, poultry, fish and eggs are most commonly associated with Salmonella. Any food that becomes contaminated and is then kept at improper temperatures can cause Salmonellosis. Salmonella are destroyed at cooking temperatures above 70° C. The major causes of Salmonellosis are insufficient cooking temperatures and contamination after cooking foods (Hatakka, 2000). Contamination of cooked foods occurs from contact with surfaces or utensils that were not properly washed after use with raw products. If *Salmonella typhimurium* is present on raw or cooked foods, multiplication can be controlled by refrigeration below 5° C. The symptoms range from mild diarrhea to severe pain. The symptoms can occur 12 hours to 3 days after ingestion of the infected food (Dewell *et al.*, 1999).

### 2.7.3 Enteropathogenic *Escherichia coli*

Enteropathogenic *E. coli* is a significant cause of diarrhea in developing countries and localities of poor sanitation (Alterkruse *et al.*, 1997). In the U.S.A it has been associated with "travelers' diarrhea." There are at least four subgroups of enteropathogenic *E. coli*: enterotoxigenic, enterinvasive, enterohemorrhagic and enteropathogenic. Each strain has
different characteristics of clinical implications of poisoning. The major source of the bacteria in the environment is mostly the feces of infected humans, but there may also be animal reservoirs mostly the gastrointestinal of most animals. Feces and untreated water are the likely sources for contamination of food (Alterkruse et al., 1997).

Some common carriers of this pathogen are unpasteurized milk and undercooked meat. *E. coli* is more likely to contaminate ground beef than steaks or other cuts of meat because bacteria on the surface can end up inside the patty when the meat is ground. Current research reveals that unpasteurized apple ciders can also harbor *E. coli* (Dewell et al., 1999). Symptoms include watery diarrhea within 1-8 days of exposure, which progresses to bloody diarrhea. Nausea, vomiting, and fever also occur as the infection progresses. *E. coli* infections can lead to kidney damage and can be life-threatening in children (Dewell et al., 1999).

### 2.7.4 *Listeria monocytogens*

Due to its widespread distribution in the environment, ability to survive for long periods of time under adverse conditions, ability to grow at refrigeration temperatures, *Listeria* is recognized as an important food-borne pathogen (Kelly et al., 2001). Immuno-compromised humans such as pregnant women or the elderly are highly susceptible to virulent *Listeria*. *Listeria monocytogenes* is the most consistently isolated pathogenic species causing Listeriosis (Kelly et al., 2001). In humans, ingestion of the bacteria is marked by a flu-like illness or symptoms usually mild and go unnoticed which leads to development of a carrier state. It is commonly found in unwashed fruits and vegetables, soil, and water (Dewell et al., 1999). *Listeria monocytogenes* causes severe diarrhea, flu-like symptoms, and even encephalitis and meningitis (Dewell et al., 1999). Death is rare in healthy adults; but in immuno-compromised adults and infants the mortality rate may reach 30 percent. *Listeria monocytogenes* grows in a pH range of 5.0 - 9.5 in growth medium. The
organism has survived the pH 5 environment of cottage cheese and ripening cheddar (Wilson, 2003). It is salt tolerant surviving concentrations as high as 30.5 percent for 100 days at 4.2°C, but for 5 days if held at 37°C (Kelly et al., 2001). It is noted that refrigeration temperatures do not stop *Listeria monocytogenes* growth but is capable of doubling in numbers every 1.5 days at 5°C. This is due to the fact that the low temperature hinders the lag phase of their growth, thus product rotation rule; First in First out (FIFO) is important in prevention of *Listeria monocytogenes* food poisoning (Hatakka, 2000). Heat greater than 74°C inactivates *Listeria* organisms from post-process contamination and environmental sources. This is a critical control point for many foods (Kelly, 2004). The pathogen is extremely dangerous to pregnant women because it can harm the unborn fetus.

### 2.7.5 *Vibrio parahaemolyticus* and *Vibrio cholerae*

*Vibrio parahaemolyticus* is found on sea foods, and requires the salt environment of sea water for growth. It is sensitive to cold and heat. Proper storage of perishable sea foods below 5°C, and subsequent cooking and holding above 63°C, is sufficient to destroy all the *Vibrio parahaemolyticus* on sea foods (Alterkruse et al., 1997). Food poisoning caused by this bacterium is as a result of insufficient cooking and / or contamination of the cooked product by a raw product, followed by improper storage temperature. *Vibrio cholerae* on the other hand is usually water borne and spreads rapidly; it can be food borne but built-in safeguards for sewage and water control normally prevent widespread outbreaks from these sources.

### 2.7.6 *Campylobacter jejuni*

*Campylobacter jejuni* is the most common cause of diarrhea and abdominal cramps. Other symptoms include fever, chills, and headaches. Symptoms start within 2-11 hours of exposure and can last 7-14 days (Dewell et al., 1999). *Campylobacter* can lead to the life-
threatening Gullian-Barre syndrome (Nachamkin et al., 1999). Unpasteurized milk, contaminated water, and poultry are common carriers of this pathogen.

2.7.7 *Clostridium botulinum*

*Clostridium botulinum* is a rare, anaerobic bacterium that produces a toxin that is unusually heat resistant. Symptoms occur within 4-36 hours after ingestion of the harmful toxin and include weakness, double vision, fatigue and diarrhea as a clear sign. The *Clostridium botulinum* toxin impairs the central nervous system and can be fatal if not treated properly in 3-10 days (Dewell et al., 1999). Although this type of severe food poisoning is rare, the mortality rate is high (Solomon and Lily, 2001). Sources of *Clostridium botulinum* include soil, water, and home-canned vegetables (Dewell et al., 1999).

2.7.8 *Clostridium perfringens*

Illness attributed to *Clostridium perfringens* is caused by an anaerobic toxin that is found on the surfaces of meat and poultry; however, it is not as serious as *Clostridium botulinum*. It is often called the “cafeteria bug” because the usual sources include food that is improperly cooked or reheated, cooled slowly, or not kept at the correct temperature, such as when food is left out on the cafeteria line (Dewell et al., 1999). Symptoms occur within 8-15 hours after ingestion and include intense abdominal pain, gas, with diarrhea as a clear sign (Rhodehamel and Harmon, 1998).

2.7.9 **Food borne viral diseases**

Food-borne viral infections are also responsible for food associated illnesses in humans. Viruses are very different from the bacteria and parasites, in the way they cause food borne illnesses (Schlundt, 2001). They are transmitted to humans via foods as a result of direct or
indirect contamination of the foods with human feces (Cliver, 1997). Some common food-borne viral infections are caused by the Norovirus, Rotavirus, and Hepatitis A (Koopmans et al., 2002).

Noroviruses are a group of related viruses that cause acute gastroenteritis in humans. Norovirus was recently approved as the official name for a group of viruses described as “Norwalk-like viruses.” Noroviruses are very contagious and can spread easily from person to person. Symptoms include nausea, vomiting, diarrhea, and stomach cramping. The illness begins suddenly, but is usually brief. Rotavirus is characterized by vomiting and fever with watery diarrhea and abdominal pain for 3-8 days. Hepatitis A usually causes a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia, abdominal discomfort and jaundice. The hepatitis A virus is found in the feces of infected people and is transmitted when susceptible individuals consume contaminated water or food. Water, shellfish, and salads are the most frequent sources. Also, contamination of foods by infected workers in flight kitchen is can occur. Hepatitis A vaccine offers the best protection against the viral infection (USDA, 1992).

Recently emerged food-borne pathogens include Vibrio vulnificus, Cryptosporidium parvum, and Cyclospora cayetanensis. These pathogens are newly described or newly associated with food-borne diseases (Tauxe, 1997). Some aspects of these food borne viral infections are presented in Table 1.
Table 1. Food-borne viral pathogens; source and symptoms (source USDA, 2002)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frequent sources</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>Shellfish, plankton, Finfish</td>
<td>Gastroenteritis, septic shock; can result in death</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Contaminated water and Soil</td>
<td>Diarrhea, stomach cramps, slight fever</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>Contaminated water and soil, fresh fruit, leafy vegetables</td>
<td>Watery diarrhea, loss of appetite, nausea, vomiting, muscle aches, fever, and fatigue</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>Raw oysters plankton, shellfish, and finfish</td>
<td>Septic shock</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Intestine of humans and animals</td>
<td>Diarrhea, stomach cramps and slight fever,</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>Food or water that has been contaminated by infected stool. Fresh produce</td>
<td>Watery diarrhea, loss of appetite, nausea, vomiting, muscle aches, fever, and fatigue</td>
</tr>
</tbody>
</table>

2.8 Antimicrobial sensitivity

The use of antibiotic(s) after the intake of the organism(s) may not be effective as the organisms may be susceptible or resistant to it. Resistance to antibiotics in food borne pathogens may create problems for disease or illness treatment while antibiotic susceptibility leads to healing of the illness which the organism(s) caused. Traveler’s diarrhea is a major inconvenience to visitors arriving in developing Countries from more industrialized areas (Dupont et al., 1982). Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe
bacterial infections. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades (Chui et al., 2002; Davis et al., 1999).

In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of antibiotic resistant foodborne microbes. Also, Enterococci are common components of the micro-floral in soil, on plants and in water. These organisms are particularly challenging to eliminate because of their ability to adapt to environmental stresses. Thus, it is not surprising that antimicrobial resistant variants of Enterococci have been found within probiotic formulations (Giraffa, 2002). More so, in the clinical environment, Enterococci can persist for long periods of time on surfaces and can readily be transferred among patient population (O’Connell and Humphreys, 2000).

2.9 Microbiological control of hygiene in the flight kitchen

Microbiological testing is needed within a HACCP programme for hazard identification, monitoring Critical Control Points (CCPs) and verification of the HACCP. The microbiological control includes testing of the whole production chain; with samples taken from food at reception, prepared food items, process lines and surfaces, water, ice cubes, food handlers and finally ready meals (Hatakka, 2000). Purchasing food items for the flight kitchen should be done with caution to avoid microbiological hazards linked with raw materials. In addition, microbiological testing on ready to eat meals is needed to prove that the legal requirements as well as the customers‘ specifications are met. Some flight catering companies take daily traceable counter samples from final meals representing each production batch. These are kept for up to three weeks in a freezer (LSG Hygiene Institute, 1997). In case of complaint, the respective frozen samples are tested. In order to assure the safety of meals purchased, airline companies use random sampling from final meals.
according to a test schedule. These samples are mostly collected on board and stored for reference.

2.10 Screening of food handling staff

In Kenya, food handlers are screened twice a year (Laws of Kenya, CAP 254, 1992). This must be carried out in a government medical institution or by a medical officer of health. A health certificate and health record of each food handler is kept at the food plant. Besides, no person is supposed to collect, prepare, manufacture, keep or transmit or expose for sale any foodstuffs without taking adequate measures to guard against or prevent infection or contamination thereof (Laws of Kenya, CAP 242, 1986). Many airline companies have imposed stricter rules than the legal requirements. A *Salmonella* test from flight kitchen employees after traveling abroad, although not legally required, is demanded by many airlines. Frequent microbiological tests on hands are done to control hand hygiene (Hatakka, 2000).

