T.C.
YEDITEPE UNIVERSITY
INSTUTUTE OF HEALTH SCIENCES
DEPARTMENT OF NUTRITION AND DIETETICS

DETERMINATION OF MICROBIAL LOAD IN FAST FOOD MEALS

MASTER THESIS

BUSE SARIKAYA

İstanbul- 2017

T.C.

YEDITEPE UNIVERSITY

INSTUTUTE OF HEALTH SCIENCES

DEPARTMENT OF NUTRITION AND DIETETICS

DETERMINATION OF MICROBIAL LOAD IN FAST FOOD MEALS

MASTER THESIS

BUSE SARIKAYA

SUPERVISOR

Assit. Prof. DR. İSKENDER KARALTI, PhD.

İstanbul-2017

APPROVAL

TEZ ONAYI FORMU

Kurum : Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü

Program : Beslenme ve Diyetetik Yüksek Lisans

Tez Başlığı : "Tüketime Hazır Yiyeceklerdeki Mikrobiyal Yükün Belirlenmesi"

Tez Sahibi : Buse SARIKAYA

Sınav Tarihi : 02/05/2017

Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Yüksek Lisans Tezi olarak kabul edilmiştir.

	Unvanı, Adı-Soyadı (Kurumu)	İmza
Jüri Başkanı:	Yrd. Doç. Dr. İskender KARALTI	
Tez danışmanı:	Yrd. Doç. Dr. İskender KARALTI	
Üye:	Yrd. Doç. Dr. Hülya DEMİR	
Üye:	Doç. Dr. Burhan ŞEN	
Üye:		

ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun ..18../..05../..2017..tarih ve ..2017/09-01..sayılı kararı ile onaylanmıştır.

İmza

Prof. Dr. Bayram YILMAZ

Sağlık Bilimleri Enstitüsü Müdürü

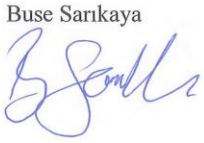
DELARATION

DECLARATION

I hereby declare that this thesis contains my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

29.05.2017

Buse Sarıkaya



ACKNOWLEDGEMENTS

I would first like to thank my supervisor Assit. Prof. DR. İSKENDER KARALTI for his valuable and constructive suggestions, guidence, support and patience during this study. All the microbial analysis of this study he conducted has been very much appreciated.

I would also like to thank to the staff of the microbiology laboratory of Yeditepe University.

I would like to extend my thanks to all the instructors of Department of Nutrition and Dietetics at Yeditepe University for their contribution to my master education. They always have been kind and supporting individually.

Finally I would like to express my profound gratitude to my family for supporting me materially and spiritually throughout my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF SYMBOLS

μm : Micron meter

μl : Micro litre

cm3 : Cubic centimeter

% : Percentage

g : Gram

ml : Mililitre

mm : Milimeter

°C : Celcius

H_sS : Hydrogen sulfide

log : logaritma

LIST OF ABBREVIATIONS

CDC : The Centers for Disease Control and Prevention

WHO : World Health Organisation

CNS : Coagulase Negative Staphylococcus

EMB : Eosin MEthylene Blue Plates

BAP : Sheep Blood Agar Plates

SS : Salmonella Shigella

CFU : Colony Forming Unit

TS : Turkish Standards

Spp : Speices

ABSTRACT

Sarıkaya B. (2017). Determination Of Microbial Load In Fast Food Meals. Yeditepe University, Institute of Health Sciences, Department of Nutrition and Dietetics, MSc thesis. İstanbul.

Fast food consuming has increased due to its practicality, taste and apperance. Furthermore food borne illness is one of the most frequent public health problem in both develop and developing countries. Food safety of fast foods including meat, poultry and fish which are sold by small scaled shops and vendors have become issue of concern by science reseachers. In this study, 50 of samples that were used fast foods such as hamburger, döner were purchased from a local fast food restaurants in Istanbul/Kadıköy and analized in the terms of microbiological quality and food safety. In the study, speices of pathogen microoganisms which were found in the samples were defined by microbial methods. Samples were incubated on culture media and kept at the 37 °C for 48 hours in order to detect microorganisms. Gram staining method was used to differentiate Gram-negative and Gram-positive bacteria. Biochemical tests were used for the species which could not be defined by direct microscopic count method. Catalase test was used to differentiate *Staphylacoccus/ Streptococcus*. Coagulase test was used to identify of *S.aureus*. Oxidase test was used to identfy Gram-negative bacteria speices. VITEC 2.0 ve API®/ID32 C microbiology identification systems including a variety of microbial identification systems and cards were used as extented indentification. In the study, no growh of microorganism was detected in 17 (34%) samples in 50. From 33 samples which were pathogenic microorganisms isolated, , 3- 3,47 log cfu/g *Coagulase Negative Staphylococcus* was isolated in 2 samples (4 %), 3-4 log cfu/g *S.aureus* was isolated in 8 samples (16 %), 3 -6 log cfu/g *Streptococcus* was isolated in 8 samples (16%), 4,6 log cfu/g *Salmonella spp.* was isolated in 1 sample (2%), 3-6 log cfu/g *Klebsiella pneumoniae* was isolated in 5 samples (10%) and 3-4,90 log cfu/g *E.coli* was isolated in 6 samples (12%), 3 log cfu/g *Enterobacter spp.* was isolated in 1 sample (2%), 12 cfu/g *Aspergillus spp.* was isolated in 3 samples (6%), 9 cfu/g *Penicillum spp.* was isolated in 9 samples (18%), 1 cfu/g *Rhizopus spp.* was isolated in 1 sample (2%) and 2 cfu/g *Rhodotorula spp.* was isolated in 2 samples (4%). Total microbial colony count was detected between 3 log cfu- 6,0047 log cfu/g. Total microbial load is log 4,93 cfu/g in all samples, 4,69 log

cfu/g in beef samples, 5.10 cfu/g in chicken samples, 3 log cfu/g in fish sample on average And it is determined that cooked chicken döner has lower microbiological quality than meat döner in our study. Detection of undesired bacterias in food which are risks of health shows hygenical conditions are inadequate. Personnel hands are thought to be reason of contaminations. In the light of the results of this study indicates microbial criterias must be well noticed in the stages from the selection of raw materials, storage, preparation to service.

Key words: Microbial load, fast food, food safety, foodborne pathogens.

ÖZET

Sarıkaya B. (2017). Fast Food Tipi Besinlerdeki Patojenlerin İncelenmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik ABD, Master Tezi. İstanbul.

Hazır besinlerin tüketimi günümüzde pratikliği, çekici tat ve görüntüsü sebebiyle artış göstermektedir. Bununla birlikte besin kaynaklı hastalıklar gelişmiş ve gelişmekte olan ülkelerde sıkça rastlanan halk sağlığı sorunları arasındadır. Küçük işletmelerde ve sokakta satılan et, tavuk, balık içeren hazır besinlerin güvenilirliği pek çok bilim araştırmacısı için merak konusu olmuştur.Bu çalışmada İstanbul/Kadıköy merkezde bulunan farklı işletmelerden seçilen, hayvan etleri içeren 50 adet hamburger, döner ve benzeri pişmiş hazır yiyecekler mikrobiyolojik kalite ve besin güvenilirliği yönünden incelenmiştir. Çalışmada numunelerden alınan örneklerde bulunan patojen mikroorganizmalar mikrobiyolojik yöntemlerle tür düzeyinde tanımlanmıştır. Toplanan numunelerdeki mikroorganizmaları tespit etmek amacıyla, kültür medyalara inkübe edilen örnekler, 37 °C'de 48 saat bekletilmiştir. Tanımlamada Gram boyama yöntemi kullanarak Gram positif- Gram negatif bakteriler ayırt edilmiştir. Direk mikrokopik incelemeler sonucunda tayin edilemeyen türler için ayırt edici biyokimyasal testler uygulanmıştır. *Staphylococcus/ Streptococcus* ayrımında katalaz testi kullanılmıştır. S.aureus tanımlamada koagulaz testi, Gram negatif bakteri tayininde oksidaz testi kullanılmıştır. Tür düzeyinde daha ileri mikrobiyal tanımlama için, birçok sayıda mikrobiyal tanımlama sistemlerinden ve tanımlama kartlardan oluşan VITEC 2.0 ve API®/ID32 C ticari tanı sistemleri kullanılmıştır. Çalışmada 50 örnekten 17'sinde patojen mikroorganizma üremesi tespit edilmemiştir. Patojen mikroorganizma varlığının tespit edildiği 33 örnekten 2'sinde (%4) 3-3,47 log kob/g *Koagulaz Negatif Staphylacoccus*, 8'inde (%16) 3-4 log kob/g *S.aureus*, 8'inde (%16) 3-6 log kob/g *Streptococcus*, 1'inde (%2) 4,6 log kob/g *Salmonella spp.*, 5'inde (%10) 3-6 log kob/g *Klebsiella spp.*, 6'sında (%12) 3-4,90 log kob/g *E.coli* ve 1'inde (%2) 3 log kob/g *Enterobacter spp.*, 3'ünde (%6) 12 kob/g *Aspergillus spp.*, 9'unda (%18) 9 kob/g *Penicillium spp.*, 1'inde (%2) 1 kob/g *Rhizopus spp.*ve 2'sinde (%4) 1 kob/g *Rhodotorula spp.* tayin edilmiştir. Toplam koloni sayısı 3 log kob/g – 6.0047 kob/g arasında bulunmuştur. Toplam mikrobiyal yükün ortalaması tüm örneklerde 4,93 kob/g, et örneklerinde 4,93 kob/g, tavuk örneklerinde 5.10 kob/g , balık örneğinde 3

log kob/g. Tavuk örneklerinin et örneklerine göre mikrobiyal kalitesinin daha düşük olduğu bulunmuştur. Bu çalışmada örneklerde, besinlerde istenmeyen ve sağlık açısından ciddi risk oluşturan mikroorganizmaların saptanması hijyenik koşulların yetersiz kaldığını göstermektedir. Bulaşların özellikle besin hazırlayıcıların ellerinden kaynaklandığı düşünülmektedir. Sonuçlar tüketime hazır besinlerinde hammadde seçiminden, depolama, hazırlama ve servis aşamalarına kadar mikrobiyal kriterlere daha dikkatli uyulması gerektiğini göstermiştir.

Anahtar kelimeler: Mikrobiyal yük, hazır besinler, besin güvenilirliği, besin kaynaklı patojenler.

1 INTRODUCTION AND AIM

Foodborne diseases occur in both developed and developing countries and become widespread health problem by growing[1]. According to The Centers for Disease Control and Prevention (CDC) estimated datas, foodborne diseases are cause of illness 1 in 6 Americans (aproximately 48 million people). And these illnesses are resulting in 128.000 hospitalization and 3.000 deaths[2]. Foodborne diseases are generally transmitted through ingested foods. Foodborne diseases consist of broad group of illnesses by enteric pathogens, chemical contaminants, parasites and biotoxins[1].

Accessing is getting easy to high number of fast food restaurants. That results in greater consumption of fast foods[3]. Diet and fast food opportunities and their relation have become an interest in food enviromental research[4]. Outbreaks of foodborne illnesses result in considerable cost to individuals, the food industry, economy in general and community health systems. So food safety is directly critial for human health and indirectly to economy[5].

Microorganisms are living organisms. They are microscopic sized: Bacteria, virus, yeast and mold (These can defined as fungi), algae and protozoa. Food handling has various stages such as storage, production, serving and comsumption. While food is being processed in these stages, many pathogenic microorganisms may cause contamination[6]. Foodborne pathogens have been recognized by many years. Still some new kind of pathogens occour. The main pathogens are *Escherihia coli O157:H7, Salmonella Typhimurium Definitive Type 104, Arcobacter butzleri, Helicobacter pylori. Listeria monocytogenes, Enterococcus, Campylobacter jejuni, Salmonella enteritiditis, Vibrio vulnificus, Mycobacterium avium subsp. paratuberculosis, Enterobacter sakazak*ii have been recognized as pathogens for many years but they have been determined to be primarily foodborne in the past two decades. Many factors are playing important role in the epidemiology of new emerging and reemerging foodborne pathogens. These factors are dietary habits, new changes in health sector, economical and technological developments, changes in the pathogens, poverty and pollution. Food vehicles of transmission, increasing in travel and migration, demographic changes and trade in food and animal feed can be added[7].

Food safety regulatory agencies are responsible to control broad range of food commodities. These agencies regulate multiple commodities effectively. Thus they should develop policies to distribute resources by considering their magnitudes of the risk of the illness. Each of the commodities reveals to the broad consumer population[8].

The study aimed to detect of potantial risk of fast foods, food hygiene of restaurants, microbiological quality of foods, microorganisms contaminating with foods, through identifying pathogens from fast foods which were collected from 50 different restaurant.

Result of the study can shed light on awareness of microbial effects in foods under low hygiene conditions, designing methods to provide better hygiene conditions, food regulations by applying standards in food production.

1.1 INTRODUCTION

Foods of plant origin and foods of animal origin carry a microflora on the surface of their parts. Besides this, animals have an intestinal microflora. Contamination of both plants and animals may occur from outside sources. It is reported that in the inner health tissues of animals and plants a few or none microorganisms live.

Animals are collected and slaughtered, the fruit and vegetable are harvested, fish and other producsts are caught from natural waters, milk is drawn. All food have their own usual microflora. After initial handling, further contamination begins and it continues during processing and preparing till servicing.

Food can be contaminated by each other or by equipment pieces. Air, water and ingredients, dust may be added to their total contaminants. When food is handled personally, there is always risk of addition of human pathogens[9].

Muscle food is popular in human nutrition because of their nutrient density and palability. Poultry products are sold in fast food markets. Consumption of processed meat has been gaining popularity rapidly. During the preparation of fast food, many different cooking methods and several types of spice treatment have been processing[10].

Döner kebab is a traditional food which is produced with beef, lamb or chicken. That product is popular in fast food outlets, restaurants in Turkey and other Middle Eastern countries. Also it is served in Europe, Canada and Usa due to immigration and transmission of cultures.

During production of the döner kebab, meat (1-6 mm thickness), minced meat, and tallow (2-4 mm thickness) are marinated during 3-6 hours. Spice mixture is added during marination such as white pepper, black pepper, allspice, thyme, cumin. According to demands of costumer or manufactures ingredients may be different. Salt, onion, onion powder or onion juice, tomato juice or tomato sauce, grated tomato, olive oil, vinegar or lemon juice, milk or milk powder, egg, yoghurt, grape juice or white sugar also can be added to ingredients for the marination. After marination, meat, tallow and minced meat are put on a döner kebab stick in order and shaped into a cone[11]. The döner stick with meat is being grilled on one side (by turning) in vertically and it is cooked then it is cut as thin slides. The period time is about 1-2 days. Inner side of the döner meat remains raw because it is heat treated to only the side which is required. During grilling, temperature reaches 40 °C. The flora is associated with ripening and hygienically, it is important for microorganisms to multiply[12].

Several factors play role in microbiological quality of products which are purchased by the consumer. These are efficacy of the cooking process, quality of the raw materials, sanitation of the place where döner is produced and personal hygiene. Potential problems may appear if low quality meat is used or the product is undercooked. When a left over döner is reheated, it becomes even more hazardous[10].

2 GENERAL CHARACTERISTICS OF MICROORGANISMS

Every determined type of the plant or animal microflora is effected by environmental conditions. Every food can be contaminated from outside sources on the way from field to the processing, storage, serving, transport and distbution as well. There are many different types of microorganisms in everywhere, soil and water, air, on foods, and in the digestive tract of both animals and humans. While the majority of the microorganisms have beneficial functions in the environment, in branches of food industry, such as wine production, bakery, beer production, dairy products, some other kind of microorganisms cause unwanted spoilage by contamination of food with pathogens. That further results in food safety problems.

The mostly isolated microorganisms on food are divided into three groups practically: Mold, yeast and bacteria. Molds are supposed to cause spoilage of foods, They are used restrictedly (for example mold ripened cheese). Yeasts are supposed to ferment sugars to ethanol and carbondioxide. Due to their ability of fermenting, they are the most widely used in food industy. Some types of yeasts are grown in the industry (such as baker's yeast), and some can be used as protein sources mainly in animal feed.

The most common isolated bacteria from foods can be divided into groups accourding to end product of the fermantation: Acetic acid bacteria, lactic acid bacteria, propionic acid bacteria.

Systematic classification of bacterias is divided according to their physiological and morphological properties (e.g. gas forming bacteria, aerobic and anaerobic bacteria, , etc). Lactic acid bacteria is widely used in the dairy industry. Acetic acid bacteria is widely used in vinegar production. Many bacteria causes spoilage. Some of them are pathogens such as *Salmonella spp. and Staphylococcus*[6].

2.1 CLASSIFICATION OF MICROORGANISMS

All kind of organisms are divided in three domains:

1. Bacteria (Their cell walls contain a protein-carbohydrate complex called peptidoglycan)

2. Archaea (Their cell walls lack peptidoglycan if exist)
3. Eukarya, in two domains (Organisms which exist in foods) as follows:
    a. Protists (slime molds, protozoa, and algae)
    b. Fungi (unicellular yeasts, multicellular molds, and mushrooms)[13]

2.2 MOSTLY ISOLATED MICROORGANISM IN FOODS

2.2.1 BACTERIA

They are unicellular. Their size about 0,2 x 10 μm[6]

1. Prokaryotic (Their genetic material is not surrounded by a nuclear membrane)
    - Prokaryotes consist of the bacteria and archaea
3. Bacteria has seen in one of several shapes:
    a. *bacilli* (rodlike),
    b. *coccus* (spherical),
    c. *spiral* (corkscrew or curved),
    d. Some are star-shaped or square.
4. Individual bacteria can form pairs, chains, clusters, or other groupings.
5. They are enclosed in cell walls largely composed of peptidoglycan (carbohydrate and protein complex).
6. Bacteria reproduce by binary fission (division into two equal cells).
7. Most bacteria use organic chemicals which are derived from dead or living organisms for nutrition.
8. Some bacteria produce their food by photosynthesis[13].
9. Some bacteria can derive nutrition from inorganic substances[13].
10. Bacteria can be motile or nonmotile[6].

11. Bacteria can utilize food nutrients and grow in suitable conditions depending on wide range of temperatures, oxygen, ph and water activity[6].

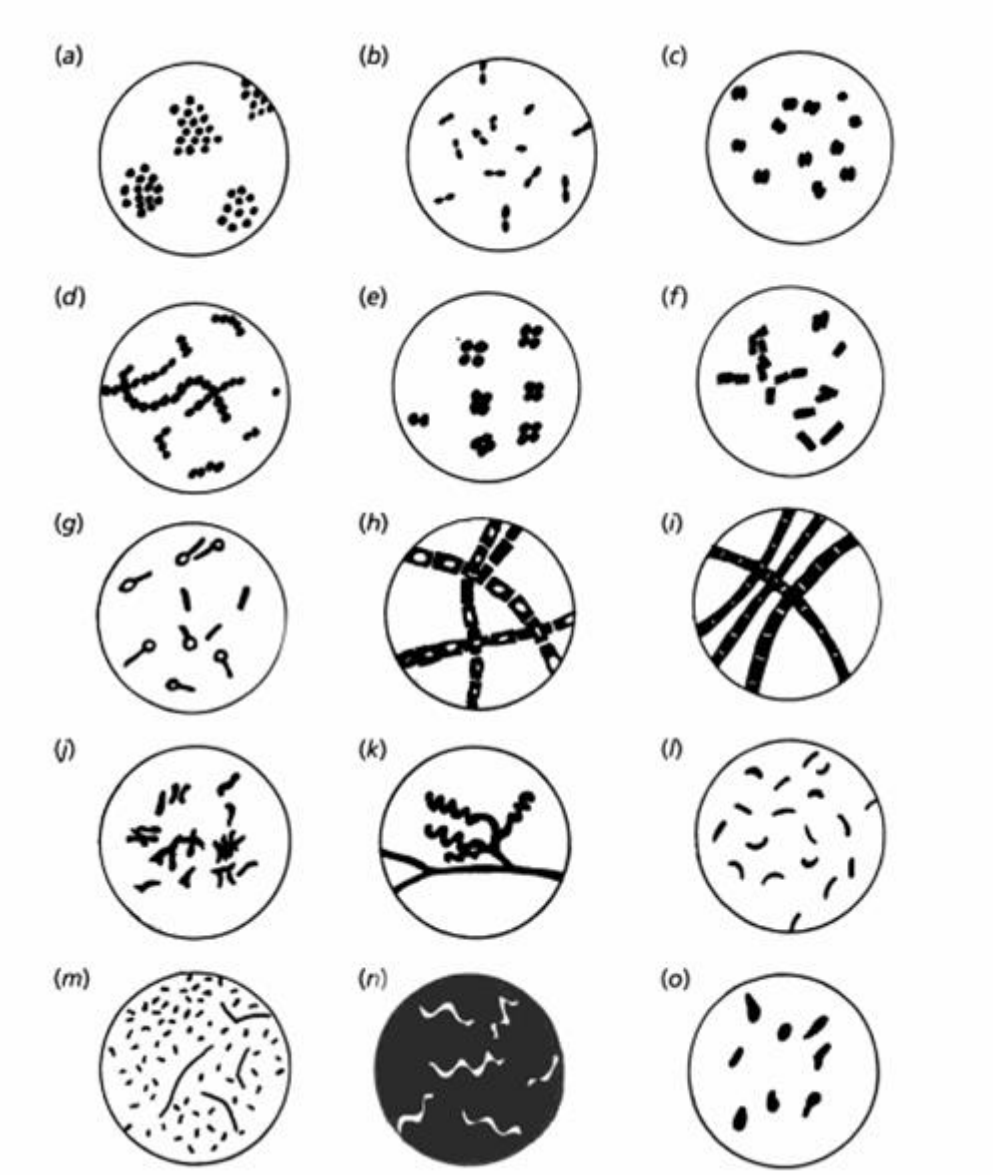
Types of microorganisms	Minimum (°C)	Optimum (°C)	Maximum (°C)
Psychrophiles	0	15-25	30
Mesophiles	10	37	43
Thermoplies	25	50- 65.5	85

Table 1: Temperature range for growth of microorganisms[14]

Microorganism	Minimum water activity
Normal bacteria	0.90
Normal yeast	0.88
Normal moulds	0.80
Halophilic bacteria	0.75
Dryness resistant moulds	0.05
Osmotic pressure resistant yeast	0.61

Table 2: Minimum water activity for growth of microorganisms[14]

2.2.1.1 *MICROSCOPIC* APPERANCE OF BACTERIA



(a*) S.aureus* (b) *Streptococcus pneumonie* (c) *Neisseria gonorrhoeae* (d) *Streptococcus pyogenes sarcina spp*. (f) *Colostridum perfringens* (g) *Colostridum tetani* (h) *Bacillus anthracis* (i) *Beggiatoa spp.* (j) *Corynebacterium diphtheriae* (k) *Streptomyces viridochromogenes* (l) *Vibro cholerae* (m) *Haemophilus influenzae* (n) *Borrelia recurrentis* (o) *Bacteriods of Rhizobium tifolii*.

Figure 1: Microscopic Apperance of Bacteria[15]

2.2.1.2 MOSTLY ISOLATED BACTERIAL PATHOGENS IN FOOD

Salmonella spp.

Salmonella spp. are from *Enterobacteriacea* family, Gram-negative, nonspore, rod shaped, facultative anaerobic, catalase-positive, oxidase-negative and motile. Growth temperature ranges from 2°C to 48 °C. *Salmonella spp*. naturally exist in gastointestinal tract of animals and birds. They can cause an illness called Salmonellosis.

Both animal and and animal food are important carrier of *Salmonella spp.* Meat, fish, fish products, shellfish, vegetable, fruits, chocolate, potato, salad, eggs, milk, cheese, poultry, ice-cream, restaurant foods, orange juice and tomatoes are associated with Salmonellosis[16].

S.aureus

S.aureus is a Gram-positive, coccus, forming sphericial to ovoid cells, appears as grape shape, catalase-positive, oxidase-negative, mesophilic, facultative anaerobic bacterium. Growth temperature ranges from 7°C to 48°C. Poisonings which are caused by S.aureus is a common reason of gastroenteritis in all of the world.

Humans are the main vehicles of the *Staphylococcus*. They exist naturally on the skin, mouth, throat and nasal cavity of healthy people. 30-50 % of healthy people are reservoir of *S.aureus* and 40-50 % of them are enterotoxins. Infected wounds contain *S.aureus* widely. *S.aureus* also exists in air, soil, water, sewage, plant, meats, poultry and dairy products. Protein rich foods are generally associated with Staphylococcal foodborne outbreaks. These are meat, canned beef, bacon, salami, chicken, tuna, ham, sausages, meat, fish, cheese, butter, chocolate, pudding, ice-cream[17].

C.botulinum

C.botulinum is Gram-positive, anaerobic, straight or slightly curved rods, exists as a single cell or in a small chains, produces endospores and motile. They generally exist in soil. Growth temperature ranges from 3,3 °C to 45 °C. Spores are heat resistant at 115 °C but cells are killed at moderate temperature with the process such as pasteurization.

They produce different type of botulism toxins. Human botulism is associated with foodborne and a kind of botulism (type E) is associated with fish.

They are widely distributed in soil, fresh water and intestinal tract of the animals. Most of the fruits and vegetables can be contaminated by C.botulinum. These are asparagus, beans, cabbage, carrots, celery, corn, onions, potatoes, turnips, olives, apricots, cherries, peaches and tomatoes. Low acid canned foods, meat products without adequate cooking can be associated with botulism. Home preserved foods, commercial foods and restaurant foods, ready to eat foods, minimally processed foods are packaged in modified atmospheres can cause botulism outbreaks as well[18].

E.coli

E.coli is from *Enterobacteriaceae* family, Gram-negative, catalase-positive, oxidase-negative, fermentative, motile, nonspore forming, short rod, anaerobic and mesophilic bacterium. Growth temperature ranges from 7°C to 50 °C. They are normal habitants of both human and animal intestinal tract. E.coli can be used as a monitor of fecal contamination. They are enteric pathogens in food and water. Most *E.coli* strains are harmless but some are pathogenic. The main carrier of this bacteria is distinguished as feces of human. *E.coli* infections are associated with meat, meat products, fish, poultry, milk, dairy products, vegetables and water. The main reason of infections occurs by fecally contamination from food handlers, feces from animal during slaughter and processing and water[19 20].

Campylobacter spp.

They are from *Camphylobacteriacea* family, Gram-negative, nonspor forming, catalase-positive and oxidase-positive, small and spirally curved and motile bacteria. Growth temperature is 37 °C. They can survive in refrigerated foods but they are sensitive to freezing. *Camphylobacter spp*. exist widely in intestinal tract of wild and domestic animals. Surface water, vegetables, meats, poultry, eggs, mushrooms are contaminated with directly fecal material from animals and infected handlers or directly from the sewage and water. *Campylobacter spp.* can be easily transferred person to person or animal to person. Main reasons of the *Campylobacter spp.* infections are derived from undercooked chicken meat, raw or poorly pasteurized milk, contaminated ready to eat foods and contaminated water[21].

Brucella spp.

Brucella spp. are from *Brucellaceae* family, Gram-negative, catalase-positive and oxidase-positive, facultative, non-motile, nonspore forming, short oval rods, aerobic bacterium. They occur as single or in pairs or short chains. Growth temperature ranges from 10 °C to 40 °C. It is killed by heat process at 60 °C in 10 min. *Brucella spp.* cause an illness called Brucellosis also known in the name of Mediterranean fever, Malta fever.

People who are working with animals and meats can easily be infected with *Brucella spp*. They can survive in milk and raw meats for a long time[6].

Klebsiella pneumoniae

Klebsiella pneumoniae is a Gram-negative, rod shaped, non-motile, lactose fermentive, facultative anaerobic bacterium[22]. It is mainly associated with urinary tract, respiratory and wound infections [23]. When *Klebsiella pneumoniae* enters in respiratory tract causes pneumoniae or in blood causes bloodstream infection[24]. It is resistant to most of antibiotics. It is ubiquitous in nature. It is generally found in intestinal tract of humans and animals [25]. Main vehicles are hospital personnels. They can easily spread in air currents in hospitals.

Coagulase Negative Staphylococcus (CNS)

The defination of *Coagulase-Negative Staphylococcus* is still in process to differentiate from S.aureus as being nonpathogenic or less pathogenic. Determining is not easy because they represent heterogenous group. It is associated with nasocomial (hospital) infections. In addition to this, it is referred as food-saphrophytes [26]. They are found in skin, mocous membranes of human and animals. *Coagulase-Negative Staphylococcus* arise from long term indwelling central venous catheter use, practice of parenteral nutrition and many other factors such as hands of medical personnels. [27].

Streptococcus

Streptococcus are Gram-positive, catalase-negative (differentiate from *Staphylacoccus*), nonspore forming, nonmotile, facultative or strict anaerobes. They occur in pairs or chains. They are found in normal flora. They are classified on hemolysis ability on agar. They are divided into three groups: γ hemolytic (no hemolysis), α-hemolytic (incomplete), β-hemolytic (completely clearing of red cells). They are widely found in nature. %5-15 of humans carry *S.pyogens* or *S.agalactiae* in the nasopharynx.

They are particularly found in mouth and intestinal tract, genital tract and the skin. They are associated to many infections including femaile genital tract, brain, pulmonary and abdominal abscess[28].

Enterobacter spp.

Enterobacter spp. are Gram-negative pathogens. They rarely cause disease. They occur at hospital infections. They grow well at 4 °C. They cause infections of skin and soft tissues, respiratory tract, urinary tract, bone and joints, central nervous system, gastrointestinal tract [29].

Listeria monocytogenes

Listeria monocytogens is a Gram-positive, facultative anaerobe, rod shaped bacteria[30]. *L. monocytogenes* is decribed as human pathogen causing meningits and sepsis [31 32]. *L. monocytogenes* can grow at low temperatures. Growth temperature ranges from 1 °C to 45 °C [33]. The cause of enfection is defined through ingestion of contaminated food[34]. Compared to other foodborne pathogens, *L. monocytogenes* is able to grow at refrigeration temperatures with high salt and low moisture conditions[35].

Clostridium perfringens

C.perfringens is Gram-positive, spore forming bacterium. They are found in environment and intestinal tract of humans and animals. *C. perfringens* are mostly found in raw meat and poultry and grow under low oxygen conditions. *C. perfringens* produce toxins and causes illness. *C.perfringens* can survive at high temperatures in food from 12 °C to 60 °C [36].

2.2.2 FUNGI

Fungi absorbe nutrients from external source to obtain their food. There are thousands types of fungi. Fungi absorb food from substances: Wood, decaying organic matter, soil, living organisms and other organisms. They differ from tiny single-celled organisms which is invisible to see with naked eye to largest living multicellular organisms. There are three major group of fungi. These are distinguished such as mold, yeast and mushroom.

A widely large group of fungi play main role in all natural ecosystems. Fungi decompose organic matter, break down plant and animal residues and wastes into their chemical components. Fungi have important role in recycling of minerals and carbon. Certain types of fungi, including many types of mold, produce antibiotics and hormones which are used in medicine and enzymes which are used in certain manufacturing process. Every type of fungi is not benefical. Some have bad effects on damaging agricultural crops, causing diseases both in animals and humans and having hazardous effects by producing toxins in food.

Fungi are eukaryotic (Their distinct nucleus have cell's genetic material is surrounded by a nuclear membrane)

1. Fungi can be unicellular or multicellular
2. Multicellular fungi look like plants (such as mushroom) but they do not process photosynthesis.
3. Certain fungi have cell walls which consist of chitin.
4. The unicellular fungi (yeast) are oval microorganisms that larger than bacteria.
5. Molds are the most typical fungi which are generated of visible masses of hyphae (filaments) called mycelia.
6. Fungi use organic chemicals for producing energy but they can not process photosynthesis.
7. Fungi reproduce by both sexually or asexually.
8. Fungi absorb solutions of organic material from environment such as soil, plant or animal host, fresh water and sea water to obtain nutrients.
9. Slime molds show characteristic properties both of fungi and ameobas[37].

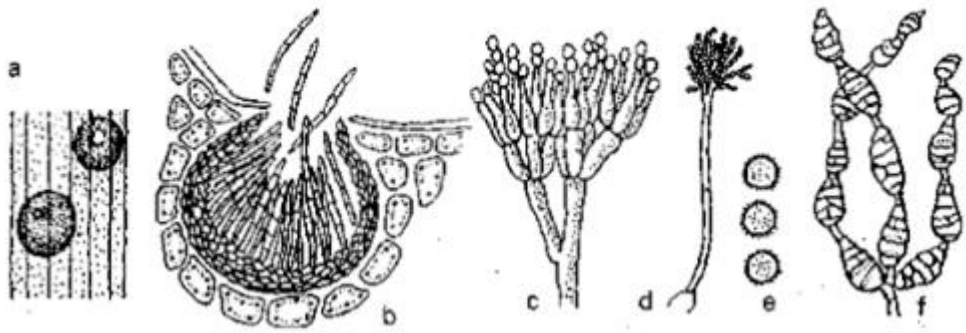


Figure 2: Examples of some microscopic fungi of the group Deuteromycetes [38]

a- pycnidia of *Phoma spp*.; b- secton through the pycnidium of *Septoria spp*. with conidia, c- conidiophore of *Aspergillus niger* with conidia, e- conidia of *Aspergillus spp.*; f- conidiophore of *Alternariatenuis* with conidial chanis

2.2.2.1 MOLDS

1. Molds are eucaryotic, multicellular and filamentous.
2. Molds are nonmotile.
3. Molds grow on foods (They have cottony apperance, branching and flamentation).
4. A mold contains a mass of branching filaments (called hypa, and whole mass of hyphae is called as mycelium). Septate hyphae is comprised of hyphae which is divided with cross walls while nonseptate hypha is comprised of hyphae without cross walls)
5. Hyphae can be reproductive and vegetative.
6. Hyphae plays role in absorption of water.
7. When mold grow, surface hyphae buds reproductive hyphae which produce sexual spores with extending in the air or asexual spores.
8. Taxonomic classification of mold is formed by size, colour, spores and shape.
9. Separated mold spores locate on a suitable substrate and send germ tubes that elongate into hyphae, during the asexual life cycle. (Mycelium is produced with continued growth)
10. A single colony of mold can have 500 spore-bearing structures [39].

2.2.2.1.1 MOSTLY ISOLATED MOLD FROM FOOD SAMPLES

Molds cause about a million diseases of plants, including about more than half of the major crop diseases (70 %) , and that results in an economic loss of billions of dollars each year[39].