2.11 Hazard analysis critical control point (HACCP) Concept.

The concept hazard analysis critical control point (HACCP) is a systematic approach to the identification and assessment of food safety hazards and defining means of their control. As a management tool, HACCP provides for a structural approach to identifiable hazards that directly affect safety of food. The system focuses on prevention at every step of the production line rather than detection of unsafe food products at the end of production. It provides an efficient right – first – time approach to food processing, thereby reducing end product monitoring including microbiological testing (Codex, 1993). It is not only cost effective, but also a powerful system, which assures food safety while increasing competitiveness at the same time. Flight caterers must demonstrate their HACCP system by documenting the relevant system elements according to codex Alimentarius 1997 principles (Codex, 1993). Although there are more than 250 types of food-borne diseases, most can be
prevented if certain precautions are taken. Use of good personal hygiene, cooking foods thoroughly, and keeping foods at the correct temperatures during serving and storage are rules that should be followed. Everyone is at risk for food-borne illness, but there are certain individuals who are at greater risk than others. Pregnant women, children, the elderly, and those with compromised immune systems are at an increased risk to illnesses associated with food (Carole et al., 1990). The main idea behind HACCP is that it is possible to identify potential hazards and faulty practices at an early stage in food production, processing or preparation and storage before consumption. These hazards can then be controlled in order to prevent or minimize risk to health of the consumer or economic loss from food spoilage.

HACCP involves the identification of hazards associated with any stage of food production, processing or preparation, the assessment of related risks, and the determination of steps where control is critical to achieving safety (National Advisory Committee on Microbiological Criteria for Foods NACMCF, 1992). HACCP system is important for maintaining food safety in food businesses, yet it seems that HACCP system is not implemented widely in airline catering establishments.

Many food establishments in Kenya have implemented HACCP although they face challenge on its maintenance. (Ombui et al., 2001). As such, gaps develop and the likelihood of food contamination increases. Further, prerequisite programs, defined as those procedures that address operational conditions providing the foundation for the HACCP system may not be in place. There have been no documented studies examining the extent to which food safety prerequisite programs are implemented in airline catering business. However, prior to effectively implementing HACCP, catering establishments should already have in place various practices including ingredient and product specifications, staff training, cleaning and disinfectant regimes, hygienically designed facilities and be engaged in good hygienic practices (GHP) (WHO, 1993). However, HACCP programmes cannot
guarantee that all foods will be safe especially because it is a system that is run by the food handlers themselves and any small negligence of the food handler could result to a food safety lapse. HACCP programmes must include a written document that describes how food safety concern will be controlled in a specific process at a specific location. The specific process and specific location must comply with established GMPs and SSOPs as HACCP foundation programmes before the HACCP programme can be developed and implemented (FDA, 2000; Schmidt and Rodrick, 2003).

The development of HACCP systems in food establishments can be made simple and less time consuming by spreading the entire procedure over a period of time. A caterer for instance, can start with auditing of suppliers and establishment of control and monitoring procedures for receipt of products. This may only take a fortnightly meeting of the catering manager, chefs and other food handlers for a given period of time. In addition, this would help to ensure that one step is functioning effectively before the next is embarked upon (Eheri et al., 1997). The study done by Walker and Jones (2002) claimed that poor implementation of prerequisites for food safety caused sicknesses in this food business and they suggested that the establishment of PRP could provide a solid foundation to develop HACCP.

2.12 Pre-requisite programmes

These are programmes such as good manufacturing practices and good hygiene practices that must be working effectively within a commodity system before HACCP is applied. If these pre-requisite programmes are not functioning effectively then the introduction of HACCP will be complicated, resulting in a cumbersome, over-documented system. These include the following; training, premises and equipment, maintenance, cleaning and sanitation, waste management, product recall/withdrawal and traceability and others as identified during the risk assessment (Codex, 1993). PRP includes various practices in
kitchen, such as ingredient and product specifications, staff training, cleaning and sanitation procedures, hygienically designed facilities, proper storage of items and pest control (Walker et al., 2003). Hazards that have little or no risk, or unlikely to occur, can often be monitored and controlled by standard operation procedures (SOPs; routine employee hygiene practices, cleaning procedures) and good manufacture practices (GMP) and need not necessarily be critical control points addressed by the HACCP system (Mc Swane et al., 2003).

2.13 Industry’s Regulatory specifications

The AEA has issued recommendations for microbiological analyses and limits for aircraft food as is indicated in Table 2. Bulk items, such as hot meats, which have been portioned after heat treatment should not exceed the value of 5.0 x 105 cfu/g for total count and 1.0 x 103 cfu/g for coliforms. For items that have been handled (e.g. slicing, cutting) after heat treatment, small values of less than 10 total count and coliforms are permitted. Although the results of the total count and coliforms can be higher than the limit values, the food is not considered to be unsafe, but according to the AEA (1996) an investigation of food production practice is advised. If the AEA limits for Escherichia coli, S. aureus, Bacillus cereus, Clostridium perfringens and Salmonella spp. are exceeded, the food must be considered to be unsafe. Monitoring meals for indicators may reveal food processing or food handling errors but it is not advisable or valid to predict the safety of food based on these indicators alone (Tompkin, 1983; Sofos et al., 1999). In Kenya, the standards regulatory body Kenya Bureau of Standards (KEBS) is mandated in establishing these limits although local based airline caterers opt to go for stringent international standards.
Table 2. AEA food microbiological limits

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Total counts cfu/g</th>
<th>Coliforms cfu/g</th>
<th>E.coli cfu/g</th>
<th>S.aureus cfu/g</th>
<th>Salmonella /25g</th>
<th>C.perfrigens cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk items</td>
<td>5.0x10^5</td>
<td>1.0x10^3</td>
<td>10</td>
<td>1.0x10^2</td>
<td>D</td>
<td>1.0x10^3</td>
</tr>
<tr>
<td>Cold meal</td>
<td>1.0x10^6</td>
<td>1.0x10^4</td>
<td>10</td>
<td>1.0x10^2</td>
<td>D</td>
<td>1.0x10^3</td>
</tr>
<tr>
<td>Blanched items</td>
<td>1.0x10^6</td>
<td>1.0x10^4</td>
<td>10</td>
<td>1.0x10^2</td>
<td>D</td>
<td>NA</td>
</tr>
<tr>
<td>Cheeses</td>
<td>NA</td>
<td>1.0x10^4</td>
<td>10</td>
<td>1.0x10^2</td>
<td>D</td>
<td>NA</td>
</tr>
</tbody>
</table>

2.14 International food safety policies

Following the recurrence of serious events of food contamination across the globe, food safety has become a matter of ever increasing international concern and the World Health Organization has defined food borne diseases as a global public health challenge (Negri, 2009). Recent events concerning food contamination in China, the United States, Canada, Italy, and Ireland have contributed to bringing food safety issues back in the spotlight of public opinion. Some of these events, which have found a wide echo in international media, have triggered a worldwide alert that evoked the concerns raised by the high profile “food scares” of the near past (mainly bovine spongiform encephalopathy and avian influenza). As a result, global governance of public health challenges posed by food borne hazards has been put high again on the international agenda of governmental agencies and international organizations (Negri, 2009). In the wake of a trend towards more efficient food safety policies, the 2007 Beijing Declaration on Food Safety (WHO, 2007) gives voice to the global community’s concern that a comprehensive and integrated approach be adopted, prompting all stakeholders to take cooperative and concerted actions and strengthening links between the different sectors involved. The Declaration, in fact, recognizes that “integrated food safety systems are best suited to address potential risks across the entire food-chain from production to consumption” and that “oversight of food safety is an essential public health function that protects consumers from health risks”. In this perspective, it mainly urges States to develop transparent regulation to guarantee safety
standards; to ensure adequate and effective enforcement of food safety legislation using risk-based methods; to establish procedures, including tracing and recall systems in conjunction with industry; to rapidly identify, investigate and control food safety incidents and to alert the World Health Organization (WHO) of those events falling under the revised International Health Regulations. In short, the Declaration expresses the need to understand food safety as both a national and an international responsibility (WHO, 2007)
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Design

This was a descriptive cross sectional study. The study focused on analyzing aircraft meals and describes the microorganisms found in the foods. Samples were taken at various points along the production line. It also involved administration of a questionnaire to assess food safety knowledge, attitude and practices.

3.2 Study site

The study was conducted at the Jomo Kenyatta International Airport (JKIA) food unit. JKIA serves a population of 5 million persons yearly. It is located in Embakasi, a suburb to the south west of Nairobi 15 km from CBD. Its Latitude is 1° 19’48” S and Longitude 36°55’30” E. (Figure 2a, 2b and 2c). The Jomo Kenyatta International Airport in Nairobi is a major terminus for Kenya Airways, and other international airlines. The unit provides catering services to all commercial airlines coming to Nairobi including cargo flights and chartered flights. The total population served is approximately 15000 daily (KAA, 2010).
Figure 2a. Map of Kenya

Figure 2b. Map of Embakasi

Figure 2c. Map of JKIA
3.3 Food samples for analysis

Food produced for the airlines from the food production unit were sampled and analysed.

3.4 Sampling

3.4.1 Sampling method

A total of 361 meals were collected along the production process. Convenient and purposive sampling techniques were used and meals were divided into 4 categories namely (i) Starter dishes such as hors d'oeuvre, canapés, prawn cocktail-dishes that require a fair amount of handling during preparation and which are served without reheating; (ii) Main courses mainly meals to be served hot; (iii) Cold desserts; and (iv) Snack meals which include sandwiches, vol-au-vents, and tartlets.

3.4.2 Sample size

A pathogen detection rate of 50% from food samples (Alterkruse et al., 1997) and 5% significance level was assumed. Applying the formula by Daniel (1999), 361 food samples was calculated as shown below:

\[ n = \frac{NZ^2P (1-P)}{d^2 (N-1) + Z^2P (1-P)} \]

\[ N = 15000 = \text{Total meals produced in one day} \]

\[ Z = 1.96 = \text{Standard error from the mean} \]

\[ P = 0.5 = \text{Pathogenic detection rate} \]

\[ d = 0.05 = \text{Absolute precision} \]

\[ n = \frac{15000 \times 1.96^2 \times 0.5 \times (1-0.5)}{0.05^2(10000-1) + 1.96^2(1-0.05)} \]

\[ = 361 \text{ food samples} \]
3.5 Data collection

3.5.1 Isolation and identification of organisms

3.5.1.1 Preparation of media and homogenate

Twenty five grams of each category of food samples 25 degrees Celsius was ground and
diluted in 225 ml of peptone water with 0.9% NaCl (w/v) and homogenized for 1 minute in
a stomacher.