Mucor spp.

They are widely food spoilage organisms. They are found especially in stored grain, dung hay, vegetables, fruits, milk, both in animals and humans and in soil. They are also used for fermantation of sufu.

Rhziopus spp.

They are distributed worldwide. But especially they are found in tropical and subtropical areas, grain, soil, water, vegetables and fruits. They are used in production of tempeh soya bean as a fermenting agent. They are reported to produce toxin but there is no strong evidence for its referred in mycotoxicoses[39].

Aspergillus spp.

Some *Aspergillus spp.* play role as plant and animal pathogens. They grow in any member of the genus on living host. They cause the animal disease called Aspergillosis. *Aspergillus spp.* spores commonly exist air currents. Depending on environmental conditions they spread to both short and long distances. They are able to spread in air easily and they can grow anywhere under proper conditions such as food and water are available. The term 'ubiquitous' is commonly used for molds to describe[40].

Penicillum spp.

The *penicillium spp.* are well known for antibiotic production (*P.chrysogenum* is Penicillin from the mold). Many spices of *Penicillum* play role in degradation of large organic matters in the bioshere. In addition to this, they destroy foods and other materials and cause many diseases in plants. Mycotoxins that are poisonous to both humans and animals are procuded by *Penicillium spp*. as well. Some enzymes of *Penicillium spp.* that are useful in the food industry whereas others has harmful effects[41].

Botrytis spp.

Botrytis cinerea and other *Botrytis spp.* are main pathogens of nursery plants, ornamental, field, vegetables, orchard crops, stored and agricultural products. They are used by agrochemical companies and reseachers[42].

Geotricum spp.

Geotricum spp. are limited to habitants of high water availability. The conidia have a very low heat resistance. They are significant pathogen of citrus fruits. They cause spoilage during post harvest and storage[43].

Fusarium spp.

As result of the *Fusarium spp.* contamination, serious yielf losses and degradation of wheat quality occur[44].

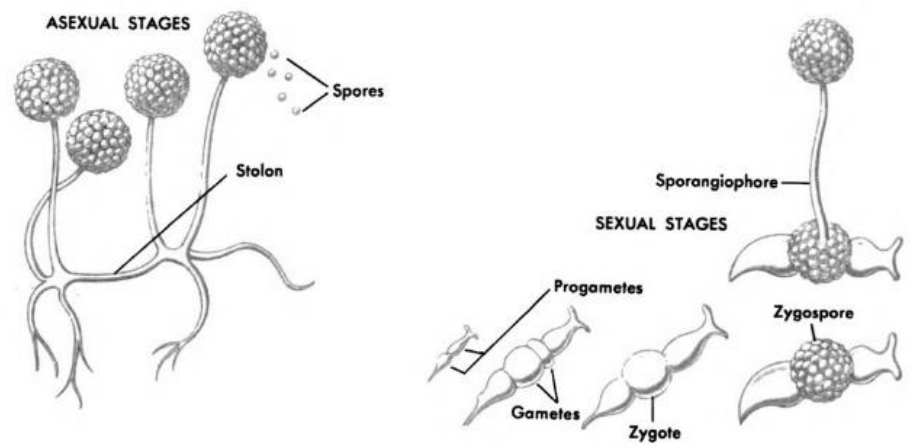


Figure 3: *Rhizopus* is a bread mold reproduce by both asexually and sexually[45]

2.2.2.2 YEAST

Yeast widely exist in nature. They are nonmotile. They can be in various shapes such as elongated, lemon, spherical or cylindirical shaped differ in size (about 10 μm). The nucleous is surrounded by nuclear membrane. They reproduce asexually by buddying mostly. A few species reproduce by both fissuon and budding. True yeast (*Ascomycotina*) reproduce sexually by ascospores while false yeast (*Fungi imperfecti*) do not produce ascospores or other sexual spores such as *Rhodotorula spp., Cryptococcus spp. and Candida spp.* Yeast may be fermentative, oxitative or both. The oxidative yeast can grow as film, pellice on the surface (termed as film yeast) while fermentative yeast grow in liquid and produce carbondioxide.

Yeast are distributed to many respiratory diseases in human[6]. Yeast cause foodborne illness and also spoilage in food products as well.

Spoilage is result of initial contamination of the food product in set of intrinsic and extinsic conditions which allow spoilage flora to proliferate. It is estimated that 1/3 of all the food are lost to spoilage or other kinds of waste for human consumption[6].

2.2.2.2.1 MOSTLY ISOLATED YEAST FROM FOOD SAMPLES

Saccharomyces spp.

They are oval, round and long cells. They include important yeast in food industry. They naturally exist in fruit and vegetables. They ferment sugar and produce alcohol and CO_2. Important species is *S.cerevisia*.

Zygosacchoromyces spp.

They ferment sugar. They cause spoilage in foods including intense sugar such as honey, marmalade, jam, syrup.

Debaromyces spp.

They exist in milk products. They cause spoilage in salt brine meats.

Brettanomyces spp.

They cause spoilage in pickles

Candida spp.

They exist in ground meat and poultry.

Rhodotorula spp.

They cause spoilage in poultry, fish, shrimps and ground meat. They grow surface of the butter[46].

2.3 SIGNIFICANCE OF MICROORGANISMS IN FOODS

There are many factors which determine the microbiological quality of meat. These are temperature of slaughter place, the spread of contamination during slaughtering and processing, storage and distribution. All edible tissues may be exposed to contamination with microorganisms from outside of the animal during slaughtering. The carcass of the animals comprise several types of microorganism, mainly bacteria which come from themselves (hair, skin, feathers, respiratory tract and gastrointestinal tract, etc.) pasture (water, soil, feed and manure) and environment. Normally carcass contains

various enteric pathogens such as *Esherichia coli, Salmonella enteritidis, Yersinia enterocolitica, Camphylobacter jejuni.* In addition to this *S.aureus* and *C.perfiringers* may be present in low levels. Generally the incidence of *Salmonella spp.* contamination from fecals is high in carcass of chicken and birds.

The ground meat and chilled meat can be contaminaed with microorganisms from equipments, human handling, air, water and carcasses. These meats may have mesophilic microorganisms such as *Bacillus, Clostridium, Lactobacillus, Micrococcus, Enterococus*, coliform and other Enterobacteriaceae. Mesophiles grow in high temperatures. Mainly psychrotrophic microorganisms cause spoilage in raw meats are *Clostridum laramie, Achromobacter, Leuconostoc, Lactobacillus, Serratia, Alteromonas, Pseudomonas, Moraxella, Acinetobacter, Proteus* and coliforms. These pathogens may cause spoilage in meats which are kept at low temperature range -1 °C to 5 °C. Main psychrotrophic pathogens in meats are *Y. Enterocolitica, Listeria monocytogenes, Aeromoas hydrophilia and Brochothrix themospacta.* Ground meat is able to contain 10^{4-5} microorganisms per gram. The psychrotropic aeroboes grow fast in foods which are storaged under aerobic conditions. Especially Gram-negative rods such as *Alteromonas, Alcaligenes, Proteus, Pseudomonas*. Aerobic packaged meat mainly contain anaerobes such as *Clostriduim* and facultative anaerobes such as *Leuconostoc, Brochothrix, Serratia, Lactobacillus* and some coliforms. The determination which of the main microorganism will exist in the meat depends on ph of the meat (about 5,6 in beef and 6,0 in birds), high protein and low carbonhydrate content.

Bacteria grow faster than mold and yeast. Psychrotropic molds on chilled meat contain species of *Mucor, Rhizopus, Cladosporium, Thamnidium and Penicillium.* Yeast contain species of *Candida, Torulapsis, Rhdotorula* and *Crytococus*. The response of the microorganisms to freezing is different. Some vegatative cells and lots of spores are highly cold resistant such as *Streptococus, Micrococcus, Staphylococcus*. Main of the other nonspore forming organism response are different at the temperatures of freezing. Killing or injury temperature levels differ from -2 °C to 10 °C whereas -27 °C causes lethal effect. The occurance of injury is very important for public use. Injured foods can recover and grow after thawing and cause poisoning or spoilage even if it is difficult to detect in a frozen product.

To reach internal temperature to 71°C or higher kills most of the microorganisms but thermodurics such as *Enterococus*, *Lactobacillus*, *Micrococcus* and *Clostridium* spp. can survive at these temperatues. Heated meat can contain microorganisms in the number of 10^{1-2} per g. Further heating, microorganisms can contaminate during following processing from contact with food handlers, air, equipment and water[6].

3 GENERAL INFORMATION ABOUT FOODBORNE

3.1 PATHOGENS REASONS OF FOODBORNE DISEASE

The main factors that are attributed to foodborne diseases include one or more of the followings:

Cross-contamination
Adequate cooking
Storage at room temperature
Consumption of raw foods
Contaminated by infected food handlers
Contaminated ingredient use
Poor general hygien
Failure in processing
Expending storage time
Contaminated equipments
Use of left-overs
Inadequate hot holding
Inadequate rehating
Inadequare thawing

These factors should be considered for improving general hygiene by reseachers. Main of them are within the control of the consumer[6].

Table 3: Agents causing foodborne diseases[6]

Agents	**Examples**
Bacteria	*S. aureus, C. botulinum, C.jejuni, C. perfringens, B. cereus, Y. Enterocolitica, V. parahaemolyticus, V. cholerae, Salmonella spp.*
Viruses	Hepatitis, Parvovirus, Rotavirus, Norwalk viruses.

Molds	*A. flavus* (aflatoxin), *P.patulum* (patulin)
Marine protozoa	*Gonyaulax tamarensis* (paralytic shellfish poisoning), *Gambierdiscus toxicus* (ciguatera poisoning).
Parasites (helminths, protozoa, amebae) Chemical Piron	*Trichinella spiralis, Taenia solium, T. Saginata, G. Lamblia, E. Histolytica*. Heavy metals, e.g., head, zinc, copper, mercury, arsenic. Insecticides, herbicides, fungcides, cleasers, disinfetants. vCJD
Toxic plants	Raw or undercooked bean (Phaselous vulgaris lecxtin) Apricot kernels (lucide- amygdlain), green potatoes (solanine).
Toxic food animals	Puffer fish (tetrodotoxin), red whelk poisoning (tetramine) Scombroid fish poorly processed (histamine)

Table 4: Common associations for foodborne pathogens[6]

Epidemiology	**Pathogens**
Travel to a developing area	- Enterotoxigenic *E. coli* (ETEC) - *Salmonella* (e.g., *S. typhi*) - *Shigella* (e.g., *S. dysenteriae*) - *Campylobacter* - *E. histolytica*
Consumption of raw or undercooked foods of animal origin	- *Salmonella* (e.g., undercooked eggs, meat and chicken) - *Campylobacter* (e.g., undercooked chicken, milk)

	- *E. coli O157:H7* (e.g., undercooked beef, especially hamburger) - *T.gondii* (e.g., undercooked pork) - *T. spiralis* (e.g., undercooked meat) - *Y. enterocolitica* (e.g., undercooked meat, milk)
Exposure to untreated water	- *Shigella* (e.g., lakes contaminated with human feces) - *E. coli O157:H7* (e.g., lakes contaminated with feces) - *Cryptosporidium* (e.g., water contaminated with feces, including swimming pools, since the oocytes are resistant to chlorine) - *Giardia* (e.g., water contaminated with feces)
Contact with animals	- *Crytosporidum* (e.g., petting zoos) - *Salmonella* (e.g., reptiles) - *E. coli O157:H7* (e.g., farm animals)
Undercooked or raw shellfish	- *Vibro species* - *Hepatitis A virus* - *Norwalk* and *Norwalk-like viruses*
Ready-to-eat meats	- *L. monocytogenes*
Food contaminated by infected food handlers	- *Hepatitis A virus* - *Norovirus* - *Rotavirus* - *Salmonella* - *Shigella* - *Staphylococcus aureus*
Recent antibiotic exposure	- *Colostridium difficile* - *Salmonella* - *Campylobacter*

4 MATERIALS AND METHODS

4.1 MATERIALS

For detection experiments involving food samples that we used fast foods were purchased from a local fast food restaurants in Kadıköy. 50 samples were analyzed in total. Meats were kept frozen until used at -18 °C. 1 cm^3 samples were extracted in 5 ml of sterile water in tubes and homogenated. Then, 100 µl was inoculated on to mediums.

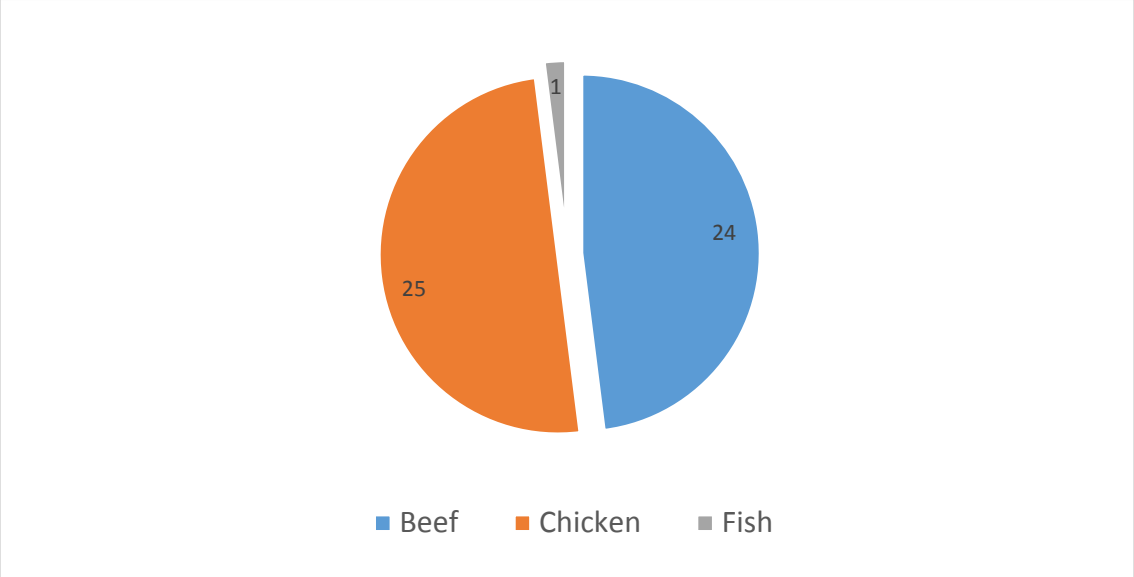
Figure 4: Samples before inoculation

Table 5: Samples of the Study

1.	Chicken Schnitzel	26.	Chicken Döner
2.	Hamburger	27.	Chicken Nudget
3.	Chicken Döner	28.	Chicken Döner
4.	Chicken Döner	29.	Wet Hamburger
5.	Fish	30.	Hotdog
6.	Hamburger	31.	Hamburger
7.	Hotdog	32.	Chicken Roast
8.	Chicken Schnitzel	33.	Hotdog
9.	Hamburger	34.	Chicken Schnitzel
10.	Hamburger	35.	Chicken Schnitzel
11.	Chicken Schnitzel	36.	Chicken Tenders
12.	Hamburger	37.	Hotdog
13.	Chicken Schnitzel	38.	Hamburger
14.	Wet Hamburger	39.	Hamburger
15.	Hamburger	40.	Chicken Döner
16.	Hamburger	41.	Chicken Döner
17.	Chicken Schnitzel	42.	Hamburger
18.	Chicken Döner	43.	Hamburger
19.	Chicken Nudget	44.	Wet Hamburger
20.	Hamburger	45.	Hamburger

21.	Wet Hamburger	46.	Chicken Döner
22.	Chicken Döner	47.	Chicken Döner
23.	Chicken Schnitzel	48.	Deboned Crispy Chicken
24.	Hamburger	49.	Chicken Burger
25.	Wet Hamburger	50.	Chicken Döner

Figure 5: Samples of the Study



Graph 1: Number of Samples

4.2 METHODS

4.2.1 CULTURE MEDIA

Microorganisms grow under proper conditions. Suitable food, water, suitable temperature and ph, adequate concentration are required. In order to obtain a culture microorganisms in the labrotory, the easiest way is to buy suitable grown medium from biological seller. Then sterile with distilled water according to maker's instructions. A proper growth medium contain all the needs which microorganisms to grow[47].

In this study 50 of sample incubated in Sheep Blood/ EMB Agar and Salmonella-Shigella (SS) at 37 °C in incubator and in Sabouraud's Dextrose Agar in room temperature under proper conditions.

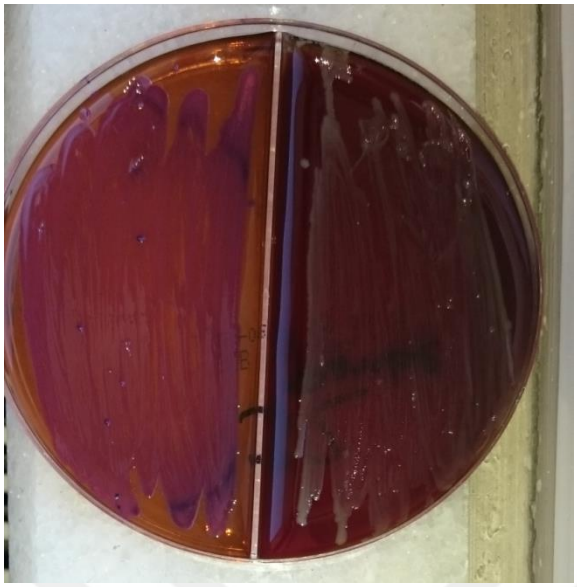


Figure 6: Incubation Method: Spread plate technique

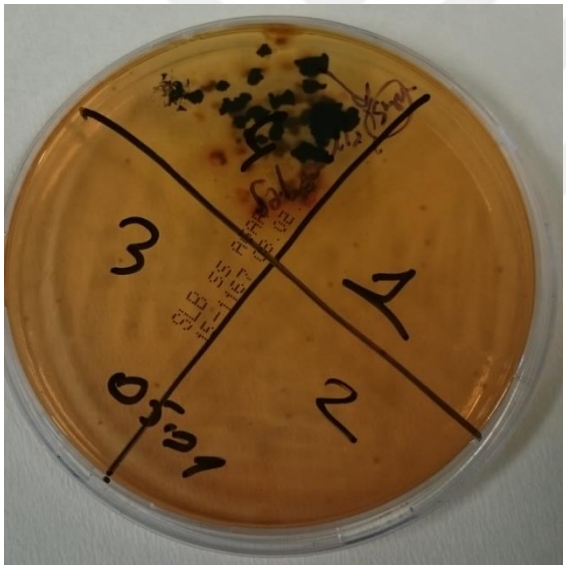


Figure 7: Culture in SS Agar: Spread Plate Technique

Eosin Methylene Blue (EMB) Plates

EMB plates have a dye material which is a ph indicator that changes colours when ph changes. This is helpful for detection because during fermantation (ph decreases) , acids are always produced. Organisms which are able to fermante lactose (EMB plates contain carbonhydrate) form dark colonies whereas others which are not capable of fermenting form clear or light colonies. Additionally, the dye is bacteriostatic (decelerate

the growth) against Gram-positive species. Because of that EMB plates are differential (depending on lactose fermentation) and selective (againts Gram-positive cells)[48].

Sheep Blood Agar Plates (BAP)

Some bacteria are able to process of hemolysis (breaking down red blood cells) while others are not. Sheep's blood agar plates (or the blood of some animal) help to test hemolytic ability. Hemolysis can be seen as clearing of the agar (blood red clour dissapears) surrouning colonies. It is used to differentiate *Stahylococcus* and *Streptococcus*[48].

SS Agar (Salmonella Shigella)

SS agar is used for the selective differentiation and isolation of pathogenic enteric bacili (especially *Salmonella spp.*). This medium is not used for the primary isolation of Shigella species. Lactose fermenting bacteria are seen as small pink red colonies such as *Escheriachia coli* or *Klebsiella pneumoniae* while lactose-non fermenting baceria are seen as colourless colonies such as *Salmonella spp.*, *Shigella spp.*, *Proteus spp*. Presence of H_sS (produced by *Salmonella spp.*) turns the center of the colonies into black colour[49].

Sabouraud's Dextrose Agar

Sabourau's dextrose agar is the most useful medium to detection of fungi. Enriched medium enchances typical sporulation and supplies more characteristic colonial morphology[50].

4.2.2 IDENTIFICATION METHODS FOR BACTERIA

4.2.2.1 GRAM STAINING TECNIQUE

Living bacteria are generally colourless and invisible because their lack of contrast with water. Thus staining is necessary to make them visible. So it becomes easy to observation of intracellular structures and overall morphology.

Danish bacteriologist Christian Gram is the founder of staining technique (in 1884). This technique divides bacteria into two groups as Gram-positive and Gram-

negative. The procedure is based on capable of the micoorganisms, during decolorization with alcohol, remain purple colour of crystal violet. Gram-positive bacteria remain purple (not colorized). After decolorization, safrain, a red counterstain, is added to import a pink colour to the decolorized Gram-negative bacteria. Staining process can be applied to all bacteria and many fungi.

The methods of Gram-staining are the followings respectively:

1. Covering the smear with crystal violet for 30 seconds.
2. Washing off the stain briefly by using distilled water.
3. Addition of Gram's iodine solution for 1 minute.
4. Decolorisation of ethyl alcohol for 10-20 seconds. Decolorization occurs when solvent gets colorless from the slide.
5. Rinsing the slide with water for few seconds.
6. Covering the smear with safranin for 30 seconds.
7. Washing again and blotting dry with bibulous paper and air dry.
8. Viewing the slide under oil immersion[51].

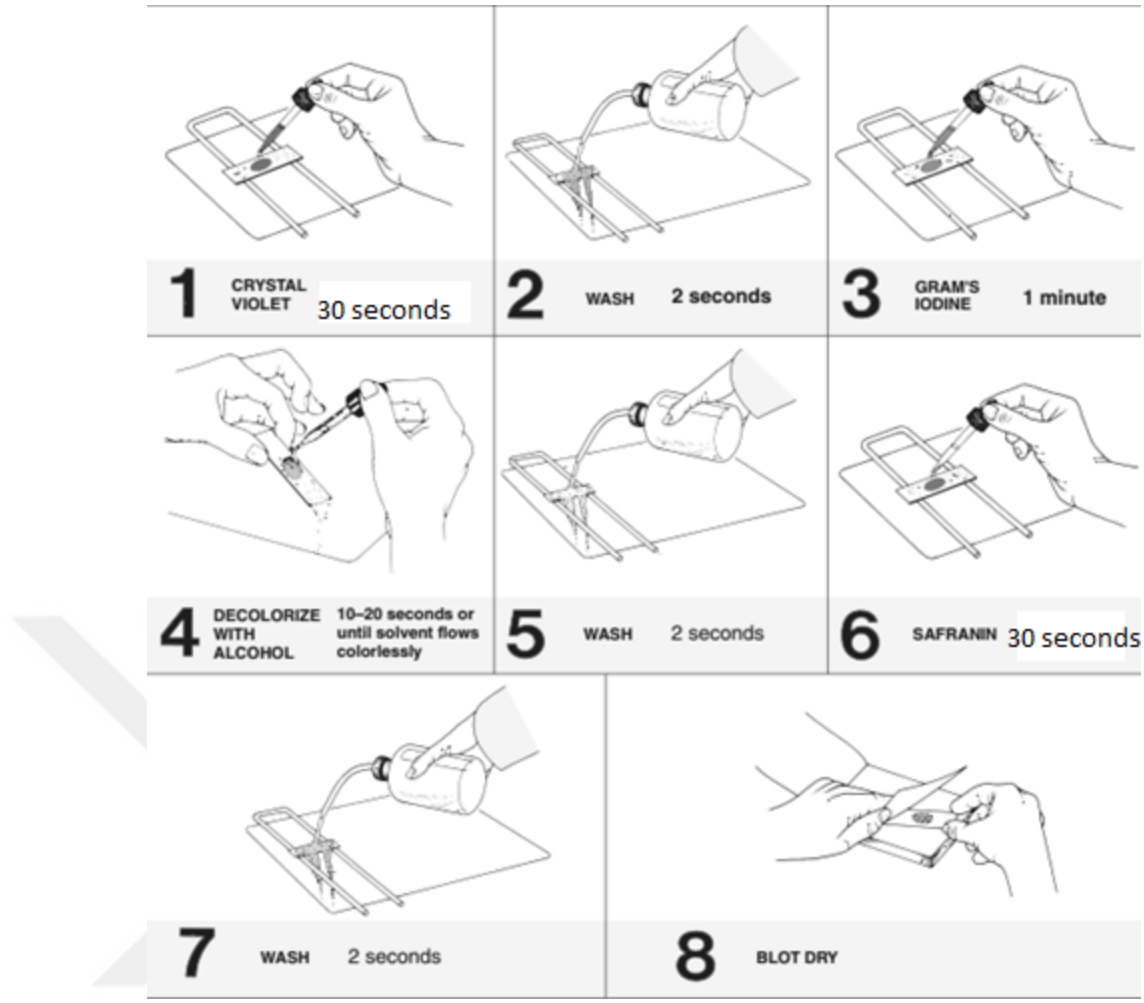


Figure 8: Gram staining method[51]

Figure 9: Gram Stainig Solutions

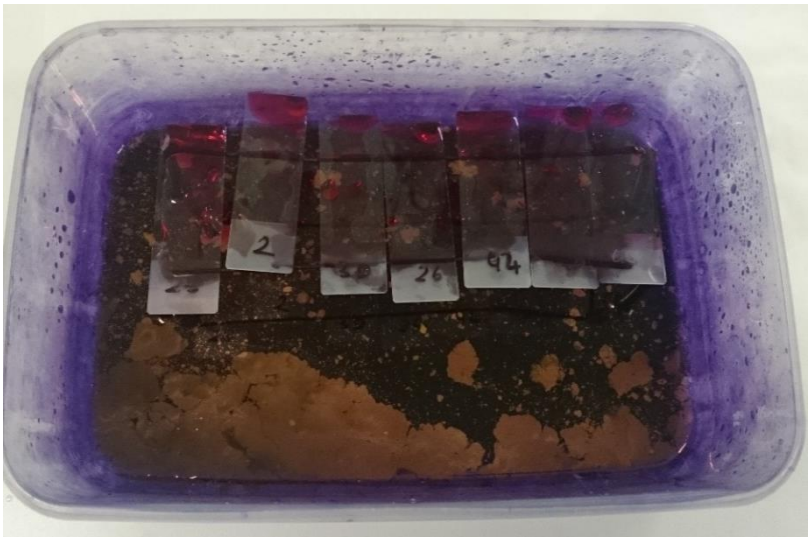


Figure 10: Slides on gram staining

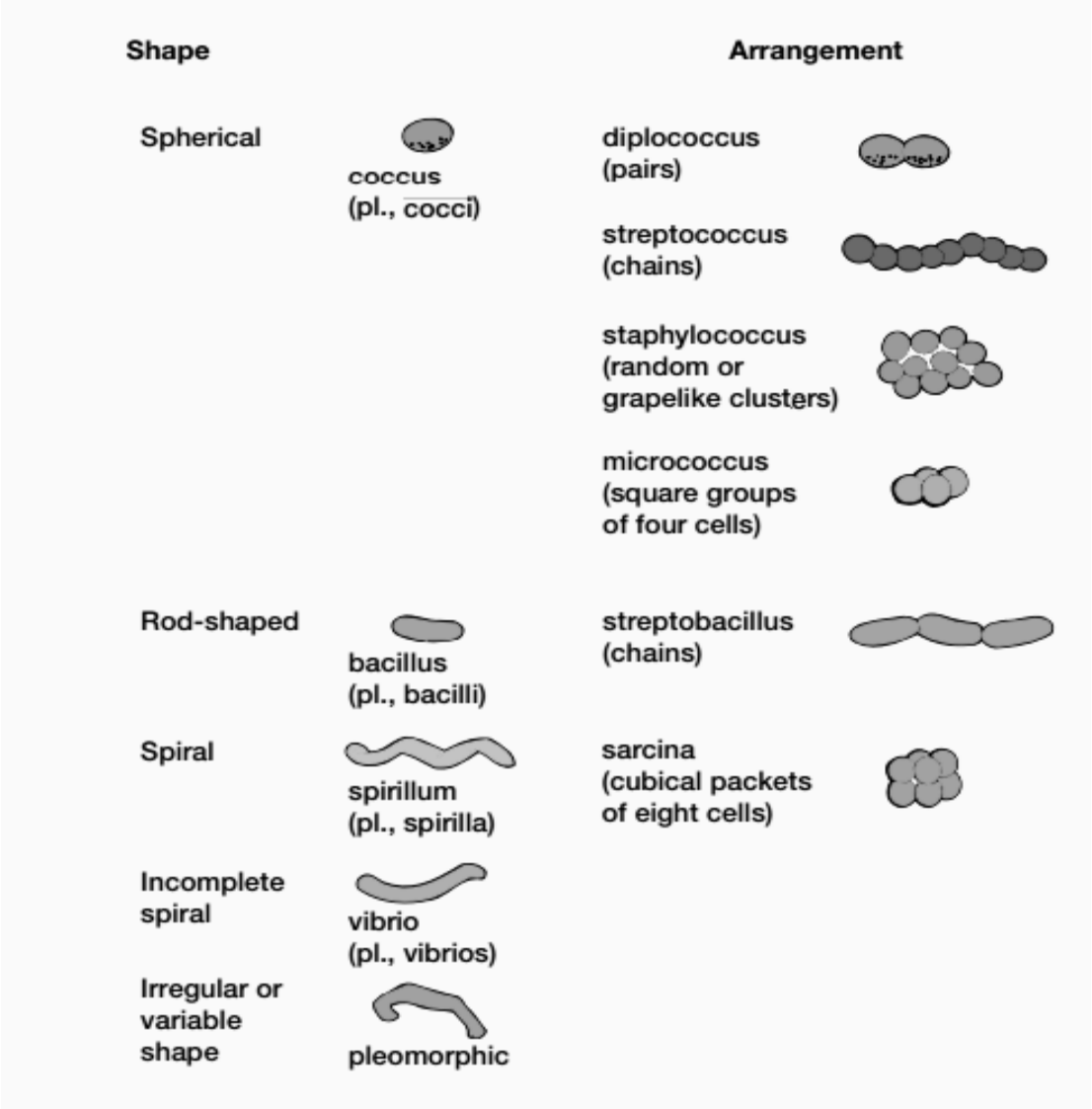


Figure 11: Common Bacterial Shapes [52]

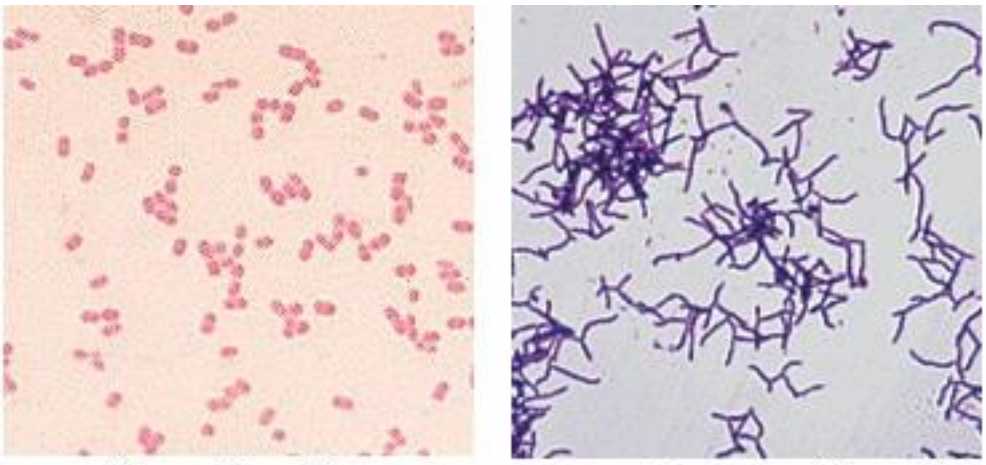


Figure 12: Microscopic apperance after Gram staining [53]

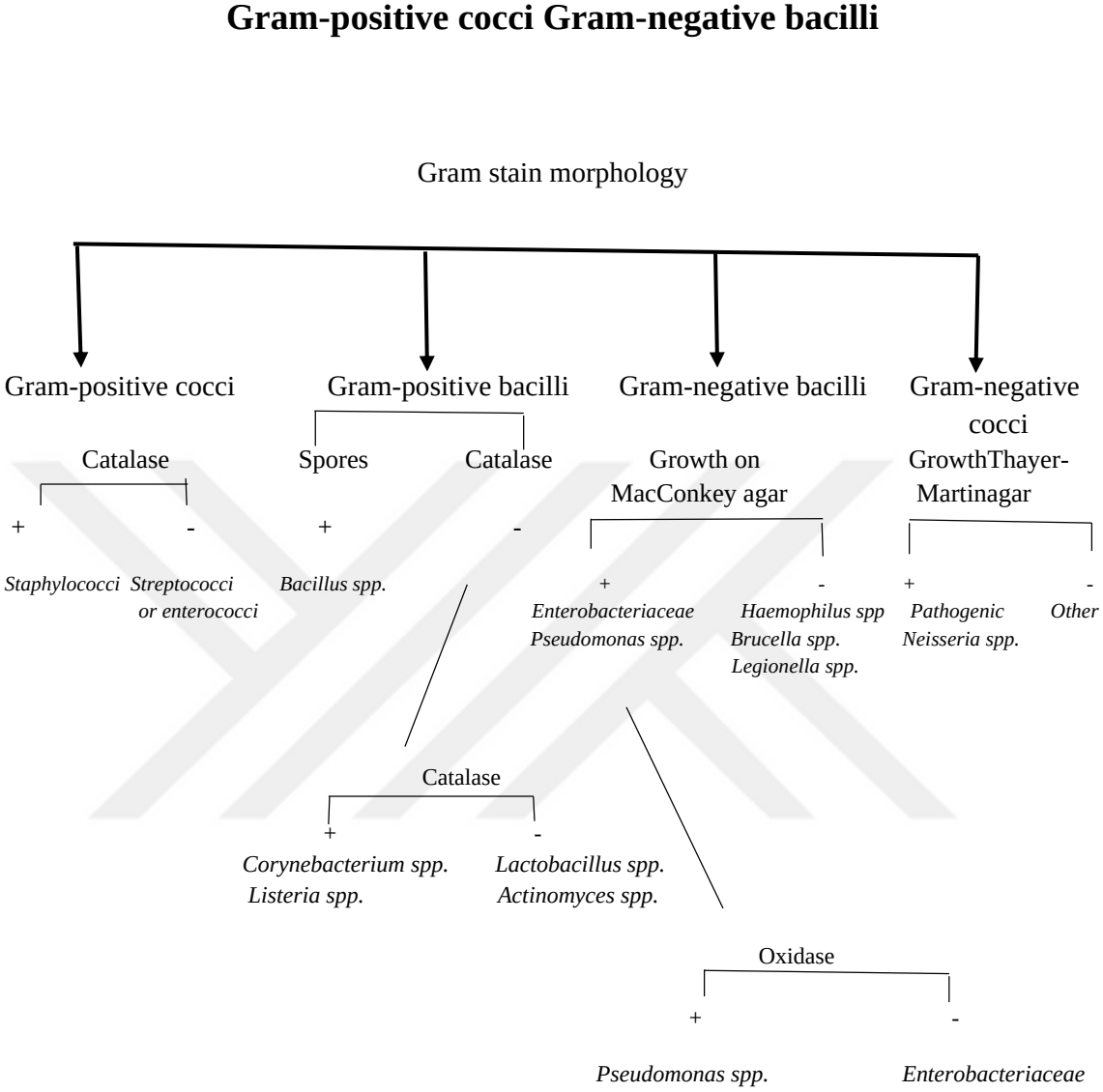


Figure 13: Gram staining morphology [54]

4.2.2.2 GENERAL CLASSIFICATION OF BACTERIAS ON BASIS GRAM STAINING

Gram-negative, aerobic basilli and cocci

Pseudomance type: They are spoilage factor of meat, poultry, fish and egg which are storaged in cold temperatures. They form green, black and brown pigments on the surface of the foods.