After dilutions, 1 ml duplicate samples was spread on aerobic count plates and 1 ml on
blood agar and incubated aerobically at 30° C for 3 days to determine aerobic plate counts
(APC). Single colonies were re-streaked on Mueller Hinton agar for purity. All the media
used were prepared as explained in Appendix 4.

3.5.1.2 Enumeration of Coliforms and *Escherichia coli* (KS 05 220)

The homogenate was prepared by weighing 25 g of each food sample and 225 ml of
buffered peptone water which was blended at 15000 rpm using Waring commercial
blendor. Further serial dilutions were made using 9 ml buffered peptone water thus $10^2 -
10^6$. For presumptive coliform counts, lauryl sulphate tryptose (LST) broth was used in sets
of three bottles per dilution and incubated at 37° C for 24 hours and 48 hours. All turbid
bottles with gas formation were considered positive  and sub-cultured onto Violet Red Bile
Agar (VRBA). One loopful of the culture was picked onto LST and incubated at 44° C for
48 hours and also sub-cultured on Eosin Methylene Blue agar (EMB) and Sorbitol Mac
Conkey agar. Gas positive LST bottles were recorded  and the number of coliforms
calculated from the McCradys table (Cruikshank, 1972). Greenish metallic colonies from
EMB were considered positive for *E. coli* while colourless colonies on Sorbitol Mac
Conkey agar were typed with specific *E. coli* 0157 antisera. All the characteristic colonies
from VRBA and EMB were identified and confirmed using biochemical tests and API 20 E
(Biomireux, France).
The API 20 E is a commercial test system (Appendix 5 and Appendix 6) that is used for the identification of Enterobactericea and other Gram-negative rods. It uses 23 miniaturised biochemical tests. The strip consists of 20 micro tubes containing dehydrated substrates, which is inoculated with a bacterial suspension in reference to McFarland 0.5 standard and incubated at 37°C for between 18 to 24 hours. During incubation bacterial metabolism produces colour changes that are either spontaneous or shown by addition of specific reagents.

### 3.5.1.3 Detection of Salmonella and Shigella (KS 05 220)

The homogenate was prepared as in section 3.5.1.1 and incubated at 37°C for between 18 to 24 hours. For enrichment, 10 ml of homogenate sample was added onto 100 ml Rappaport vassiliadis enrichment broth and another 10 ml of homogenate put in each of into 100 ml selenite cystine and incubated at 37°C aerobically overnight. The enrichments were sub-cultured onto Xylose lysine decarboxylase agar (XLD) and incubated at 37°C for between 18 to 24 hours. The XLD was examined for clear non-lactose fermenting colonies and, or non-lactose fermenting colonies with black pigmentation. The colonies were picked and sub-cultured on Triple Sugar Iron agar (TSI) and incubated aerobically at 37°C for between 18 hours to 24 hours. The TSI was examined for characteristic reactions that included alkaline slant / acid butt, little or no gas with no hydrogen sulphide production for salmonella and alkaline butt for Shigella. Shigella was non-motile. Absence of Salmonella was confirmed by urease positive test through polyvalent Flagellar (H) test that were incubated and showed agglutination while Shigella colonies were confirmed though agglutination of polyvalent Shigella antisera and API 20 E (Appareil et procedes d identification montalien vercien biomerieux, France).
3.5.1.4 Enumeration of *Staphylococcus aureus* (KS 05 220)

A homogenate was prepared as shown in section 3.5.1.1 and serial dilutions made with 9 ml buffered peptone water to make dilutions of $10^1 - 10^6$. Then 0.1 ml of each dilution was sub-cultured onto Baird and Parker agar and incubated at $37^0$ C for between 24 to 48 hours. Colonies that were black surrounded by clear zone were suspected to be *S. aureus* and were counted. The colonies were tested for coagulase activity by inoculating into plasma and incubating at $37^0$ C for 2 hours. The inoculations were then checked visually for clot formation and when negative they were further incubated for 4 hours and 24 hours for the test to have been considered negative. A firm clot which does not move when the tube is tipped on its side (4+ coagulase reaction) was considered a positive test of *S. aureus*.

3.5.1.5 Enumeration of *Listeria monocytogenes* (KS ISO 10560)

The homogenate was prepared by weighing 25 grams of the food sample and 225 ml of *Listeria* enrichment broth and incubated at $30^0$ C for 24 hours. The selective enrichment was subcultured onto *Listeria* selective agar at $37^0$ C for a further 48 hours. Five presumptive listeria colonies of each morphological type were sub-cultured in horse-blood agar from listeria chromogenic agar and confirmatory test carried by performing single stab inoculation to facilitate haemolysis detection and give discrete colonies. They were then incubated at $37^0$ C for 24 hours and examined for purity, colonial morphology (characteristic black-zoned colonies) and presence of β-haemolysis. A negative gram stain confirmed absence of *Listeria*.

3.5.1.6 Enumeration of *Clostridium perfringens* (KS 05 220)

Food homogenate was prepared as indicated in section 3.5.1.1 and serial dilutions of $10^2 - 10^6$ were made with 9 ml buffered peptone water. Then 1 ml of each dilution was transferred onto selective *Perfringens* agar and Sheep blood agar and incubated anaerobically at $37^0$ C for 24 hours. Colonies of Clostridia (black colonies) were not
identifiable from *Perfringens* agar and absence of formation of double zoned haemolysis on Sheep blood agar.

### 3.5.1.7 Enumeration of *Yersinia enterocolitica* (KS ISO10273)

Food homogenate was prepared as described in section 3.5.1.1 and serial dilutions of $10^2$ - $10^6$ were made with 9 ml buffered peptone water. One ml of each dilution was poured onto *Yersinia* selective agar base and incubated at 37°C for between 18 to 24 hours. Biochemical tests that included catalase +ve, VP –ve, lactose –ve and API 20 E confirmed typical dark red colonies of *Y. enterocolitica* resembling bull’s eye.

### 3.5.1.8 Enumeration of *Vibrio parahaemolyticus* (KS 05 459)

Food homogenate was prepared by adding 25 g of the food sample and 225 ml of alkaline peptone water to make a fine homogenate. Further dilution of $10^2$ - $10^6$ was made with 9 ml of alkaline peptone water. Then 10 ml was transferred onto two sets of double strength alkaline peptone and 1 ml transferred onto single strength alkaline peptone water and incubated aerobically at 35°C for between 18 to 24 hours. All the alkaline peptone water samples were sub cultured onto Thiosulphate Citrate Bile salt Sucrose Agar (TCBS) and incubated at 35°C for between 18 to 24 hours. The absence of characteristic bluish/green colonies was confirmed by inoculating onto TSI agar, which was further incubated at 37°C for 18 hours. Further absence confirmation was through TSI agar that was examined for characteristic alkaline/acid, gas production, and H₂S reaction.

### 3.5.2 Antibiotic susceptibility tests

Kirby-Bauer agar disk diffusion technique (Bauer *et al.*, 1966) was used in testing the microbial antibiotic susceptibility. At least 4 to 5 well-isolated colonies of the same morphological type from an agar plate were selected and transferred using a wire loop to a
tube containing 4 to 5 ml of tryptic-soy broth. The broth culture was allowed to incubate at 35°C for three hours until it achieved or exceeded the turbidity of 0.5 McFarland standards.

Alternatively, the inoculum was prepared by making a direct saline or broth suspension of colonies that were selected from an 18 to 24 hour nutrient agar plate. The turbidity was adjusted with sterile saline or broth to compare with 0.5 McFarland standards. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile non-toxic (pyrogen free) swab on an applicator was dipped into the adjusted suspension, rotating the swab several times and pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The dried surface of a Muller-Hinton agar plate was then inoculated by streaking the swab over the entire sterile agar surface, repeating this procedure two more times, and rotating the plate 60° each time to ensure an even distribution of inoculum. Using a sterile forceps, the dispensing apparatus was then placed in the appropriate disks evenly on the surface of the agar plate. The plate was then inverted and placed in an incubator at 35°C aerobically within 15 minutes after disks were applied. After 18-24 hours of incubation, each plate was examined and the diameters of the zones of complete inhibition measured, including the diameter of the disk, to the nearest millimeter using a ruler. The *E. coli* ATCC 26922 organism was used for quality control of growth and disc potency. The zone sizes were then interpreted by referring to the National Committee for Clinical Laboratory Standards (NCCLS) 2004 breakpoints, and the organism reported to be susceptible, intermediate, or resistant. Quality control organisms such as *E. coli* ATCC 25922 and were tested periodically to validate the accuracy of the procedures. The antibiotics used were based on the representative of different antibiotic classes (1st, 2nd, 3rd and 4th generation drugs) and were as follows; Chloramphenicol C-30, Ampicillin AMP – 25, Tetracycline TE-25, Gentamicin CN – 10, Sulfamethoxazole/ Trimethoprim SXT – 25, Augmentin (Amoxicillic/Clavulanic Acid) Aug – 30, Kanamycin K-25, Cefuroxime CXM-30, Aztreonam AZT-30, Ceftazidime CEFT-30, Cefixime CEFI -30, and Cefotaxime CEFO-30.
3.5.3 Questionnaire design

A self-administrable questionnaire was developed for this study with 50 questions including “I do not know” for the purpose of minimizing the possibility of selecting the correct answer by chance (Appendix 1). The reliability of the questionnaire was also determined by pre-test on 50 staff of the catering unit. The reliability coefficient of knowledge test was 0.74. As a result of the item analysis, several test questions were modified to improve clarity. In addition, six questions were related to the demographic characteristics of 197 respondents (department, category of staff, gender, age, and number of years worked). The questionnaire was administered to all the 197 food handlers. The questions were designed and structured in four groups: (1) Food safety knowledge (2) food safety practices (3) attitude; and (4) comprehension of HACCP system and prerequisite programmes within the food chain. Respondents completing the questionnaire remained anonymous. Each questionnaire took approximately 10 minutes to complete. The study was conducted from June to December 2008.

3.6 Quality Control

Quality control was carried out to ensure reliability and reproducibility of results. This included the following; strain testing, selection skill and control of inoculum’s density in broth and control of culture during storage period and use of aseptic measures while inoculating at any stage and pre-testing of the data collection tools including machine verification and validation. Replicate tests were also carried out.
3.7 Variables

Dependent variable: Food quality.

Independent variable: Isolation technique, food safety system in place, staff knowledge, attitude and practices, isolated microorganisms and type of food.

3.8 Data Analysis

Data on the microbiological quality of foods was summarized using descriptive statistics such as frequencies and percentages and presented in graphs and tabular form. To examine the relationship among and between the variables, cross tabulations and the $\chi^2$ test, Pearson correlation coefficient were used. The questionnaire was structured to contain demographic characteristics and assess food safety knowledge, practices and attitude. Statistical significance was set at $p < 0.05$ using Statistical Package for Social Sciences (SPSS) version 11.5. A structured questionnaire was used to assess food safety knowledge, practices and attitude, and thus establish the controls that fail along the process chain.