Halococcus type: They form red pigments on salty meat and fish and cause spoilage.

Alcaligenes type: They exist in poultry. They cause spoilage of meat.

Gram-negative, facultative anaerobe bacilli

Escheria type: They naturally exist in intestinal flora of human and animals. Indicator of fecal contamination.

Salmonella type: They are natural inhabitans of the gastrointestinal tract of domestic and wild animals and birds. Salmonellas are responsible for a number of different diseases. The most important one is typhoid.

Enterobacter type: They naturally exist in human intestinal flora. Also they exist in water, soil, sewage and plants.

Shigella type: They exist in intestinal flora. They contaminate with dirty water.

Erwinia type: They form acid from carbonhydrates.

Proteus type: They are factor of putrefaction in meat, fish and eggs.

Gram-positive cocci

Micrococcus type: They are saprophyte. They are not pathogenic.

Staphylcoccus type: They are naturally exist in skin, nose and saliva. They are resistant to heat.

Streptococcus type: They are generally pathogenic for human and animals. They are used as starter culture in food technology.

Lactococcus type: They exist in milk.

Enterococcus type: They naturally exist in human and animal intestinal flora. They are resistant to heat and so they are distinguished from Streptococcus. They are indicator of fecal contamination.

Leuconostoc type: They are undesirable in sugar industry.

Endospore forming, Gram-positive bacilli and cocci

Bacillus type: They have benefits in food industry (Fermation of sugar, pasteursation of foods).

Clostridium type: C.botulinum, C. perfringens are common species. They are spoilage factor of canned foods in inadequate heat process.

Nonspore Gram-positive regular shaped bacilli

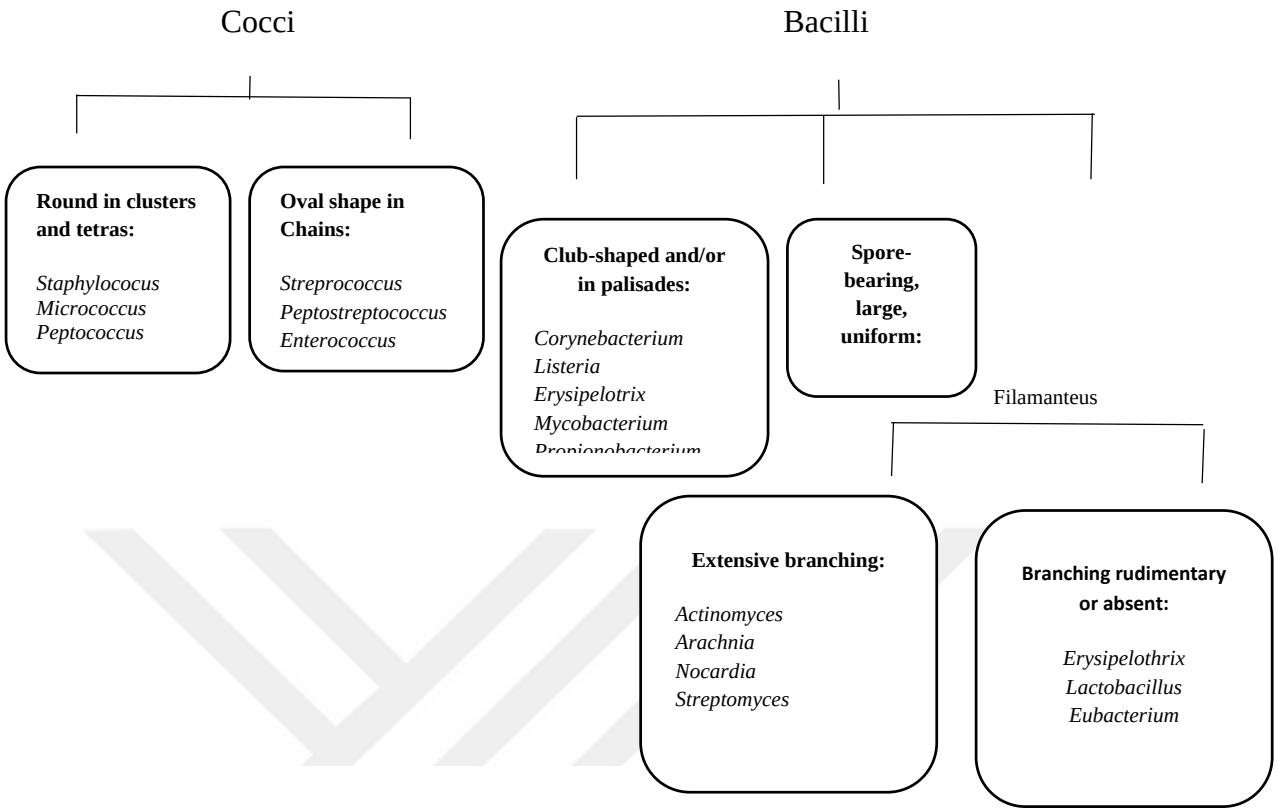
Lactobasillus type: L.bulgaricus exist in milk and yoghurt. They are used as starter culture with *S.thermophilus*.

Listeria type: They exist in soil, plants, sewege.

Nonspore gram positive irregular shaped bacilli

Corynebacterium type: They naturally exist in human intestinal flora. Also they exist in water, soil, sewage and plants[46].

Gram-positive



Gram-negative

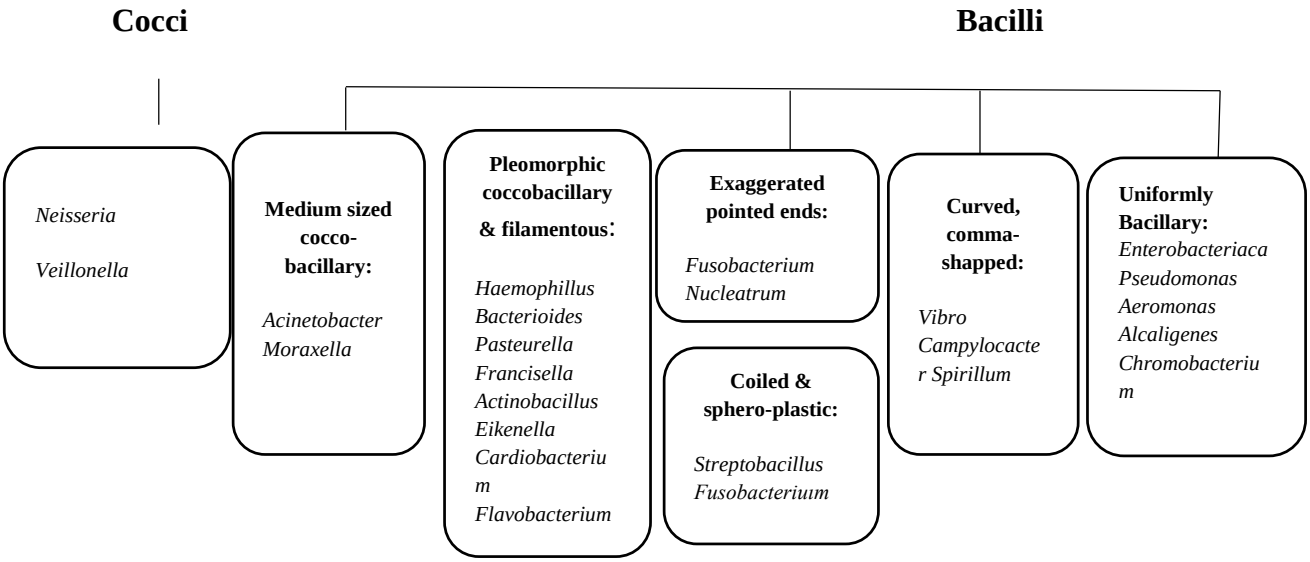


Figure 14: Classification of Bacteria according to Gram staining and shape[55]

4.3 WET- MOUNT TECHNIQUE

The method of wet mount technique is direct examination of living bacteria (prokaryotic) and yeast (eukaryotic) and determinig their natural shapes and sizes, mobility and reactions against to various chemicals. With that method, a drop of the sample is poured on a clean slide and then addition of cover slip. Its is quick and easy. Main problems of this technique are drifting out of fluid and evaporation. So its required to do microscopic observations after specimen prepared promptly[56].

4.4 THE RATIONALE MICROBIAL TESTS

The microbial tests are requited to monitor food quality. There are several different tests and they depend on the food being anaylzed. The test can be list as the followings:

1- Total numbers of microorganisms are estimated.
2- Numbers of indicator organisms are estimated.
3- Food spoilage organisms are estimated.
4- Numbers of food poisoning and foodborne pathogenic organisms are estimated
5- Metabolic products of microorganisms are estimated (This is required for further anaylzes)[57].

4.4.1 BIOCHEMICAL TESTS

Microbial tests include the incolation of a food with specific microbial hazards and reveal their growth during various stages such as survival, storage and death[58].

CATALASE TEST

The catalase test is used to distinguish catalase-positive *Staphylococcus* from catalase-negative *Streptococcus*. The catalase enzyme is produced by bacterias which are available of respiration by using oxygen and proctects them from the toxins procuded by oxygen metabolism. Catalase positive bacterias contain strict aerobes and facultative

anaerobes, they are all able to respire oxygen as a terminal electron acceptor though. Catalase-negative bacteria can be anaerobes or facultative anaerobes which do not respire oxygen as a terminal electron acceptor but only ferment (such as *Streptococcus*)[59].

COAGULASE TEST

Coagulase test is used in the purposed of distinguish *S.aureus*. Coagulase is an enzyme produced by *Staphylococcus aureus* which converts fibrinogen (soluble) to fibrin (insoluble) in plasma. It is specific to *S.aureus* because other *Staphylococcus* do not produce coagulase[59 60].

OXIDASE TEST

The oxidase test is used to distinguish bacteria which produce cytochrome c oxidase (an enzyme of the bacterial electron transport chain). Since all bacterias are available to use oxygen as a terminal electron acceptor thus they are oxidase positive and aerobic. This test is used to differentiate *Enterobacteriaceae* and Nonfermantative Gram negative bacteria. That does not mean that they are strict aerobes. Oxidase-negative bacteria can be anaerobic, aerobic or facultative, the oxidase negative results just indicate that these organisms do not have the cytochrome c oxidase which oxidizes the test reagent. They can respire by using other oxidases in electron transport[59].

4.5 TOTAL BACTERIAL LOAD

The growth and and maintanence of microorganisms on media have been a common practise in microbiology. Only viable cells which are capable of growing are counted. Each living bacteria in suspension will form a colony after incubation. The total bacteria count is the number of bacteria that form countable colony forming units (CFUs) on standart methods after kept at 36 °C for 48 hours[61].

4.6 MICROBIAL IDENTIFICATION DEVICES

VITEC 2.0

VITEK 2 (bioMe´rieux, Inc. Hazelwood, MO) is a microbiology identification system that monitors an optical signal by individual biochemical reactions. The system is full automated and includes a variety of microbe identification cards. The unknown organism is inoculated with standardized suspension before each self-contained card is incubated and read by instrument's internal optics. Organisms identification is processed by comparision of results to known species-specific reactions in the VITEK 2 database[62 63].

Figure 15: VITEC 2.0 microbial identificatiion device[62]

API®/ID32 C

API is revolutionised completely for the bacteriology. The API range presents a standardised and miniaturised version of the existing techiques that were difficult to interpret and complicated to perform up till now. API makes bacterial identification simplier, fast and reliable. Today API/ID32 has the most extensive range available. It

contains 15 indenfication systems that includes all groups of bacteria encountered in industrial microbiology labrotories[64].

Figure 16: Apperance of API®/ID32 C stribe[65]

5 RESULTS

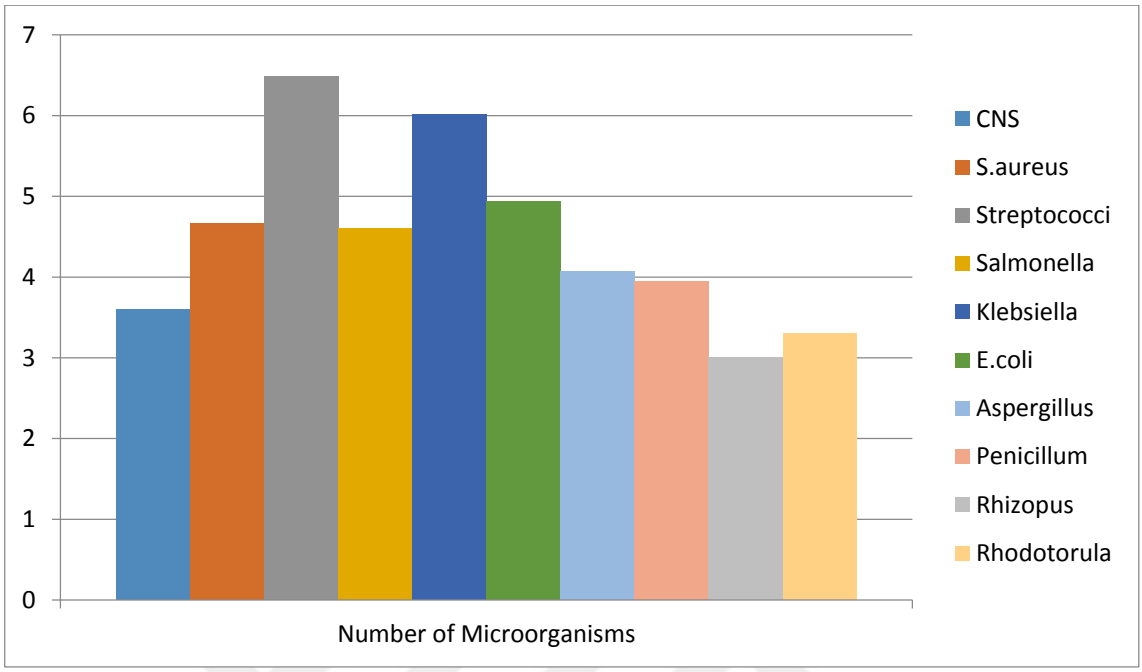
Table 6: List of isolated microorganis

1	No-growth	0	0
2	*Coagulase Negative Staphylococcus*	3,47 log cfu/g	3,6 log cfu/g
	S.aureus	3 log cfu/g	
3	*Streptococcus*	3 log cfu/g	3 log cfu/g
4	*Salmonella spp*.	4,6 log cfu/g	6,01 log cfu/g
	Klebsiella pneumoniae	6 log cfu/g	
	E.coli	3 log cfu/g	
5	*Klebsiella pneumoniae*	3 log cfu/g	3 log cfu/g
6	*E.coli*	3 log cfu/g	3,30 log cfu/g
	Streptococcus	3 log cfu/g	
7	No-growth	0	0
8	*Aspergillus niger*	1 cfu/g	1 cfu/g
9	*Coagulase Negative Staphylococcus*	3 log cfu/g	3,30 log cfu/g
	S.aureus	3 log cfu/g	
10	No-growth	0	0
11	*S.aureus*	3,90 log cfu/g	3,90 log cfu/g
12	*E.coli*	3 log cfu/g	3 log cfu/g
13	*Penicillium spp.*	1 cfu/g	1 cfu/g
14	*S.aureus*	3 log cfu/g	3 log cfu/g
	Penicillium spp.	1 cfu/g	
15	*S.aureus*	3 log cfu/g	3 log cfu/g
16	*S.aureus*	3,95 log cfu/g	3,95 log cfu/g
17	No-growth	0	0

18	*Streptococcus*	6 log cfu/g	6,0030 log cfu/g
	E.coli	3 log cfu/g	
	S.aureus	3,7 log cfu/g	
19	No-growth	0	0
20	*Penicillium spp.*	1 cfu/g	6,0004 log cfu/g
	Streptococcus	6 log cfu/g	
	Klebsiella pneumoniae	4 log cfu/g	
21	*E.coli*	3 log cfu/g	3,77 log cfu/g
	S.aureus	3,69 log cfu/g	
22	Ungrowth	0	0
23	*Penicillium spp.*	1 cfu/g	1 cfu/g
24	No-growth	0	0
25	*Streptococcus*	4,30 log cfu/g	4,30 log cfu/g
26	*Klebsiella pneumoniae*	3,30 log cfu/g	3,30 log cfu/g
	Penicillium spp.	1 cfu/g	
27	No-growth	0	0
28	No-growth	0	0
29	*S.aureus*	4 log cfu/g	4 log cfu/g
30	*Rhodotorula spp.*	1 cfu/g	1 cfu/g
31	No-growth	0	0
32	*E.coli*	4,90 log cfu/g	5 log cfu/g
	Klebsiella pneumoniae	4,30 log cfu/g	
33	*Streptococcus spp.*	4,90 log cfu/g	4,90 log cfu/g
34	No-growth	0	0
35	*Aspergillus flavus*	1 cfu/g	1 cfu/g
36	No-growth	0	0
37	*Penicillium spp.*	1 cfu/g	1 cfu/g

38	*Streptococcus*	4,17 log cfu/g	4,17 log cfu/g
39	*Rhizopus spp.*	1 cfu/g	1 cfu/g
40	*Rhodotorula spp.*	1 cfu/g	1 cfu/g
41	*Penicillium spp.*	1 cfu/g	3 log cfu/g
	S.aureus	3 log cfu/g	
42	*S.aureus*	3 log cfu/g	3,30 cfu/g
	Enterobacter spp.	3 log cfu/g	
	Penicillium spp.	1 cfu/g	
43	No-growth	0	0
44	*Aspergillus niger*	10 cfu/g	10 cfu/g
45	No-growth	0	0
46	*Streptococcus*	6 log cfu/g	6 log cfu/g
47	No-growth	0	0
48	No-growth	0	0
49	*Penicillium spp.*	1 cfu/g	1 cfu/g
50	No-growth	0	0

Graph 2: Number of microorganisms



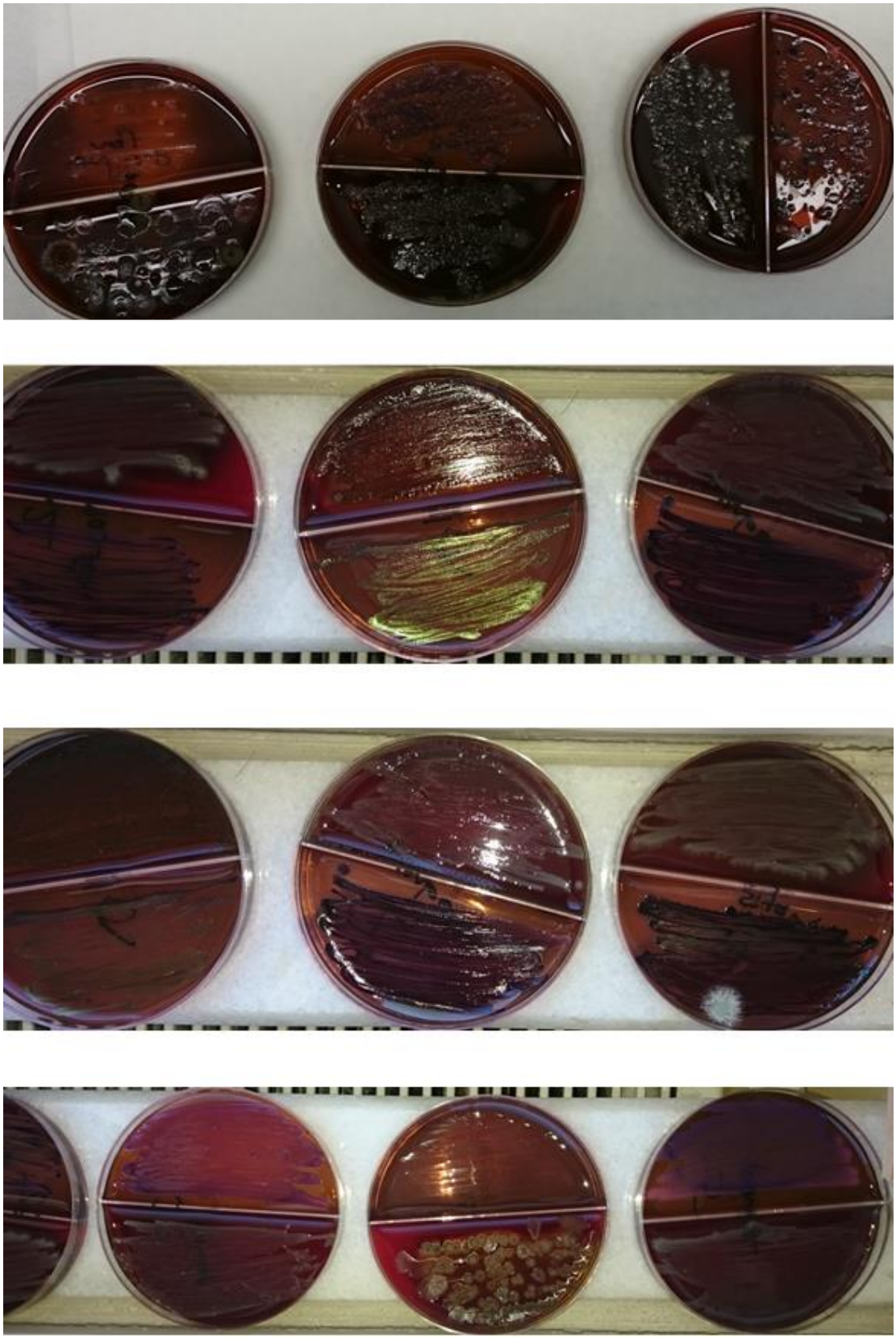
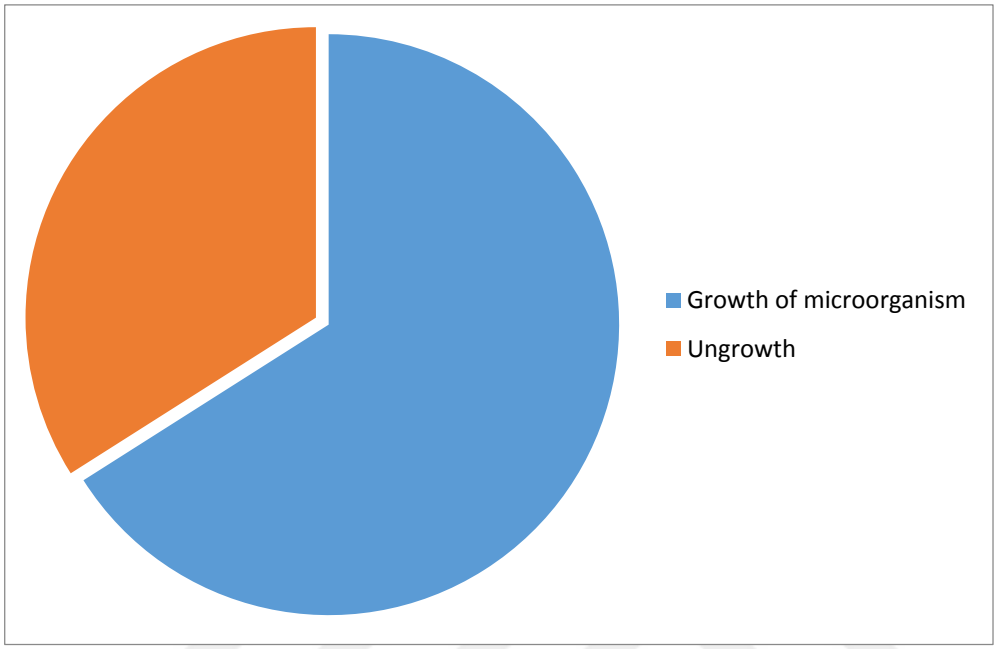


Figure 17: Apperance of incubated samples

5.1 EVALUATION OF ANALYSIS RESULTS

We isolated pathogen microorganisms from 33 samples in 50 samples in total (66%). 34% of the samples are detected no-growth.



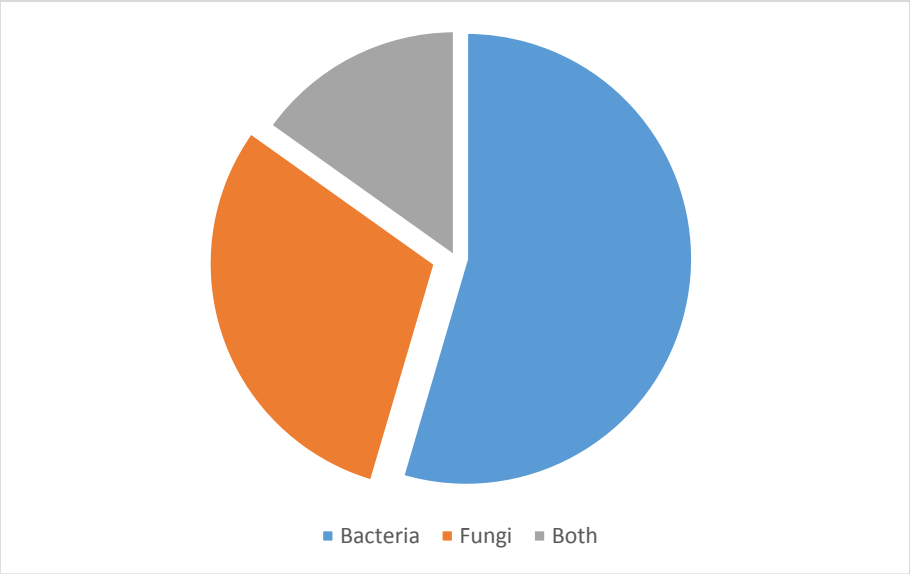
Graph 3: Growth/ no-growth ratio or microorganisms of the samples

Beef	6
Chicken	11

= 17

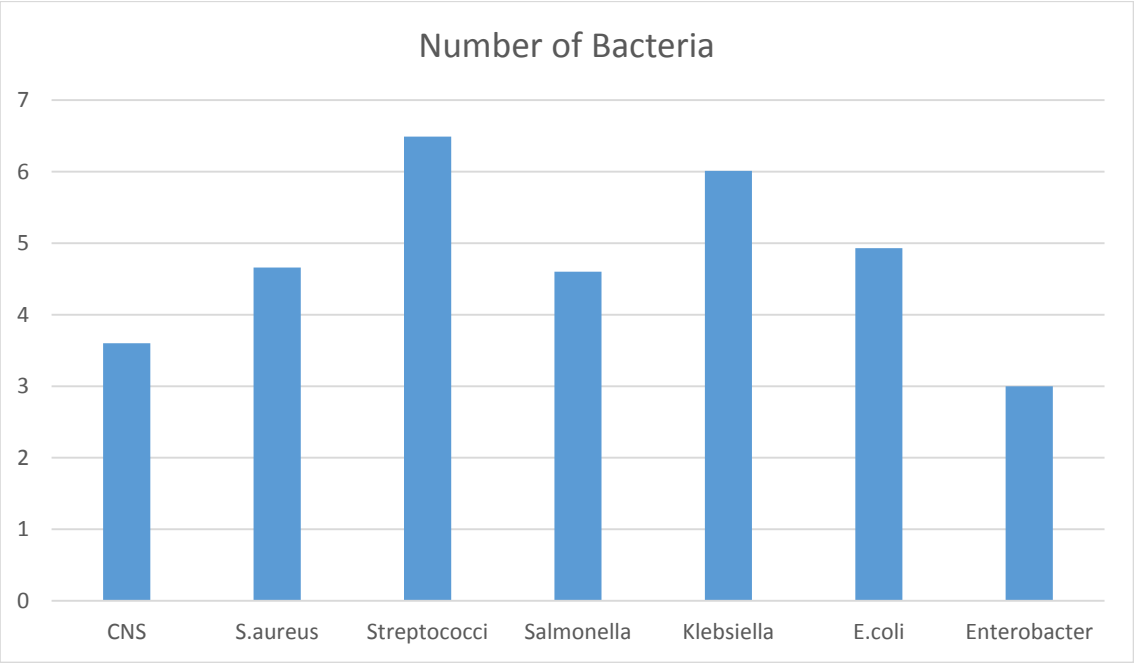
Table 7: Beef/ chicken ratio of the no-growth samples

6 beef sample and 11 chicken sample are detected no-growth in 17 samples. From 33 samples which pathogenic microorganisms isolated, 18 of them were from bacteria species, 10 of them were from Fungi species, 5 of them were from both Bacteria and Fungi species. This corresponds to 69 % of the samples are derived bacteria origin, 45 % of the samples are derived from fungi origin.



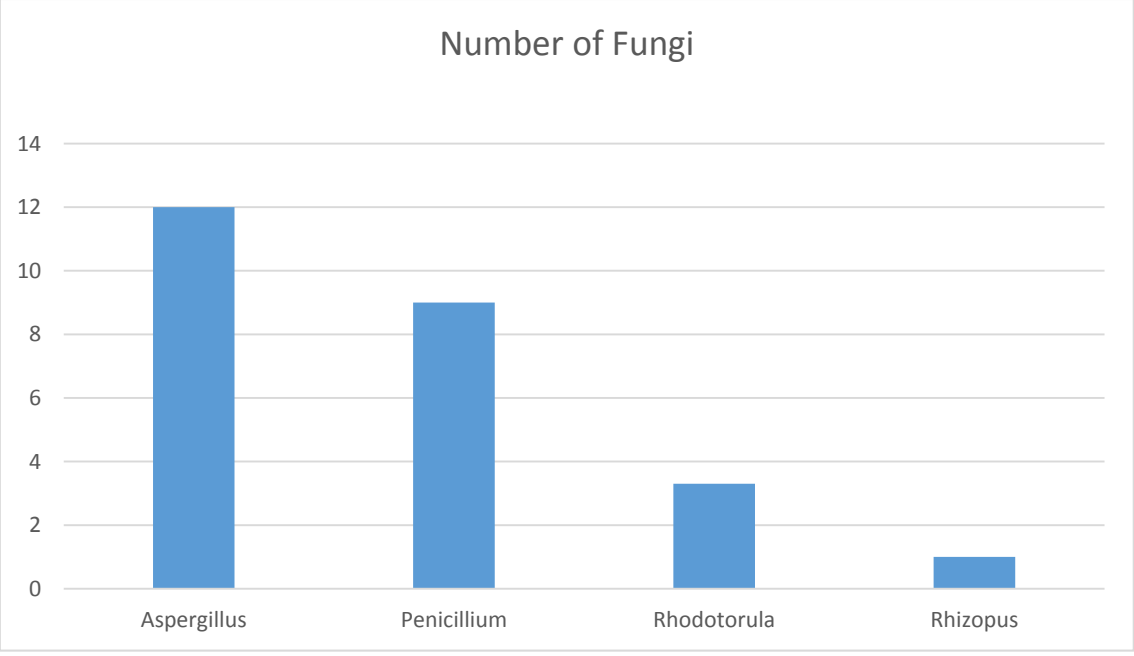
Graph 4: Bacteria/ Fungi ratio which were detecred from growth samples

We isolated *Coagulase Negative Staphylococcus* in 2 samples (4 %) , *S.aureus* in 11 samples (22 %), *Streptococcus* in 8 samples (16%), *Salmonella spp.* in 1 sample (2%), *Klebsiella pneumoniae* in 5 samples (10%) and *E.coli* in 6 samples (12%), *Enterobacter spp.* in 1 sample (2%) in bacteria speices.

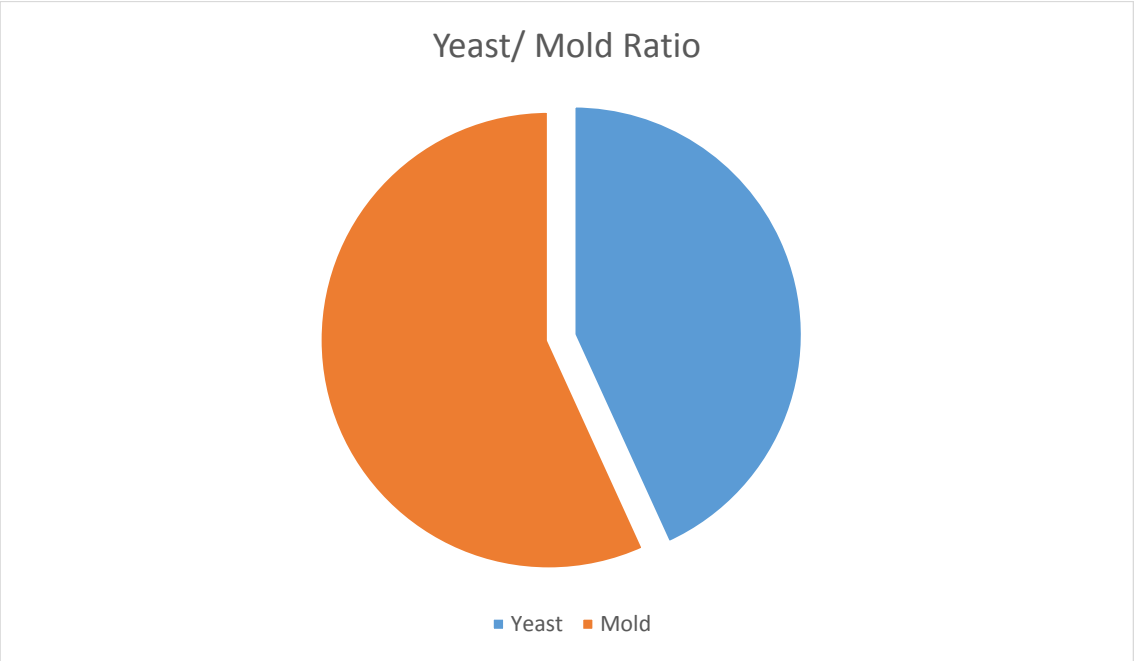


Graph 5: Number of bacteria which were detecred from samples

We isolated *Aspergillus spp*. in 3 samples (6%), *Penicillum spp*. in 9 samples (18%), *Rhizopus spp*. in 1 sample (2%), *Rhodotorula spp*. in 2 samples (4%),



Graph 6 : Number of Fungi which were detecred from samples



Graph 7: Yeast/ mold ratio which were detected from samples

Total microbial colony count was detected between 3 log cfu- 6,0047 log cfu/g. Total microbial load is 4,93 log cfu/g, 4,69 log cfu/g in beef samples, 5.10 log cfu/g in chicken samples, 3 log cfu/g in fish sample on average.