3.9 Ethical Consideration

Ethical consent was obtained from the food production unit personnel department. Further consent was sought from Kenyatta University. Benefits of the study will go directly to the catering unit including, recommendations for improvement, gaps identified in the process chain and need correction and report on microorganisms of importance isolated that require attention.
CHAPTER FOUR: RESULTS

4.1 Prevalence of bacteria in food samples

A total of 361 food samples were collected throughout the facility. *Klebsiella ozaenae* was the most prevalent isolate 86 (25.3%). Three hundred and forty micro-organisms were isolated (Table 3) with a number of pathogenic isolates identified namely, *Staphylococcus aureus* 4 (1.2), *Shigella flexineri* 12 (3.5%) and *Escherichia coli* 34 (10%).

Table 3. Bacterial species isolated from food samples.

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas caviae</em></td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>55</td>
<td>16.2</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Enterobacter sakazakii</em></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>34</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Hafnia alvei</em></td>
<td>17</td>
<td>5.0</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Klebsiella ozaenae</em></td>
<td>86</td>
<td>25.3</td>
</tr>
<tr>
<td><em>Klebsiella rhinocoleromatis</em></td>
<td>54</td>
<td>15.9</td>
</tr>
<tr>
<td><em>Providencia alcalifaciens</em></td>
<td>14</td>
<td>4.1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>13</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td>25</td>
<td>7.4</td>
</tr>
<tr>
<td><em>Shigella flexineri</em></td>
<td>12</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>340</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Three categories of microorganisms were isolated from the 361 food samples (Fig 3), namely; Bacteriological counts usually expressed as the total viable count (TVC) and is used as a microbiological indicator of food quality (60.3%), Indicator organisms which are present in very large numbers in environments inhabited by pathogens (21.5%), and Pathogenic micro-organisms (18.2%) that should never be present in hygienically handled and effectively processed high-risk food.

![Chart showing frequency of isolated class of microorganism](image)

Figure 3. Frequency of isolated class of microorganism

**4.1.1 Prevalence of bacteria isolated from sampling source**

Samples were analyzed from source point (Fig. 4) and food samples from the supplier (samples collected once the supplier has just delivered to the unit) and had not been processed in the catering unit were the most contaminated in terms of microbial load (Pathogenic, Indicator and Total counts) at (42.4%) followed by samples processed at the supplier (31.2%) then the ones sampled as airline (19.4%) and lastly bulk at 7.0%.
4.2 Prevalence of bacterial species isolated from food samples

Hot meals were less contaminated 59 (16.3%) compared to cold meals 248 (68.7%) thus underscoring the importance of processes such as cooking. The frequency of *E. coli* featured more in cold (13%) isolates than in hot meals (4%) isolates. Bacterial strains that are capable of causing food borne diseases (*Staphylococcus aureus, Shigella flexineri*) were isolated in cold meals (16%) and none in hot meals. *E. coli* was isolated in both classes of meals.

4.2.1 Isolates from hot meals

Table 4 shows the prevalence of isolates from hot meals. Pathogenic isolates of *Escherichia coli* were isolated in food samples such as in beef 13 (37.1%), chicken 4 (67%) and in blanched vegetables 4 (23.5%). Pork which was processed in a different room was least contaminated as compared to other hot meals (1.7%).
Table 4. Prevalence of bacterial isolates from hot meals

<table>
<thead>
<tr>
<th>Food (n)</th>
<th>Isolate</th>
<th>Frequency of isolated bacteria</th>
<th>Cumulative Percentage of bacterial contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef (26)</td>
<td><em>Enterobacter</em></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter sakazakii</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella ozaenae</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Serratia liquefaciens</em></td>
<td>2</td>
<td>59.3</td>
</tr>
<tr>
<td>Chicken (26)</td>
<td><em>E. coli</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em></td>
<td>1</td>
<td>10.2</td>
</tr>
<tr>
<td>Pork (14)</td>
<td><em>Klebsiella</em></td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Vegetables (26)</td>
<td><em>Enterobacter</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella ozaenae</em></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Providencia</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>alcalifaciens</em></td>
<td>1</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td><em>Serratia liquefaciens</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2.1.1 Contamination according to type of hot meal

The results of bacterial contamination in hot meals are as shown in Fig. 5. Cooked beef products were the most contaminated category of hot meal (59.3%) of the hot meals. These
included fried beef, beef shwarma, beef curry and roasted meat. Vegetables accounted for 28.8%, chicken 10.2% and lastly pork products at 1.7%.

Figure 5. Proportion of contaminated hot meals.

4.2.2 Isolates from cold meals

A total of 248 (68.7%) cold meals were contaminated (Table 5), with starters being the most contaminated category of cold meal which had a 62.7% of isolated bacteria. In starters the largest contamination came from *Klebsiella ozaenae*, 37 isolates (25%). Some of the pathogenic isolates were *E.Coli* 13 isolates (9%), *Pseudomonas* 5 (3.4%) *Shigella flexineria* 12 isolates (8.1) and *Yersinia enterolitica* 5 isolates (3.4%). For desserts *Klebsiella ozaenae* 37 (51.3%) was isolated and had the highest frequency. In snacks *Klebsiella ornithinolytica* had the most prevalence, 12 isolates (43.9%). Four pathogenic strain of *Staphylococcus aureus* (14.3%) were isolated from snacks (chicken sandwich) which is a supplier product. In addition the *Klebsiella* species was isolated more accounting for 47 % of all isolates from the cold meals.
Table 5. Prevalence of bacterial isolates from cold meals

<table>
<thead>
<tr>
<th>Food (n)</th>
<th>Isolates</th>
<th>Frequency of isolated bacteria</th>
<th>Cumulative Percentage of bacterial contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starters (90)</td>
<td>Citrobacter freundii</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacter</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacter cloacae</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hafnia alvei</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella oxytoca</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ozaenea</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ornithinolytica</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Providencia alcalifaciens</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pseudomonas</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serratia liquefaciens</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shigella flexineria</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yersinia enterocolitica</td>
<td>5</td>
<td>62.7</td>
</tr>
<tr>
<td>Dessert (90)</td>
<td>Enterobacter</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hafnia alvei</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ozaenea</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ornithinolytica</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Providencia alcalifaciens</td>
<td>4</td>
<td>26.9</td>
</tr>
<tr>
<td>Snacks (68)</td>
<td>Enterobacter</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ozaenea</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella ornithinolytica</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pseudomonas</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>10.4</td>
</tr>
</tbody>
</table>

4.2.2.1 Contamination according to the type of cold meal

Starters were the most contaminated amongst the cold meals that were sampled accounting for 62.7%. This was followed by desserts (26.9%) and lastly the snack meals (10.4%) Fig. 6.
In addition, raw materials from vegetable suppliers were more contaminated than from other suppliers ($\chi^2 = 121.948$, df = 4, p = 0.0001). However, this food once it had undergone processing, the bacterial load reduced.

There was a significant relationship in the type of microorganism isolated and the type of cold meal type ($\chi^2 = 143.844$, df = 26, p = 0.001). The microorganisms isolated from cold meals were more likely to have originated from starters than from the rest of the cold meals. The starters had more bacterial load of indicator microorganisms than the rest, significantly showing that they were more likely to be contaminated with pathogenic microorganisms ($\chi^2 = 18.439$, df = 2 p = 0.001).

Though not significant, beef classified as hot meal had a larger percentage of contamination than other hot meals. *Klebsiella* Spp was the most isolated microorganism accounting for 80% of all microorganisms isolated.
4.3 Antibiotic susceptibility

The diameter of zones of inhibition were measured and recorded in millimeters, (Fig. 7). Susceptibility results were interpreted as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The isolates were from various food samples as shown in Appendix 3.

Kanamycin had the least resistance on the isolate (1.1 %) that was *E. agglomerans* isolated from an orange goblet. Gentamicin had the second lowest resistance at 3.4%. There was a statistically significant relationship in these two ($\chi^2 = 41.572$, df = 4, p = 0.0001) where the microorganisms that were resistant to Kanamycin were more likely to be resistant towards Gentamicin. Most of the isolates were resistant to Augmentin (81.8 %) and Ampicilin (84.1%). In addition there was a significant relationship between these two ($\chi^2 = 88.0$, df = 4, p = 0.0001) in that those isolates that were resistant to Augmenteine were likely to be resistant to Ampicilin.
This relationship was also evident between tetracycline and Cufuroxime ($\chi^2=128.905$, df = 4, $p = 0.0001$) and also between tetracycline and Chloromphenicol ($\chi^2=120.323$, df = 4, $p = 0.0001$). A statistically significant relationship was observed in the resistance and sensitivity between Tetracycline and Cefixime ($\chi^2 = 151.988$, df = 4, $p = 0.0001$) and tetracycline and Ceftriaxone ($\chi^2 = 151.988$, df = 4, $p = 0.0001$). *A. caviae* 1 (100%) was susceptible to all antibiotics except Augmentin while *E. agglomerans* 5 (100%) was resistant to Ampicilin and Augmentin. *E. coli* 6 (100%) was resistant to Augmentin but susceptible to Kanamycin. Fig. 8 gives a visual display of the percentages.
Figure 8. Antibiotic sensitivity profile of the drugs.

KEY
AZT - Aztreonam
CEFT - Ceftazidime
CEFI - Cefixime
CEFO - Cefotaxime
C - Chloramphenol
CXM - Cefuroxime
CN - Gentamicin
K - Kanamycin
AUG - Augmentin
SXT - Sulphamethoxazole
TE - Tetracycline
AMP - Ampicillin
4.4 Control of the food safety system

The catering unit had in place good measure of food safety controls that included personal hygiene policy, HACCP, use of color coding and cleaning and sanitation. However as shall be seen in the results some of these controls are never followed and gaps were identified that need immediate attention to prevent any chance of food poisoning.

4.4.1 Demographic characteristics of the food handlers

Of the 197 employees taking part in the research, 81 (41.1%) classified themselves as cold kitchen, 44 (22.3%) hot kitchen, 30 (15.2%) stores, 26 (13.2%) bakery, 12 (4%) catering and 4 (2%) as cleansing. Majority of the respondents (64%) were male. Average number of years worked was 7 years (SD = 4.496). Of the 197 respondents, 86.8% were unionisable while 13.2% were in management. More than 70% of the respondents were below the age of 30 years. Majority of the respondent 27 (13%) had worked for 4 years while 3 respondents had worked for 17, 22, and 28 years. These staff all worked in the cold kitchen.

4.4.1.1 Education level

The study looked at education level of the food handlers in the food production chain. Figure 9 shows the education level of the majority respondents, (88.8%) had finished secondary education or had a college diploma. Staff who had finished form six accounted for 10.7% while only 1 (0.5%) respondent had qualified at the university level.
Figure 9. Frequency of respondents’ education level with gender

Though majority (72.5%) of the college diploma workers did not comply with food safety best practices, there was no significant difference in the education level and compliance to food safety regulations \( \chi^2 = 3.6, \text{df} = 3, p = 0.308 \). However, this is contrary to food safety knowledge where there was significant difference between education level and food safety knowledge \( \chi^2 = 14.2, \text{df} = 6, p = 0.035 \). Staff with higher level of education or equivalent were likely to know more on food safety best practices. However, there was no significant difference between college trained and university trained staff.

4.4.1.2 Gender

Of the respondents who answered the questionnaire, 64% were male while 36% were female. There was no significant difference between the gender with regard to compliance \( \chi^2 = 0.438, \text{df} = 1, p = 0.508 \) and food safety knowledge \( \chi^2 = 0.208, \text{df} = 1, p = 0.901 \).
4.5 Food safety practices of the food handlers

To evaluate the handling practices of the food handlers and assess where personal hygiene controls were compromised, the use of tissue paper and its availability in the toilets was considered an important measure for prevention of food contamination. This is on the basis of the fact that oral fecal mode of transmission of pathogens is a food contamination method. There was a significant relationship between the number of years worked and action taken when there was no tissue paper in the toilet \( \chi^2 = 87.987, \text{ df } = 57, \text{ p } = 0.005 \).

Fig. 10 illustrates that majority of the staff who had worked less than 10 years were more likely to inform the supervisor if there was no toilet paper than use hand paper tissue or any other paper.

![Figure 10](image)

Figure 10. Frequency of the respondent toilet practice with regard to no. of years worked

A relationship was also established between staff food safety knowledge and how long it took to wash their hands \( \chi^2 = 14.689, \text{ df } = 6, \text{ p } = 0.032 \).
Staff that with post secondary education were likely to take 20 seconds which is the acceptable standard in airline catering than those who had no post secondary training. These also included the university trained staff. They also knew when to wash their hands especially after handling products that could cross contaminate ready to eat foods. An interesting relationship was also evident between the last time staff had a food safety training and how often staff portioned food in excess. Staff who were recently trained (3 months) were likely to never portion in excess compared to those who had this training one year or more prior ($\chi^2 = 65.411, \text{df} = 9, p = 0.001$). Food portioning is an important aspect as returning food that was in excess into the bulk holding container re-contaminates the food. In addition all staff who had been trained recently (within a period of three months) were likely to use a thermometer more than those who had been trained more than a year before ($\chi^2 = 123.160, \text{df} = 3, p = 0.001$). A relationship was established on frequency of changing hands gloves and the departments ($\chi^2 = 38.995, \text{df} = 10, p = 0.001$), with food handlers’ in the stores department likely to change their gloves more frequently than other departments.

In addition college/ diploma trained personnel (68%) were likely to use canned products without blast chilling as compared to other groups ($\chi^2 = 22.053, \text{df} = 9, p = 0.009$). The same group also were likely to shake hands more in production than any other group ($\chi^2 = 14.273, \text{df} = 6, p = 0.027$). Table 6 shows different responses to questions on food handling practices.
Table 6. Food handling practices likely to contaminate food

<table>
<thead>
<tr>
<th>Statements</th>
<th>Never (%)</th>
<th>Rarely (%)</th>
<th>Sometimes (%)</th>
<th>Always (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of hand washing</td>
<td>87.8</td>
<td>8.1</td>
<td>4.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Frequency of practicing “clean as you go” policy</td>
<td>79.7</td>
<td>16.2</td>
<td>4.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Frequency of portioning excess food</td>
<td>16.2</td>
<td>51.8</td>
<td>19.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Frequency of washing gastonorm</td>
<td>68.5</td>
<td>19.8</td>
<td>7.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Frequency of blast chilling canned products</td>
<td>23.8</td>
<td>12.2</td>
<td>23.9</td>
<td>40.1</td>
</tr>
<tr>
<td>Frequency of shaking hands in production</td>
<td>0.0</td>
<td>40.1</td>
<td>7.6</td>
<td>52.3</td>
</tr>
<tr>
<td>Frequency of checking color code before serving</td>
<td>95.9</td>
<td>4.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Frequency of recording food temperature</td>
<td>71.6</td>
<td>28.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Frequency of changing gloves</td>
<td>76.1</td>
<td>19.8</td>
<td>0.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Frequency of changing apron</td>
<td>20.3</td>
<td>31.0</td>
<td>20.3</td>
<td>28.4</td>
</tr>
</tbody>
</table>

4.6 Food Safety Compliance of the food handlers

The respondents who were likely to consult with the management or totally reject non-conforming products were likely to comply with the food safety best practices ($\chi^2 = 8.485$, df = 2, p = 0.014), Figure 11. In addition a statistically significant relationship was demonstrated in respondents who failed to take any action against cross contamination in the cold rooms in that they were less likely to comply with best practices ($\chi^2 = 8.395$ df = 3, p = 0.039). Respondents taking more than 45 minutes to assemble food or did not know what corrective action to institute were less likely to comply with the food safety best practices ($\chi^2 = 7.489$, df = 2, p = 0.024).
4.7 Attitude of food handlers

The respondents with negative attitude were more likely to have less food safety knowledge \( (\chi^2 = 185.649, \ df = 1, \ p = 0.001) \) (the right color for chopping boards, expiry dates of products, thawing procedure and cleaning and sanitation) and were less likely to comply with the food safety best practices wearing of gloves, vegetable sanitization and avoidance of cross contamination \( (\chi^2 = 197.00, \ df = 1, \ p = 0.001) \).
5.1 Bacterial species isolated from aircraft food

The organism diversity present in the various airplane bound food samples was surprisingly complex. Total viable counts were the most isolated compared to pathogenic and Indicator microorganism and these are mostly associated with food handlers and this could be linked with the fact that most of the food handlers (87.8%) admitted never to wash hands upon entering production area while 76.1% also admitted to not change their gloves during their entire work shift. According to Martínez-Tomé et al., (2000) the hands of food handlers as well as their protective clothing should be kept clean and food handlers should avoid contact with food whenever possible. The usual indicator organisms used by the food microbiologists belong to the Enterobacteriaceae family, many of which live in the intestine of human and animals (Sprenger, 1995). For many foods, especially those that are ready-to-eat, the cleanliness of food contact surfaces is likely to be identified as being critical to food safety (Moore and Griffith, 2002). The most frequently isolated microorganism was Klebsiella ozaenae that falls under total viable count and this is attributed to the cleanliness of the food contact surfaces.

The organism that was detected with the best known source of origin was Escherichia coli and this is attributed to the fact that some food handlers did not wash their hands upon entering the food production area and using alternative ways when tissue paper is unavailable. According to De Wit and Rombouts, (1992), Escherichia coli is normally absent from hands and the presence of Escherichia coli is thought to give a better indication of faecal contamination (enteric pathogens in particular) than the entire group of Enterobacteriaceae.
The only pathogenic microorganism that was isolated of concern was *Staphylococcus aureus*. Notwithstanding that low numbers are extremely unlikely to cause food poisoning with regard to pathogenicity (Sprenger, 1995), food contaminated with this organism is potentially hazardous and is evidence that the food has not been handled hygienically. *Staphyloccocus aureus* is the predominant species involved in staphylococcal food poisoning outbreaks, arising due to the handling of cooked foods by persons who carry enterotoxigenic *Staphylococci* in their noses or on their skin. (Angelillo *et al.*, 2000) and (Portocarrero *et al.*, 2002). *Staphylococci* are ubiquitously distributed in man’s environment and strains present in the nose often contaminate the back of hands, fingers and face and nasal carriers could therefore easily become skin carriers (Desmarchelier *et al.*, 1999), (García *et al.*, 1986; ), (Genigeorgis, 1989) and (Gorman *et al.*, 2002).

Microorganisms on the human skin can be divided into two groups: permanent and transitory; and the only pathogenic microorganism in the permanent group of bacteria associated with the human skin is *Staphylococcus aureus*. According to Moore *et al.*, (2001) an inadequately cleaned surface can, if in contact with food, lead to cross-contamination and contribute to a product’s microbial load, which might result in a decreased shelf life. Further, the presence of *Staphylococcus aureus* usually indicates a breakdown in personal hygiene or the handling of food which is in line with a study by Sprenger, (1995), where 87.8% of staff did not wash their hands upon entering food production area. The study by Spranger (1995) also was in line with this study, where 53.5 % answered they always shook hands while in food production area. Such practices propagate spread of bacteria through cross contamination.
5.2 Bacteriological quality of meals

5.2.1 Hot meals

Hot meal samples (16.3%) exceeded the limit of AEA standard for *E. coli* counts of 11%. The numbers of samples having a higher count than AEA guidelines were 11. A previous study by Hatakka, M., (2000) found a higher proportion of hot meals exceeding these values (15%) for total counts and (13%) for *Escherichia coli*. The following reasons may account for the high bacteria counts in the hot meals; i) Critical aspects controlling the bacterial level in hot meals are chilling, ii) The time-temperature combination during portioning and packing, iii) The temperature during storage in the flight kitchen, and iv) Transport to the aircraft and the storage on board before serving. Considerable differences in the means of total bacteria and *Escherichia coli* counts indicate differences in the hygienic levels between departments. However, undercooked food items such as deep-frozen blanched vegetables and steaks are commonly used in hot meals. This may be one important factor contributing to the high counts of total bacteria, *Escherichia coli*, coliforms and *Enterobacteriaceae*.

5.2.2 Cold meals

A high total bacteria count 76.6 % reflects compromised microbiological quality of cold meals, partly because appetizers, salads and desserts often include raw items such as fresh vegetables, fruits or garnishes, and they normally contain a high count of total bacteria. In addition these items require a fair amount of handling during preparation and contamination may occur through this handling.

The use of farm manure during planting and subsequent farming stages could also account for the high numbers of contamination of fruits and vegetables such as *E. coli*. 
The use of sausages and cheeses produced using starter cultures as items in appetisers increases bacterial count. Many of the cold dishes (30%) in this study had higher *Escherichia coli* counts than the AEA standard permits (AEA, 1996). The occurrence of *E. coli*, especially in such high values as $1.0 \times 10^6$ cfu/g detected, indicates contamination and poor microbiological quality. Raw items are commonly used for appetiser and salad dishes. The highest contamination rates were found in these dishes. This could be as a result of inadequate sanitization of fruits and vegetables and inadequate cleaning and sanitation as 79.7% of the respondents said they did not practice the “clean as you go” policy. The source here is attributed to the raw supplies from the supplier and failure in cleaning and sanitation programme.