	Number of Samples	Total Microbial count
Beef	25	4,69 log cfu/g
Chicken	24	5,10 log cfu/g
Fish	1	3 log cfu/g

Table 8: Total microbial count of the samples on basis of raw material

6 DISCUSSION AND CONCLUSION

6.1 DISCUSSION

In Microbial analyses, 3- 3,47 log cfu/g *Coagulase Negative Staphylococcus* is detected in 2 samples (4%). The occurence of *Coagulase Negative Staphylococcus* in food is unsafe[66]. CNS is mosly found in blood of human. In a study investigating issues about isolating of CNS showed that 109 (2,6%) blood cultures was CNS positive in 4234 blood cultures which are collected from patients[66.] Detecting CNS in foods may be indicator or contamination personnel hands with open wound or knife wounds during handling.

3-4 log cfu/g *S.aureus* is detected in 8 samples (16%). *S.aureus* is one of the main pathogens causing many infections in human including in infective endocarditis, skin and tissue infections, pleuropulmonary, osteoarticular and device related infections[67]. *S.aureus* is found approximately 30% of the human. Human nasal cavity and skin are the main vehicle of *S.aureus*[68]. Contamination route may be though nose to hands. Heat processing in meat products is generally adequate for *S.aureus*. But contamination of *S.aureus* after process can be multiply and become hazardous. The main contamination factor is personnel hands [69]. This may arise from personnels without gloves during process of döner such as cutting, scaling, servicing. Also *S.aureus* can survive in case of inadequate heat process [70].

3 -6 log cfu/g *Streptococcus* is detected in 8 samples (16%). In another study reported that 5.3-6.2 log cfu/g *Staphylococcus was isolated in 40 döner samples in Germany*[71]. *Streptococcus* genus is considered to found in normal flora in humans and animals. *Streptococcus* species can contaminate to food pharynx and hand lesions of food handlers. *Streptococcus* cause foodborne illness with symtoms such as sore throat, enlarged tonsils, coughing, coryza and pharryngeal.[72]

4,6 log cfu/g *Salmonella* is detected in 1 sample (2%). Researchers marked the association between Salmonella and döner kebabs and several microbiogical problems could occur in all the world[73]. A study about hygienic quality of döners in Elazığ reported that Salmonella was detected in 26.6 % of the samples[74] . A study on prevalence, antimicrobial resistance and genetic diversity of Salmonella detected from ready to eat

foods in China reported that 3 sample was Salmonella positive, (2.0 %) in the 152 chicken samples[75]. Another study about hygienic quality of döner samples in 60 samples which are purchased from 5 different fast food markets in Tekirdağ showed all of the raw kebabs contained *Salmonella spp.* also 80% of the cooked chicken and 40 % of the cooked beef samples contained pathogens.[76] For a study on prevalance of food borne pathogens in ready to eat meat in seven different regions in China regions showed 32 samples was *Salmonella spp.* positive in 4047 (0.79%) . In a study on ready to eat foods in Jordan reported that *Salmonella spp.* was found 0.5% in overall samples.0.8% in 550 ready to eat chicken and 0.2% in 478 ready to eat beef products [77]. A study reported that *Salmonella spp.* was detected 16% in ready to eat foods in the microbiological survey in purchased foods in Johannesburg. South Africa[78]. In a study findings show that 1.5% of the ready to eat food was *Salmonella spp.* positive [79]. In another study *Salmonella spp.* was detected from 1.2 of smoked salmon, 2% of cooked ham samples, 1.5 % of frozen chicken croquettes, 11.1% of cured dried sausage samples [80]. *Salmonella* is mostly found in feces of human and main vehicles of *Salmonella spp*. are humans. Contamination occurs ingestion of contaminated food and water[81]. This indicates dirty hands of food handler. According to TS11869 and Turkish Food Codex, no *Salmonella* is allowed in raw döner [11].The main reason of *Salmonella* contamination is associated with consumption of fast foods which are purchased from street vendor[79].

3-6 log cfu/g *Klebsiella pneumoniae* is detected in 5 samples (10%). *Klebsiella spp.* are mainly distributed in the nature. They are commonly found in environment, surface water, sewage, soil and on plants and surface of humans [82 83 84 85 86]. *Klebsiella spp.* is pathogen of humans. In a study is investigating issues about *Klebsiella spp.* showed carriers were hands (42%), pharynx (19%), feces (77%) of hospital patients[87]. *Klebsiella spp.* causes contamination in retail meats. It is also main pathogen in fresh vegetables causing foodborne illness. A study showed that *K. pneumoniae* was detected in 65% of raw vegetables in hypermarkets[88] . Detection of *Klebsiella* in meat may be indicator or contamination from feces of humans via personnel hands or cross contamination with equitments which are used with vegetables. In a study about detection of *Klebsiella* showed 4,7 % sample was positive, in meat samples including poultry and meat[89].

3-4,90 log cfu/g *E.coli* is detected in 6 sample (12%). 102 cfu/g *E.coli* was detected in samples among döner kebabs sold in Erzurum Turkey[70] A study on prevelance and numbers of E.coli in minced beef and beef burgers in supermarkets in the Republic

of Ireland reported that 43 sample were *E.coli* positive (2.80%) of the 1553 beef samples collected over 13 month period[90]. In another study from 72 cooked chicken döner, 31% of the samples was *E.coli* positive[91.] For a study on prevalance of foodborne pathogens in ready to eat meat in seven different regions in China regions 3774 samples were gathered in total in 2016. Diarrheagenic *E. coli* was detected in 40 samples (1.06%) of the samples was [92].

Detection of coliform bacterias in cooked meat is associated inadequate heat process and contamination after processing. *E.coli* is member of coliform bacteria, sensetive to heat and directly associated with intestinal contamination. Consequently, detection of *E.coli* in cook meat is indication of fecal contamination[93]. On the other hand *E.coli* level is determined in TS 11859 for raw döner is (25) n = 5, c = 2, m = 5 cfu/g, M = $5x10^2$ cfu/g and as (24) n = 5, c = 2, m = $1x10^2$ cfu/g (2 log cfu/g), M = $1x10^3$ cfu/g (3 log cfu/g) [11].

3 log cfu/g *Enterobacter spp.* is detected in 1 sample (2%). *Enterococcus* type bacterias can survive in cooked meats because they are heat resistant [94]. In a study, 14% of the samples were detected *Enterococcus* positive in the ready to eat döner samples[95]. Detection of *Enteriobacteriaceae* kind of bacterias in cooked meats is associated with inadequate heat process [96 97 98].

In the studies about microbiological quality of ready to eat döner samples, reported that 4 log cfu/g *Clostridum perfringens* was found under the surface of reheated döner[94] . In another study *C.perfringens* is detected from the 10% of the total sample[99]. *C. perfringers* was detected in 8 of the samples in 44 cooked döner in a study about döners in Munich Germany [100]. A study about hygienic quality of döners in Elazığ reported 2-6 log cfu/g of *C.perfingens* was count[73]. We did not isolate any *C.perfringens* in our study. *C. perfringens* outbreaks occur in catering services such as school cafeterias, hospitals, nursing homes in case of keeping foods inadequate temperatures long time before service.

L. monocytogenes is found in 57 ready to eat meat samples in 3774 samples in total(1.43%) in a study in China[74]. In a study in Sweden among 507 heat treated meat product samples, *L. monocytogenes* detected 1.2% [101]. 4 % of ready to eat fermentad sausages were *L.monocytogenes* positive in the study on ready to eat fermented sausages among 100 samples purchased from markets in Edmonton, Alberta, Canada

[102]. 4,6% of the samples was *L.monocytogenes* positive in the study of the Republic of Ireland in 2014 [103]. The döners which contain *L.monocytogenes* may cause risk of illness in human. We did not isolate any *L.monocytogenes* in our study. According to TS11869 and Turkish Food Codex, no *L.monocytogenes* is allowed in döner[11].

The number of total aerobic bacteria exist in meat products is an important criteria to determine of the microbial quality [100]. In this study, total microbial colony count was detected between 3 log cfu- 6,0047 log cfu/g. Total colony count is detected between 5.68 log cfu/g–4.92 log cfu/g in another study about hygienic quality of döner samples reported in Tekirdağ [10]. The limit value is ≤ 5 log cfu/g for meat products [100 104]. In TS11859 the value determined as n = 5, C = 2, m = 5 x 10^5 cfu/g (5,69 log cfu/g) , M = 5 x 10^6 cfu/g (6,69 cfu/g) [11].

9 cfu/g *Penicillum spp.* is found in 9 samples (18%) . *Penicillum* occur in many speices and they are widely distributed in nature from air and soil to food. Many fungal speices which grow on food cause deterioation as in vegetable, fruit, cold meats, sandwiches, cereals and cereal and agricultural products [105 106 107 108]. *Penicillium spp.* are mainly contaminants on considerable organic materials and produce mycotoxin. When foods including mycotoxins are ingested it become toxic for human [109]. *Penicillium* are associated with food pathogens and threaten public health, food safety and national economy of the world[110 111]. To determine contamination of *Penicillium* is not easy due to it is being ubiquitous.

12 cfu/g *Aspergillus* is found in 3 samples (6%). 2 of the samples contained *A.niger*, 1 of the sample contained *A.flavous*. Aspergillus occurance on food is unwanted due to it is mycotoxin produce. Also *A. flavus* produces aflatoxin as secondary metabolite product which is carciogenetic and immunosuppressive to human[112]. Mycotoxin delevelop in high moisture and temperature, ripen crops and poor storage conditions. In a study *Aspergillus spp.* is detected in 100% of the black peppers[113]. In another study reported *A.flavus* was detected from nasal cavity, on the contary *A.niger* was detected in nails[114] Contamination route may be from personnel nails. Spices also may be contaminant agent.

1 cfu/g *Rhizopus spp.* is detected in 1 sample (2%). Common saprophtic fungi Rhizopus spp is widely distributed on organic substances including mature fruits,

vegetables, peanuts and bread[115 116 117]. Growing temperature ranges from 25 °C to 45 °C. Humidity encourages the growth of *Rhizopus spp.*[118]. It is mostly used for fermantation agent for tempeh production[119]. Some *Rhizopus spp* are opportunistic agents of human and may be complication of diabetic ketoacidosis[120]. Some *Rhizopus spp*. is detected by biopsy of skin, nasal cavity and the lungs[121]. Contamination route may be from nose, skin to personnel hands and to food in the end. Cross contamination may occur from bread to meat aswell.

2 cfu/g Rhodotorula is detected in 2 samples (4%). *Rhodotorula* is a common environmental yeast. It is widely found in ail, lake, soil, ocean, water, milk and fruit juice[122]. *Rhodotorula* is detected in feces. It can survive in extreme conditions in gastrointestinal tract and it is suspected that whether *Rhodotorula* can pass to bloodstream from gastrointestinal tract[123]. It is uncertain that *Rhodotorula* is pathogen or opportunistic pathogen to human[124 125]. *Rhodotorula* is widely found in hospital environment and most common microorganism which is detected from hands of hospital personnels and patients. Rhodotorulosis can occur in patients who have week immune system [126]. It is possible to estimate contamination route as from environment to personnel hands and to food in the end.

Candida spp.	*C.albicans*	Other yeast	*Blastoschizomyces spp.*
	C.glabrata		*Cryptococcus neoformans*
	C:gulliermondi		*Malassezia spp.*
	C.kefyr		*Rhodotorula spp.*
	C.krusei		*Saccharomyces spp.*
	C.lusitaniae		*Trichosporon spp.*
	C.rugosa	*Zygomycetes*	*Absidia spp.*
	C.parapsilosis		*Cunninghamella spp.*
	C.tropicalis		*Mucor spp*
Aspergillus spp.	*A.fumigtus*		*Rhizomucor spp.*
	A.niger		*Rhizopus spp.*
	A.flavus	*Dematiaceous moulds*	*Alternaria spp.*
	A.terreus		*Bipolaris spp.*
Other hyaline moulds	*Acremnium spp.*		*Curvularia* spp
	Fusarium spp.		*Cladophialophora spp.*
	Paecilomyces spp.		*Exophiala spp.*
	Scedosporium spp.		*Phialophora spp.*

Graph 8: Pathogenic Fungi [127]

Table 9: Microbiological Regulation of Meat By Turkish Food Codex [128]

MICROBIOLOGICAL REGULATION BY TURKISH FOOD CODEX

Food	Microorganism/toxins/metbolits	Sampling plan		Limits	
		n	c	m	M
Meat and Meat products					
Carcass, minced meat, offals					
Cattle, sheep, goat and horse carcass, raw meat and minced meat, poultry carcass and frozend minced turkey	Aerobic colony count	5	2	$5x10^5$	$5x10^6$
	S. aureus	5	2	10^3	10^4
	Salmonella spp.	5	0	0/25 g-mL	
	L. monocytogenes	5	0	0/25 g-mL	
	E. coli O157:H7	5	0	0/25 g-mL	
Offals	*C. perfringens*	5	2	10^3	10^4
	Salmonella spp.	5	0	0/25 g-mL	
	E. coli O157:H7	5	0	0/25 g-mL	

Processed Meat					
Processed meat and poultry (chilled/ frozen)	Aerobic colony count	5	2	10^5	10^6
	S. aureus	5	2	10^3	10^4
	C. perfringens	5	2	10^3	10^4
	Salmonella spp.	5	0	0/25 g-mL	
	L. monocytogenes	5	0	0/25 g-mL	
	E. coli O157:H7	5	0	0/25 g-mL	

Meat and meat products					
Non heat-processed meats					
Cured and dried meat	Mold and yeat	5	2	10^1	10^2
	S. aureus	5	2	10^2	10^3
	Sulfide reducing *Clostridium* spp.	5	2	10^3	10^4
	Salmonella spp.	5	0	0/25 g-mL	
Fermented (sausage etc.)	Mold	5	2	10^2	10^3
	S. aureus	5	2	10^2	10^3
	Salmonella spp.	5	0	0/25 g-mL	
	L. monocytogenes	5	0	0/25 g-mL	

	E. coli O157:H7	5	0	0/25 g-mL	
Heat processed meat (sausage, salami, meatballs, döner etc.)	Yeast and Mold	5	2	10^2	10^3
	S. aureus	5	2	10^2	10^3
	C. perfringens	5	2	10^2	10^3
	Salmonella spp.	5	0	0/25 g-mL	
	L. monocytogenes	5	0	0/25 g-mL	
	E. coli O157:H7	5	0	0/25 g-mL	
Canned meat	Sterile	5	0	Madde 4-(g)	
Other					
Gelatin and Collagen	*Salmonella* spp.	5	0	0/25 g-mL	
Bouillon cube, powders, instant soups, spices, sauces					
Raw powder mixes	Enterobacteriacea	5	2	10^2	10^3
	Mold and Yeast	5	2	10^2	10^3
	S. aureus	5	2	10^2	10^3
	B. cereus	5	2	10^2	10^3
	C. perfringens (only inside meat)	5	2	10^2	10^3
	Salmonella spp.	5	0	0/25 g-mL	

Cooked powder mixes	Coliform bacterias	5	2	10^2	10^3
	S. aureus	5	2	10^3	10^4
	B. cereus	5	2	10^3	10^4
	C. perfringens (only in meat)	5	2	10^3	10^4
	Salmonella spp.	5	0	0/25 g-mL	

n = number of units comprising the sample;
c = number of sample units giving values between m and M.
m= microbiological value of the samples between number of n and c
M: the number of microorganism in the numbers of c that surpasses the level and it shows its unacceptable

The text results indicate the microbiological quality. The limits given is referred to each sample.
Enterobacteriacea and aerobic colony count
— SATISFACTORY, if all the value is < m,
— ACCEPTABLE, if all the value is between m and M,
— UNSATISFACTORY, if the value is >M.

Salmonella
— SATISFACTORY, if Salmonella isolated in a minimum of c samples
— ACCEPTABLE, if Salmonella isolated in more than c samples
— UNSATISFACTORY, if the daily mean log is >M.