The frequency of *Staphylococcus aureus* (1.2%) in this study was higher compared to previous studies (Roberts *et al*., 1989), where it was 0.3%. A considerably higher frequency of *Staphylococcus aureus* (24%) was reported by Lambiri *et al*., (1995). Cold meals need a lot of manual handling and contamination via the hands is therefore possible. This is further aggravated if staff is not washing their hands frequently as witnessed in this study a typical case in this study being the presence of contaminated pasteurized double cream with *Escherichia coli*. This cream is highly nutritious and any cross contamination from staff will quickly propagate the micro-organisms if already contaminated through poor handling.

*Shigella flexneri* was found in 0.5% of food samples in this study. This is a pathogenic micro-organism at very low doses of infection, and is usually transmitted from person to person but may also occur by consumption of contaminated water and foods including vegetables that have received little or no heat treatment. Food may become contaminated by infected food handlers who don’t wash their hands with soap after using the toilet. Vegetables can also become contaminated if they are harvested from a field with sewage contamination in it and thus the need for caterers to ensure they buy the vegetables from reputable suppliers. In addition frequent auditing of the vegetable supplier is encouraged.
Shigella can also be transmitted by flies. Flies can breed in infected feces and then contaminate food and thus the need for caterers to have an elaborate pest control programme.

Contamination of cooked items may occur during handling and portioning. The storage of contaminated food items that are inadequately refrigerated permits the multiplication of Staphylococcus aureus and enterotoxin formation. In respect to cold dishes, desserts such as custards and chocolate cakes have been implicated with aircraft disease outbreaks. Flight delays and subsequent temperature abuse was proved to be the final reason for two S. aureus outbreaks via desserts served on board (Munce, 1978) thus the need to ensure these microorganisms are prevented from the onset.

5.3 Food safety controls in the food chain

5.3.1 Hazard analysis critical control point

Hazard analysis critical control point (HACCP) is a food safety management system strategy which has been widely tested, and established as an effective means of preventing food-borne diseases (Codex, 1993 and WHO, 1993).

In this study, only 3.6% of food handlers understood the real meaning of what HACCP meant. This is slightly lower than other catering establishments in United Kingdom. HACCP is a preferred approach to retail food safety because it provides the most effective and efficient way to ensure that food products are safe (Mc Swane et al., 2003). HACCP programmes are designed and implemented to produce the safest food possible on the basis of current scientific information and practical experience.
In this study, the prerequisite programmes are managed not as per the required standard as 64% of the food handlers indicated that they never practicing the programme during their shift. The findings of this study indicated that 28.4% of managerial staff and 56.3% of basic food handlers (unionisable) have not received basic food hygiene training. In a study in UK, 55% of the 444 food handlers surveyed had undertaken formal food hygiene training, and 63% of managers had undertaken formal food hygiene training in UK food businesses (Walker et al., 2003). Food hygiene training should be a priority for both managers and staff as indicated by the fact that operations with individuals who have food safety certification were more likely to use appropriate food safety practices. Whereas, the success of a HACCP program also depends on the education and training of employees on the importance of their role in maintaining food safety, an understanding of HACCP and the related prerequisite programs, as well as a commitment from management, must be established to make HACCP successful (King, 1992). An important finding from this study was that HACCP not been widely understood and that this had a negative impact on the general food hygiene standards and food-handling practices of personnel. HACCP has yet to become a legal requirement for the Kenya’s’ food catering industry.

Since temperature treatment is frequently the critical control point in a production process, proper understanding of temperature control during food production could be a major hindrance of effective HACCP implementation (Walker et al., 2003). In this study, the most frequently observed poor food handling was related to time and temperature. Most food handlers did not take and record food temperature. In instances where food temperature was checked, it was not recorded immediately or at all. Only 36.7% of the food handlers reported to take end-point temperatures of all cooked food at any time during pre-preparation. The other common observed food safety problem was the failure to completely thaw frozen food. Food thawing at room temperature instead of controlled areas such as cold room as required by the caterer was practiced by 75.8% of the food handlers.
Walker et al., 2003, reported that less control was evident for the important stages of cooking, chilling and reheating in UK food businesses. In addition, the survey conducted by Walker et al., (2003) indicated that poor results (60%) for the implementation of HACCP in small and medium size food businesses in UK centered on their temperature control and record keepings. During the hazard analysis step, risk should be estimated.

5.3.2 Food safety knowledge, attitude and practices

In this study, 84.3% of the respondents had done a food hygiene course over one year past. It is recommended that food safety training be limited to within 3 months as respondents who had been trained within a period of 3 months were likely not to portion food in excess compared to those who had this training one year or more prior ($\chi^2 = 65.411$, df = 9, $p = 0.001$). In addition all staff who had been trained recently (within a period of three months) were likely to use a thermometer more than those who had been trained more than a year before ($\chi^2 = 123.160$, df = 3, $p = 0.001$). Food safety training is a relevant aspect and a regulatory requirement in in-flight catering.

This is a matter of public health concern, especially as human error has been suggested as a contributory factor in 97% of food borne disease outbreaks (Howes et al., 1996) and the frequency of human error is increased by lack of training (Clayton and Griffith, 2004). Thus there would appear to be a clear linkage between effective formal training, improved catering practice and prevention or significant reduction of food borne outbreaks in the catering industry (Coleman et al., 2000).

Training for caterers has been shown to improve food safety knowledge and hygiene awareness (Tebbutt, 1984 and Worsfold, 1993), and may result in improved food safety practices (Gillespie et al., 2000), (Thompson et al., 2005). However, there is considerable evidence that improved knowledge does not always translate into improved food handling behavior (Kassa, 2001; Riben et al., 1994 and Taylor, 1994).
In this study, better general formal training did not appear to be significantly linked to better food safety practices. This confirms the concerns expressed by Griffith and Clayton (2005), who reported that it is unwise to automatically assume that improved knowledge will lead to behavioral changes involving improved practice, and also suggested that other factors, including staff attitudes can limit or prevent improvements in staff practices. Griffith and Clayton (2005) also highlighted the difficulties in correcting previously gained erroneous information and well established bad hygiene practices, which may be endemic in some sectors of the catering industry. Bearing in mind the central importance of consistent high quality food hygiene practice among catering operatives, it is essential that sustained efforts be made to guarantee that all head chefs receive adequate effective hygiene training during pre-employment training, undertake periodic refresher courses, and that their knowledge in practice should be confirmed by routine inspection.

This study observed that the unit fulfilled the structural design requirements for hygiene practice, for example by providing a sensor tap hand washing sink with soap and hot water. This could suggest that basic structural requirements for the purposes of obtaining statutory licensing/approval are initially fulfilled, but on-going hygiene requirements are less carefully observed. It may also suggest that hand washing procedures, as currently practiced may not efficiently remove contaminating agents. The general role of hand washing in preventing disease is well known in the catering industry and positive attitudes to hand washing among caterers have been reported in several studies in Italy (Angelillo et al., 2000), the UK (FSA, 2002) and the USA (Walter et al., 1997).

However, observational studies suggest that knowledge is not always put into practice. Oteri and Ekanem (1989) found that less than one-third of those who reported the importance of hand washing actually washed their hands before handling food. Manning and Snider (1993), reported that 81% of respondents stated an awareness of the importance of hand washing, but only 2% were observed to wash their hands. There was lack of
knowledge among the production staff about the critical temperatures of hot and cold ready to eat foods, acceptable refrigerator temperature ranges, periodical control of refrigerators’ and freezers’ thermostat settings, and etiologic agents associated with some food-borne diseases. This appeared to be related to the delay of in-service training for food service staff as (84.3%) had their last refresher training over 1 year ago. A similar lack of knowledge about critical temperatures of hot and cold ready to eat foods, acceptable refrigerator temperature ranges and about etiologic agents, have been reported among food service staff in food service establishments in Italy, Iran and in another study in Ankara, Turkey (Angelillo et al., 2001; Askarian et al., 2004; Bas et al., 2006 and Buccheri et al., 2007). According to these studies, temperature abuse that arises to lack of food safety knowledge is attributed to bacterial contamination of ready to eat foods in most places worldwide.

5.4 Antibiotic sensitivity profile

From the current study, it was established that the most sensitive antibiotics were Kanamycin and Aztreonam. They both had 81.8% sensitivity on the micro-organism tested. Other antibiotics showed varying resistance patterns where tetracycline had 14.8%, Chloramphenicol 10.2% and Ampicillin 84.1%. In this study, a very low frequency of antimicrobial resistance in Enterobacteriaceae was found which concurs with studies done by Osterblad et al 1999. Multi resistance profiles typical of strains associated with clinical isolates were also identified in this study contrary with studies done by Osterblad et al., 1999. The prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades (Boonmar et al., 1998 a, b; Chui et al., 2002; Davis et al., 1999), possibly as a result of selection pressure created by the use of antimicrobials in food-producing animals (Aarestrup, 1999; Angulo et al., 2000; Bywater, 2004; Teuber, 2001). The coexistence of resistance genes with mobile elements such as plasmids, transposons and integrons facilitates the rapid spread of antibiotic resistance genes among bacteria (Sunde, 2005). Also, high rates to antibiotics resistance of bacteria may possibly resulted
from inappropriate or uncontrolled use of antibiotics in farming practices, so it is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from food. In addition, the use of antibiotics in animal feeds need to be regulated strongly to minimize the opportunity for organisms to develop resistance (Thi Thu Hao Van et al., 2007). Most of the organisms isolated have being reported to adapt to environmental stress and as a result, it is always a challenge to eliminate them from the environment. This fact is reflected in the physiological study of the isolates.

5.5 Conclusions

i. Aircraft bound meals at the JKIA contain microorganisms majority being *Klebsiellas* Spp and other total counts. In addition, *Staphylococcus aureus* which is pathogenic was isolated.

ii. Cold meals were more contaminated at 68.7 %, than hot meals which recorded 16.3 % contamination. Beef was the most contaminated. Only 15 % of the total meals sampled were not contaminated.

iii. Personal hygiene contributed significantly (p=0.0001) to re-contamination of the food post production. This recontamination was attributed to food handling during portioning.

iv. Aztreonam and Kanamycin were the most sensitive drugs against the bacteria isolated while augmentin and ampicilin were the most reststant.

5.6 Recommendations

To ensure the total safety of aircraft foods, the following recommendations are suggested;

i. Caterers should enhance strict measures through supervision to enhance hygiene in food production especially during portioning and production.

ii. Caterers should strive to source food products from reputable suppliers.
iii. Caterers should discourage handshaking and encourage frequent hand washing during food production.

iv. Training on food safety should be restricted to 3 months for effective food safety practices.

v. Ministry of Public Health must enforce existing food handling regulations such as the public health act and the food, drugs and chemical substances act, food hygiene guidelines and also establish regulations and standards that match or exceeds the ones already in the industry such as AEA guidelines and ensure compliance through frequent audits.

5.7 Further research

The study recommends further research in the following areas;

i) Evaluation of the hygienic status of the cutlery and casseroles used to serve the food once inside the airplane.

ii) Evaluation of the microbiological status of airplanes hand contact surfaces on board.

iii) Investigation on the microbiological quality of the airplanes water.
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Appendix 1: Questionnaire

Answer all questions. All answers will be treated in confidence and used only for structuring hygiene training programmes. You do not need to include your name. Be as honest as possible. Tick or circle as appropriate.

Date:……………………………………………
Department:……………………………………
Sex:……………………………………………..
No of years worked:……………………………
Category of staff: - (tick as appropriate) Unionisable [___] Management [___]
Age: 18-25 [___] 26-35 [___] over 35 years [___]

1. What would you do when receiving food from suppliers?
(a) Immediately store it.
(b) Check to confirm it is in good condition before storage.
(c) Check to confirm it is in good condition, records safety parameters as necessary before storage
(d) Additional comments……………………………………………………………………
(e) I do not know

2. What would you do when a product is expired?
(a) Confirm with relevant authorities for further action
(b) Accept and warn the supplier
(c) Totally reject
(d) Additional comments……………………………………………………………………
(e) I do not know

3. Where do you get the relevant information on food handling?
(a) Internet
(b) From the work place training programme
(c) My immediate supervisor/manager
(d) Nowhere to get information (e) I do not know
4. Which of the following surfaces is best in food processing area?
(a) Wooden  (b) stainless steel  (c) both wooden and stainless  (d) None of these  (e) I do not know

5. If you cut yourself when handling food what would you do?
(a) Get a pink plaster and continue working  (b) Get a plaster and throw away the food  (c) Inform the supervisor and get a plaster  (d) Just continue working  (e) I do not know

6. How often do you use disposable gloves when handling ready to eat foods?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

7. What chopping board colour is used for the following foods in the food unit?
(a) Cooked fish  
(b) Cooked meat  
(c) Raw seafood  
(d) Dairy products  
(e) I do not know

8. How often do you wash your hands when entering the production area?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

9. How would you defrost minced meat?
(a) Under running water  (b) I always receive it defrosted already  
(c) I put it in water  (d) leave it to defrost on the work top  (e) I do not know

10. How do you wash your hands?
(a) With cold water only  (b) With a combination of warm water with the available antiseptic  (c) Cold water and antiseptic  (d) I do not know

11. How would you test if minced meat is properly cooked?
(a) Use of colour  (b) Use of time it has taken  (c) Use of thermometer  (d) I ask the supervisor  (e) I do not know

12. A blue water proof dressing is recommended for dressings of cuts because:
(a) It is cheap  (b) It is lined with antiseptics  (c) It is easily visible  (d) It prevents formation of lot of pus  (e) I do not know
13. Food handlers are not allowed to eat in production areas because:
(a) Time wasted is precious (b) Over fed staff are lazy (c) Cross contamination from the mouth via hands is likely (d) There would always be quarrels over the delicacies (e) I do not know

14. The optimum growth temperature for food poisoning bacteria is?
(a) -18°C (b) 5°C (c) 63°C (d) 37°C (e) I do not know

15. How often in the past 1 year have you worked in production while experiencing diarrhoea or vomiting?
(a) Always (b) Sometimes (c) Rarely (d) Never (e) I do not know

16. What is the safest way of tasting ready to eat foods?
(a) Use of the small finger (b) Use the ladle food cooking (c) Use a spoon and wash it immediately (d) Scoop with bare hands (e) I do not know

17. How often would you wash your hands after handling food products that are raw and cooked?
(a) Always (b) Sometimes (c) Rarely (d) Never (e) I do not know

18. How would you check for doneness of food products?
(a) Visual check (b) Touch (c) Use of a timer (d) Thermometer (e) I do not know

19. What is the safest way of drying utensils?
(a) Air dry (b) Use of wiping cloth (c) Dry using sunlight (d) No need of drying (e) I do not know

20. What would you do if you find food left and exposed in the production area?
(a) Inform the supervisor (b) Assess the food and inquire if safe (c) Throw the food (d) Leave it there (e) I do not know

21. What would you do if the food you assemble has surpassed the temperature required and reads 20°C?
(a) Add dry ice (b) Continue assembling and later put in the cold room (c) Throw away the food (d) Inform the supervisor (e) I do not know
22. How often would you practise the clean as you go policy?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

23. How often would you take portioning food in excess and still return the remainder.
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

24. How often would you wash your gastronorm before portioning new food?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

25. If you used a scoop how would you wash it?
(a) Using tap water  (b) Take it for washing at the pot wash  (c) Wipe it with M-tork
(d) Never wash the scoop  (e) I do not know

26. How often would you blast chill contents from canned containers?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

27. How often would you shake hands while in the production floor?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

28. When using a chopping board and is soiled what would you do?
(a) Take it for washing  (b) Turn it on the other side  (c) Wipe it with M-tork  (d) Continue with it as it is.  (e) I do not know

29. If the food you are portioning drops on the table what would you do?
(a) Leave it on the table  (b) Return it on the plate  (c) Throw it in the dustbin
(d) Eat it.  (e) I do not know

30. If you discover foreign matter in any food that you are handling, what would you do?
(a) Remove it with bare hands  (b) Leave it and inform the supervisor  (c) Inform the supervisor  (d) Remove it safely and inform the supervisor  (e) I do not know

31. If you enter a cold room and feel the temperature is high what would you do?
(a) Continue with your work  (b) Inform the supervisor  (c) Ask your colleague to check it  (d) Call the technical department  (e) I do not know
32. If you enter the cold room and find chicken and vegetables mixed together what would you do?
(a) Continue with what you had   (b) Separate the products   (c) Inform the supervisor   (d) Ask the person responsible to have a look at it   (e) I do not know

33. How would you thaw frozen meat products?
(a) Under warm running water   (b) On top of the oven (c) In the cold room at controlled temperatures   (d) Leave it on the table to thaw   (e) I do not know

34. How long is it safe to keep hot meal foods in the cold room?
(a) 24 hours   (b) As long as it does not smell   (c) 48 hours   (d) 3 days   (e) I do not know

35. How long is it safe to keep cold meal in the cold room?
(a) 24 hours   (b) As long as it does not smell   (c) 48 hours   (d) 3 days   (e) I do not know

36. If there is a previous day food in the cold room and is required what would you do?
(a) Immediately pack the food into ovens   (b) Smell it and give it   (c) Assess the food and ask the supervisor if it can be taken (d) Refuse to give it   (e) I do not know

37. If you are opening a canned product and discover it is dented what would you do?
(a) Ask the supervisor to warn the supplier   (b) Return the can to the stores   (c) Continue opening it for usage   (d) Look for another and leave the dented one.   (e) I do not know

38. When using un-sanitized vegetables and the staff personnel for washing is absent what would you do?
(a) Return the product and wait for the staff to come (b) Wash it myself   (c) Use it as it is   (d) Inform the supervisor   (e) I do not know

39. If you sight a pest what would you do?
(a) Kill it   (b) Inform the supervisor   (c) Chase the pest away   (d) Ignore it   (e) I do not know

40. How often would you check the colour code before using a particular food?
(a) Always   (b) Sometimes   (c) Rarely   (d) Never   (e) I do not know
41. How often would you write the temperature records?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

42. If the temperature forms are missing what would you do?
(a) Inform the person responsible to get the forms  (b) Continue with the work  (c) Write the temperature somewhere  (d) Inform your colleagues on the same  (e) I do not know

43. How long would you do take to assemble food?
(a) Less than 45 mins  (b) As long as it takes to finish  (c) 1 hour  (d) I don’t know

44. If there is no soap at the toilets what would you do?
(a) Ignore the issue  (b) Try to look for the soap elsewhere  (c) Ask the cleansing staff to provide one  (d) Inform the supervisor  (e) I do not know

45. What is your average time of washing hands when in production?
(a) 3sec  (b) 5 sec  (c) 20secs  (d) 10secs  (e) I do not know

46. How often do you change your gloves?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

47. How often do you change your apron?
(a) Always  (b) Sometimes  (c) Rarely  (d) Never  (e) I do not know

48. How often have you encountered rats in production area during the last 1 month?
(a) Never  (b) Daily  (c) Weekly  (d) Monthly  (e) I do not know

49. How often have you encountered flies in production areas during the last 1 month?
(a) Never  (b) Daily  (c) Weekly  (d) Monthly  (e) I do not know

50. When did you last undergo the food safety & hygiene training?
(a) 3 months ago  (b) 6 months ago  (c) 9 months ago  (d) Over 1 year now  (e) I do not know
Appendix 2: The Food Production Chain

- DELIVERY RECEIPT
- STORAGE
- DEFROST IF FROZEN
- COOKING
- CHILLING
- ASSEMBLY
- PACKING
- DISPATCH
Appendix 3: Sensitivity profile of isolates from various food samples

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<td>I</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>2</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Dessert tiramisu &amp; chocolate sauce</td>
<td>9</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Starter lettuce tomato, carrots cucumber</td>
<td>3</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Shredded cabbages</td>
<td>8</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Chicken sandwich</td>
<td>2</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>S</td>
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</table>

**Key:**

AM(Ampicilin)  TE(Tetracyclin)  SXT(Sulphamethoxazole)  AUG(Augmentin)  CN(Gentamicin)  
CEFO(cefotaxime)  CEFT(Ceftazidine)  AZT(Aztreonam)  C(Chloramphenol)  CXM(Cefuroxime)  
CEFI(Cefixime)  K(Kanamycin)

Appendix 4: Media Preparation

1. Sulphur-indole motility decarboxylase medium (SIM OXOID CM 435)

SIM medium dehydrated 30 g and 1000 ml distilled water. Boil to dissolve completely. Dispense in 4 ml aliquots into 13 X 100 mm screw cap tubes. Sterilize by autoclaving at 121°C for 15 minutes and store at 4°C until use.

2. Simmons citrate agar (OXOID CM 155)

Simmons citrate agar 23 g and 1000 ml distilled water. Boil to dissolve completely. Dispense in 2 ml aliquots into 13 X 100 mm tubes. Sterilize by autoclaving at 121°C for 15 minutes, set to cool in a slanted position and store at 4°C. Positive control is *Klebsiella pneumoniae* ATCC 13883 while negative control is *Escherichia coli* ATCC 25922.

3. Triple sugar iron (TSI) agar (OXOID CM 277)

TSI agar dehydrated 65 g and 1000 ml distilled water. Boil to dissolve completely. Dispense into test tubes and autoclave at 121°C for 15 minutes. Position in a slant position to form a slant and a butt. Allow to solidify and store at 4°C until use. Positive and negative Controls are *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315 and *Salmonella enteritidis* ATCC 13076

4. Tryptone Soya broth glycerol medium (15%)

Tryptone Soya broth base (OXOID CM 129) 30 g, glycerol (BDH 284546F) 150 ml and distilled water 850 ml. Dispense in 1 ml amounts in cryovial and autoclave at 121°C for 15 minutes. Cool and store at 4°C until use.

5. Urea agar (OXOID CM 53)

Urea agar base dehydrated 2.4 g and distilled water 95 ml. Boil completely to dissolve, sterilize by autoclaving at 121°C for 15 minutes. Cool to 50 – 55°C. Aseptically add 5 ml sterile 40 % urea solution SR 20 (OXOID). Mix well and dispense into 2 ml aliquots in sterile containers and allow setting in slope position and storing at 4°C. Positive control is *Proteus vulgaris* ATCC 13315 while negative control is *Escherichia coli* ATCC 25922
6. Blood agar (OXOID CM 331)

Blood agar base dehydrated 40 g and 1000 ml distilled water. Boil to dissolve completely. Sterilize by autoclaving at 121⁰C for 15 minutes. Cool to 45 – 50⁰C and add 7 % sterile blood. Mix in gentle rotation and pour onto sterile petri dishes. Positive control is *Staphylococcus aureus* ATCC 25623, 1999 while negative control is uninoculated media.

7. Mac Conkey agar (OXOID CM 7)

MacConkey agar dehydrated 52g and distilled water 1000ml. Boil carefully to dissolve completely. Autoclave at 121⁰C for 15 minutes. Cool to 50⁰C and dispense into sterile petri dishes. Allow solidifying completely and store at 2 – 8⁰C until use. Positive control is *Escherichia coli* ATCC 25922 while negative control is *Enterococcus faecalis* ATCC 29212.

8. Mcfarland standard NO 0.5

Add 0.5 ml of a 1.175 % solution of barium chloride dehydrate (BACL₂H₂O) to 99.5 ml of 0.36 N (1%) sulphuric acid. Dispense 5ml aliquots into screw cap bijou bottles and seal with cap. The turbidity standard can be stored in the dark at room temperature for 6 months or more provided the bottle is sealed to prevent evaporation. The standard must be thoroughly mixed just before use preferably on a vortex mixer.

9. MRVP medium (OXOID CM43)

MRVP dehydrated 15 g and 1000 ml distilled water. Suspend the medium in distilled water. Mix well and distribute into final containers and sterilize by autoclaving at 121⁰C for 15 minutes. Store at 4⁰C until required. MR positive is *Escherichia coli* ATCC 25922 while MR negative is *Klebsiella pneumoniae* ATCC 13883. The VP positive is *Enterobacter cloacae* ATCC 23355 while VP negative is *Escherichia coli* ATCC 25922.

10. Mueller-Hinton agar (OXOID CM337)

Mueller-Hinton dehydrated medium 38 g and 1000 ml distilled water. Suspend in distilled water. Boil to dissolve and autoclave at 121⁰C for 15 minutes cooling to 50⁰C and pour into sterile petridishes. Store at 4⁰C until required. Positive controls are *Escherichia coli* ATCC
25922 and *Staphylococcus aureus* ATCC 25923 while negative control is uninoculated media.

11. Baird-Parker agar base (OXOID CM 275)

Baird-parker agar base dehydrated 63g and 1000 ml distilled water. Suspend the medium in distilled water and boil to dissolve completely. Autoclave at 121 °C for 15 minutes and cool to 50 °C and aseptically add 50 ml of Egg yolk tellurite emulsion (OXOID SR 54). Mix well before pouring into sterile petridishes and storing at 4 °C until use. Positive control is *Staphylococcus aureus* ATCC 25923, while negative is *Escherichia coli* ATCC 25922.

12. *Bacillus cereus* selective agar (OXOID CM 617)

*Bacillus cereus* agar dehydrated 41g and 1000 ml distilled water. Suspend the medium in distilled water and autoclave at 121 °C for 15 minutes. Cool to 50°C and aseptically add the contents of two vials of *Bacillus cereus* selective supplements (OXOID SR 99) reconstituted with 4 ml of sterile distilled water then add 50ml egg yolk emulsion (OXOID SR 47). Mix well and pour into sterile petri dishes and store at 4 °C until use. Positive control is *Bacillus cereus* ATCC 10876 while negative is Uninoculated media.

13. Buffered peptone water (OXOID CM 509)

Buffered peptone water agar dehydrated 20 g and distilled water 1000 ml. Suspend the medium in distilled water. Sterilize by autoclaving at 121 °C for 15 minutes. Positive control is *Salmonella typhimurium* ATCC 14028 while negative is uninoculated media.

14. Violet red bile agar (VRBBA) (OXOID CM 107)

VRBA Agar dehydrated 38.5g and distilled water 1000 ml. Suspend the medium and bring to boil to completely dissolve. Mix well before pouring into sterile petri dishes. And store at 4 °C until use. Positive control is *Escherichia coli* ATCC 25922 while negative is *Staphylococcus aureus* ATCC 25923.

15. Eosin methylene blue (EMB) (OXOID CM 69)

EMB Agar dehydrated 37.5g and distilled water 1000 ml. Suspend medium in distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 °C for 15
minutes. Cool to 60 °C and shake the medium to oxidise the methylene blue and suspend the precipitate that is an essential part of the medium. Positive control is *Escherichia coli* ATCC 25922 while uninoculated medium used as negative control.

16. Xylose-lysine-decarboxylase (XLD) AGAR

XLD Agar dehydrated 53g and distilled water 1000 ml. Suspend the medium in distilled water. Heat with frequent agitation until the medium boils. Do not overheat. Transfer immediately to a waterbath at 50 °C. Pour into sterile petri dishes as soon as the medium has cooled. Positive control is *Salmonella Typhimurium* ATCC 14028 while negative is *Escherichia coli* ATCC 25922.

17. Listeria selective agar (OXFORD) (OXOID CM 865)

*Listeria* selective Agar dehydrated 27.75g and distilled water 500 ml. Suspend medium in distilled water and gently boil to dissolve completely. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and aseptically add one vial of *Listeria* selective supplement (OXOID SR 140) reconstituted with 5 ml ethanol / sterile distilled water (1:1). Mix well and pour into sterile petri dishes and at 2 – 8 °C until use. Positive control is *Listeria monocytogenes* ATCC 19112 while uninoculated media as negative.

18. Mac Conkey broth purple (OXOID CM5a)

Mac Conkey broth purple agar dehydrated 40g and distilled water 1000 ml. To prepare single strength broth, suspend media in distilled water. Distribute into containers fitted with fermentation Durham tubes. Sterilize by autoclaving at 121 °C for 15 minutes. Positive control is *Escherichia coli* ATCC 25922 while uninoculated media used as negative.
Appendix 5: API Listeria Reading Table

<table>
<thead>
<tr>
<th>TEST</th>
<th>ACTIVE INGREDIENT</th>
<th>QUANTITY (mg/cup)</th>
<th>REACTIONS</th>
<th>NEGATIVE</th>
<th>POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIM</td>
<td>Enzyme substrate</td>
<td>0.106</td>
<td>Differentiate <em>L. innocua</em> and <em>L. monocytogenes</em> ZYM B / &lt; 3 minutes Pale Orange, Pink beige, Grey beige</td>
<td>ZYM B / &lt; 3 minutes Orange</td>
<td></td>
</tr>
<tr>
<td>ESC</td>
<td>Esculin Ferric citrate</td>
<td>0.16</td>
<td>Hydrolysis (Esculin) Pale yellow</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>AMAN</td>
<td>4-nitrophenyl-α D-mannopyranoside</td>
<td>0.045</td>
<td>A-α-manosidase Colorless</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td>DARL</td>
<td>D-Arabitol</td>
<td>0.4</td>
<td>Acidification (D-Arabitol) Red/orange</td>
<td>Yellow / yellow orange</td>
<td></td>
</tr>
<tr>
<td>XYL</td>
<td>D-xylose</td>
<td>0.4</td>
<td>Acidification (D-xylose)</td>
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<td></td>
</tr>
<tr>
<td>RHA</td>
<td>L-Rhamnose</td>
<td>0.4</td>
<td>Acidification (L-Rhamnose)</td>
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<tr>
<td>MDG</td>
<td>Methyl-α-D-gluco pyranoside</td>
<td>0.4</td>
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<td>RIB</td>
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<tr>
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<td>D-Tagatose</td>
<td>0.4</td>
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NOTE: The quantities indicated might be adjusted depending on the titer of the raw materials used. Certain cupules contain products of animal origin, notably peptones.
### Appendix 6: Reading Table For API 20 E

<table>
<thead>
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<th>TEST</th>
<th>SUBSTRATE</th>
<th>REACTION</th>
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<th>POSITIVE</th>
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<tr>
<td></td>
<td>Ortho-nitro-β-galactopyranoside (ONPG)</td>
<td>Isopropylthiogalactopyranoside</td>
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<tr>
<td>ONPG</td>
<td>β-galactosidase</td>
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<td></td>
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<tr>
<td>ADH</td>
<td>Arginine</td>
<td>Arginine dihydrolase</td>
<td>Yellow</td>
<td>Red / orange (2)</td>
</tr>
<tr>
<td>LDC</td>
<td>Lysine</td>
<td>Lysine decarboxylase</td>
<td>Yellow</td>
<td>Red / orange (2)</td>
</tr>
<tr>
<td>ODC</td>
<td>Ornithine</td>
<td>Ornithine decarboxylase</td>
<td>Yellow</td>
<td>Red / orange (2)</td>
</tr>
<tr>
<td>CIT</td>
<td>Sodium citrate</td>
<td>Citrate</td>
<td>Pale green/yellow</td>
<td>Blue green/ blue (3)</td>
</tr>
<tr>
<td>H₂S</td>
<td>Sodium thiosulphate</td>
<td>H₂S production</td>
<td>Colorless/grey</td>
<td>Black deposit</td>
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<td>URE</td>
<td>Urea</td>
<td>Urease</td>
<td>Yellow</td>
<td>Red / orange (2)</td>
</tr>
<tr>
<td>TDA</td>
<td>Tryptophane</td>
<td>Tryptophane deaminase</td>
<td>Yellow</td>
<td>Redish brown</td>
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<td></td>
<td></td>
<td>Indole production</td>
<td>JAMES/ Immediate</td>
<td>JAMES/ Immediate</td>
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<td></td>
<td></td>
<td></td>
<td>Colorless,</td>
<td>Red/ Pink, pale green, yellow Diffusion of black pigment</td>
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<tr>
<td>IND</td>
<td>Creatine</td>
<td>Acetone production</td>
<td>VP 1 + VP 2 / 10 minutes</td>
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<tr>
<td>VP</td>
<td>Sodium pyruvate</td>
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<td>Blue/ blue green</td>
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<td>Glucose</td>
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<tr>
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</tr>
<tr>
<td>OX</td>
<td>See oxidaseCytochrome oxidase</td>
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<tr>
<td></td>
<td>Potassium nitrate</td>
<td>NO₂ Production, N₁ + N₂ / 2-5</td>
<td>Yellow. ZN / 5 Red.</td>
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<tr>
<td></td>
<td>reduction to N₂ gas</td>
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