E.coli and aerobic colony count in meat
— SATISFACTORY, if one or more of the values are < m,
— ACCEPTABLE, if one or more of the values are between m and M,
— UNSATISFACTORY, , if one or more of the values are is >M or more than c/n values are between m and M.

6.2 CONCLUSION

As fast food consumption is increasing around the world, so do concerns and challenges to meat hygiene and safety. There are many previous and current reseaches about microbiological quality of fast foods.

A study reported the reasons of consuming fast foods. The main reaons can be counted as, quick (92 %), easy to access to fast food restaurants (80%) and taste (69 %). Then the reasons goes on: Fast foods make socializing people in society (33%), nutritious foods (21%), fast food restaurants are entertaining (12 %)[129].

Heat transfer depends on composition, size of the product and shape. Meats (especially fats) are considered poor heat transferring entities. Because of the shape and cooking method of the döner, inside part remain cold or warm when outside part is cooked. That causes a temperature gradient from the center to the peripheral parts and allows bacteria to multiply[130]. Thus it may not destroy pathogenic microorganisms inside part as well as out slices part due to inadequate cooking. There is no decrease of the number of microorganisms in cooked meat despite of the heat process. That is associated seconder reasons such as contamination, personal hygene, nonsterile equipments[131].

In this study some undesired bacteria like coliforms, *Salmonella spp, S.aureus, Klebsiella pneumoniae, Streptecoccus* are detected in most of the cooked döner samples. We isolated smilar pathogens with other studies [70 73 75 90 94 98 100 132]. Whereas other studies about microbiological quality of döner kebabs we did not isolate any *L.monocytogenes* and *C. perfringens* [133] [134]. Microbiological analysis of the study indicates that manufacturig processes of döner are not appropriate in terms of cooking methods and hygienic quality.

And it is determined that cooked chicken döner has lower microbiological quality than meat döner in our study. Chicken is döner associated to have higher aerobic mesophilic bacteria and coliforms than beef döners in general in a study about microbial quality of döners in Tekirdağ[10]. So hygenic and technologic rules should be applied to all the stage of processing from raw material to consumption. Cleaning, disinfection and personal hygiene must be effective and food safety systems such as HACCP must be applied.

As a result, many public health auhorities and researches are concerned about microbiological quality of fast foods sold in the stores or street vendors. Meat products become vehicles of foodborne pathogens in case of inadequate manufacturing processes. The resuts showed that hygiene is required to be paid attention in all manufacturing stages of fast food from field to table, such as selection of raw material, transfer, storage, cleaning, cooking and service.

Because of the nature reasons foodborne problems can not be seen virtually so difficult to observe food safety. On this point of view, legislation is weak. Most of the fast food restaurants are small scale, poorly organised and usually food handlers are without educuated and unaware of the potential risks of their foods. Also many consumers are unaware of food hygiene[135].

Personnel hands are thought to be main reason of contaminations. In the light of the results of this study indicates microbial criterias and hygiene must be well noticed in the stages from the selection of raw materials, storage, preparation to service.

7 REFERANCES

1**)** Havelaar AH, Kirk MD, Torgerson PR. *et al*. World health organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med*. 2015; 12(12): e1001923. doi:10.1371/ journal.pmed.1001923

2) Scallan E, Hoekstra RM, Angulo FJ. *et al*. Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis*. 2011; 17(1): 7-15. doi: 10.3201/eid1701.P11101.

3) Thornton LE, Lamb KE, Ball K. Fast food restaurant locations according to socioeconomic disadvantage, urban-regional locality, and schools within Victoria, Australia. *SSM - Population Health* . 2016; 2:1-9. doi: 10.1016/j.ssmph.2015.12.001

4) Richardson AS, Boone HJ, Popkin BM. *et al*. Neighborhood fast food restaurants and fast food consumption: A national study. *BMC Public Health*. 2011; 11: 543.

5) Egan MB, Raats MM, Grubb SM. *et al.* A review of food safety and food hygiene training studies in the commercial sector. *Food Control*. 2007; 18(10): 1180-1190.

6) Erkmen O, Bozoğlu TF. *Food Microbiology-1,* Ankara, Türkiye: İlke Publication; 2008.

7) Meng J, Doyle MP. Emerging and evolving microbial foodborne pathogens. *Bull Inst Pasteur*. 1998; 96: 151-154.

8) David J, Ebel, Eric D. *et al*. Comparing foodborne illness risks among meat commodities in the United States. *Food Control*. 2015; 54: 353-359. doi: 10.1016/j.foodcont.2015.02.018

9) Lasztity R. *Food quality and standarts*. Oxford, United Kingdom: Eolss Publishers Co Ltd; 2009.

10) Kayisoglu S, Yilmaz, İ, Demirci M. *et al*. Chemical composition and microbiological quality of the doner kebabs sold in Tekirdağ market. *Food Control.* 2003; 14: 469-474.

11) Institute of Turk Standards (1995a). Döner kebab-raw (uncooked*). The number of Turk Standards: 18859,* Ankara, Turkey.

12) Vazgecer B, Ulu H, Oztan A. Microbiological and chemical qualities of chicken doner kebab retailed on the Turkish restaurants. *Food Control*. 2004; 15: 261-264.

13) Jay JM. *Modern Food Microbiology*. Maryland: Aspen Publishers; 1998.

14) Shewan JM. Bacteriological standards for fish and fishery products. *Chemical Industrial*. 1970; 2: 299-302.

15) Schmauder HP, Heritage J, Evans EGV. *et al. Introductory Microbiology*, Cambridge University Press, 1996; 37: 234.

16) Wray C. *Salmonella*/ Properties and occurence. In: Caballero B, Trugo L, Finglas PM, ed. *Encyclopedia of Food Sciences and Nutrition*. San Diego, CA, Academic Press; 2003: 5074-5079.

17) Wong AL, Bergdoll MS, *Staphylococcus*/Properties and occurence. In: Caballero B, Trugo L, Finglas PM, ed. *Encyclopedia of Food Sciences and Nutrition*. San Diego, CA, Academic Press; 2003: 5547-5551.

18) Austin JW, *Clostridium*/Occurance of *Clostridium botulium*. In: Caballero B, Trugo L, Finglas PM, ed. *Encyclopedia of Food Sciences and Nutrition*. San Diego, CA, Academic Press; 2003: 1407-1413.

19) Boer de E, *Enterobacteriacea, coliforms and E.coli*/ Classical and modern methods for detection/enumeration. In: Robinson RK, Batt CA, Patel BD, ed. *Encyclopedia of Food Microbiology*. San Diego, CA, Academic Press; 2004: 610-617.

20) Guglielmetti P, Bartoloni A, *Escherichia coli/* Food Poisoning by Species other than *Escherichia coli*. In: Caballero B, Trugo L, Finglas PM, ed. *Enycyclopedia of Food Sciences and Nutrition*. San Diego, CA, Academic Press; 2003: 2166-2169.

21) Skirrow MB, *Campylobacter/* Properties and Occurrence. In: Caballero B, Trugo L, Finglas PM, ed. *Enycyclopedia of Food Sciences and Nutrition*. San Diego, CA, Academic Press; 2003:779-786.

22) Puspanadan, S, Afsah-Hejri, L, Loo YY. Detection of *Klebsiella pneumoniae* in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR) *International Food Research Journa*l 2012; 19(4): 1757-1762

23) Sahly H, Podschun R. Clinical, bacteriological, and serological aspects of *Klebsiella* infections and their spondylarthropathic sequelae. *Clin Diagn Lab Immunol* 1997; 4: 393-399.

24) Centers for Disease Control and Prevention (CDC) Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. *Morb Mortal Wkly Rep* 2009; 58: 256-60.

25) Podschun R, Ullmann U. *Klebsiella* spp. As nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews*. 1998;11(4): 589-603.

26) Becker K, Heilmann C, PetersClin G. *Coagulase-Negative Staphylococci*. *Clinical Microbiology Reviews*. 2014; 27(4): 870-926 doi: 10.1128/CMR.00109-13.

27) Fowoyo PT, Ogunbanwo ST. Virulence and toxigenicity of coagulase-negative *Staphylococci* in Nigerian traditional fermented foods. *Can. J. Microbiol*. 2016; 62: 572–578. 10.1139/cjm-2015-0752

28) Patterson MJ. *Streptococcus*. In: Baron S, ed. Medical Microbiology. 4th ed.

Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 13. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7611.

29) Sanders WE, Sanders CC. *Enterobacter spp*.: Pathogens poised to flourish at the turn of the century. *Clinical Microbiology Reviews*. 1997;10(2): 220-241.

30) Low JC, Donachie WA. review of *Listeria monocytogenes* and listeriosis. *Veterinary Journal.* 1997; *153*(1):9-29.

31) Ciesielski CA, Hightower AW, Parsons SK, *et al*. Listeriosis in the United States: 1980- 1982. *Arch Intern Med*. 1988;148:1416-9.

32) Gahan CG, Hill C. Gastrointestinal phase of *Listeria monocytogenes* infection. *Journal of Applied Microbiology.* 2005;98*(6):* 1345-1353

33) Acha PN, Szyfres B. *Listeriosis*. Zoonoses and communicable diseases common to man and animals. 3rd ed. Washington D.C.: Pan American Health Organization. Scientific and Technical Publication. 2003; 1(580): 168-179.

34) Schuchat A, Deaver KA, Wenger JD. *et al.* Role of foods in sporadic *Listeriosis*. I. Case-control study of dietary risk factors. The Listeria Study Group. JAMA. 1992;267:2041-5.

35) Pradhan AK, Ivanek R, Grohn YT. *et al.* Quantitative risk assessment for *Listeria monocytogenes* in selected categories of deli meats: impact of lactate and diacetate on listeriosis cases and deaths. *J Food Prot*. 2009; 72: 978-89.

36) Grass JE, Gould LH, Mahon BE. Epidemiology of foodborne disease outbreaks caused by *Clostridium perfringens*, United States, 1998-2010. *Foodborne Pathog Dis*. 2013 Feb; 10(2): 131-6. doi: 10.1089/fpd.2012.1316. Epub 2013 Feb 4.

37) Veigas J. *Fungi and Molds*. New York: The Rosen Publishing Group; 2004.

38) Gwynne Vaughan HCI, Barnes B. *The Structure & Development of the Fungi*. 2nd ed. Cambridge, UK, Cambridge University Press; 1937.

39) Medina LM, Jordano R. Petrifilm - A simplified cultural technique. In: Batt CA ed. *Encyclopedia of Food Microbiology*. 2014; 3: 9-24.

40) Rank C, Larsen TO, Frisvad JC. Functional systems biology of *Aspergillus*. In: Machida M, Gomi K, ed. *Aspergillus: Molecular biology and Genomics*. Wymondham, Norfolk, UK: Caister Academic Press; 2010: 173-198.

41) Hui YH, Khachatourians GG. Food Biotechnology: Microorganisms. *Journal of Chemical Technology and Biotechnology.* 1996; 66: 324.

42) Elad Y, Williamson B, Tudzynski P. *Botrytis*: *Biology, Pathology and Control*, Springer Publishers, 2007. doi: 10.1007/978-1-4020-2626-3.

43) Pitt JI, Hocking AD. *Fungi and Food Spoilage*, Springer US, 2009. doi: 10.1007/978-0-387-92207-2.

44) Meyer D, Weipert D, Mielke H. Beeinflussung Der Qualitat Von Weizen Durch Den Befall Mit Fusarium Culmorum. *Getreide Mehl Brot*. 1986; 40: 35-39.

45) Prell HH, Day P. *Plant-Fungal Pathogen Interaction: A Classical and Molecular View,* Springer-Verlag Berlin Heidelberg, 2001. doi:10.1007/978-3-662-04412-4.

46) MEGEP Gıda Teknolojisi, Genel Mikrobiyoloji, Ankara. 2006: 32.

47) Alderson P, Rowland M. *Making Use of Biology*, 2nd ed, Growth in Microorganism, Macmillan Education UK, 1995: 33-42. doi: 10.1007/978-1-349-13563-9_3.

48) Leininger DJ, Roberson JR, Elvinger F. Use of eosin methylene blue agar to differentiate *Escherichia coli* from Other Gram-negative mastitis pathogens, *Journal of Veterinary Diagnostic Investigation,* 2016; 3: 273 – 275. doi: 10.1177/104063870101300319.

49) Atlas RM. *Handbook of Microbiological Media,* Washington DC; CRC Press; 2010.

50) Winn W, Allen S, Janda W. *et al*. *Koneman's Color* Atlas *and Textbook of Diagnostic microbiology*, 6th ed. Lippincott Williams & Wilkins, Philadelphia; 2006.

51) Alfred BE. *Benson's Microbiological Applications*, 9th ed, McGraw Hill Publication: Mishawaka; 2005.

52) Prescott LM, Harley JP, Klein DA. *Microbiology*, 6th ed, McGraw-Hill Higher Publication: Mishawaka; 2004.

53) Thrasher, JD. Fungi, bacteria, nano-particulates, mycotoxins and human health in water-damaged indoor environments. *Journal of Community and Public Health Nursing*. 2016; 2: 2. doi:10.4172/2471-9846.1000115.

54) Tille P. *Bailey & Scott's Diagnostic Microbiology*, 13th ed. Elseiver Mosby: St. Louis; 2013.

55) Whitman W, Goodfellow M, Kämpfer P. *et al*. ed. *Bergey's Mannual of Systematic Bacteriology The Actinobacteria*, Springer-Verlag: New York; 2012.

56) Aygan A, Arikan B. An Overview on Bacterial Motility Detection, *International Journal of Agriculture and Biology*: 2007; 9: 193-196. Available from: http://www.fspublishers.org/search_list2.php?title=bacterial+motility Access: 27.03.2017.

57) Hayes R. *Food Microbiology and Hygiene*. 2nd ed. Springer US: Boston; 1995.

58) McMeekin TA. *Detecting Pathogens in Food*. 1st ed. Tasmania: Woodhead Publishing; 2016.

59) Facklam R, Elliot JA. Identification, classification, and clinical relevance of catalase-negative, Gram-positive cocci, excluding the *Streptococci* and *Enterococci. Clinical Microbiology Rewievs.*1995;8: 479-495.

60) Goldman E, Green LH. *Practical Handbook of Microbiology*. 2nd ed. CRC Press: Boca Raton; 2015.

61) Sieuwerts S, De Bok FA, Mols E. *et al.* A simple and fast method for determining colony forming units. *Letters in Applied Microbiology.2008;* 47(4): 275-8. doi:10.1111/j.1472-765X.2008.02417.x

62) Available from: http://www.biomerieux-usa.com/clinical/vitek-2-healthcare Access: 28. 03. 2017.

63) Hata DJ, Hall L, Fothergill AW. *et al*. Multicenter Evaluation of the New VITEK 2 Advanced Colorimetric Yeast Identification Card, *Journal Of Clinical Microbiology.* 2007; 45: 1087-1092.

64) Available from http://www.biomerieux-industry.com/food/api-id32-microbial-identification Access: 28. 03. 2017.

65) Karaltı İ, Çolakoğlu G. Candida Ve Aspergillus enfeksiyonlarının Real Time Pcr yöntemi İle hızlı tanısının kültür yöntemi ile karşılaştırılması Ve antifungal direncin kolorimetrik yöntemle tayini. İstanbul, Marmara Üniversitesi, 2011.

66) Hal SJV, Frostis V, Miyakis S. *et al.* Prevalence and significance of *Coagulase-Negative Staphylococci* isolated from blood cultures in a tertiary hospital. *2007:* 551-554. Published online: 08 Jul 2009 http://dx.doi.org/10.1080/00365540701877304

67) Tong SYC, Davis JS, Eichenberger E. *et al. Staphylococcus aureus* Infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*. 2015; 28(3): 603-661. doi:10.1128/CMR.00134-14.

68) Wertheim HF, Melles DC, Vos MC. *et al.* The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis.* 2005; 5: 751–762. 10.1016/S1473-3099(05)70295-4.

69) Brown, MH. *Meat Microbiology*. Applied Science Publishers Ltd. London and New York. 1982.

70) Küpeli GV, Kaya M. Yaprak dönerin mikrobiyolojik kalitesi ve kimyasal bileşimi. *Turkish Journal of Veterinary & Animal Sciences*. 2004; 28: 1097-1103.

71) J. Krüger, V. Schultz, J. Kuntzer, Doner Kebab-Utersuchung Zum Handelsbrauch in Stuttgart Fleischwirtsch, 73 (11) (1993), pp. 1242–1248.

72) Katzenell U, Shemer J, Bar-Dayan Y. *Streptococcal* contamination of food: an unusual cause of epidemic pharyngitis. *Epidemiology and Infection*. 2001; 127(2): 179-184.

73) Snnott M, Morse DL, Maguire H. *et al*. An outbreak of *Salmonella mikawasima* associated with doner kebabs. *Epidemiol Infect* 1993; 111: 473-481.

74) Dığrak M, Gür S, Özçelik S. Elazığ'da tüketime sunulan dönerlerin mikrobiyolojik kalitesi. *Kükem Dergisi.* 1995; 18: 76-77.

75) Yang X, Huang J, Wu Q. *et al*. Prevalence, antimicrobial resistance and genetic diversity of *Salmonella* isolated from retail ready-to-eat foods in China, *Food Control*. 2016; 60: 50-56.

76) Brashears MM, Chaves BD. The diversity of beef safety: A global reason to strengthen our current systems. *Meat Sci.* 2017 Apr 5. pii: S0309-1740(17)30213-9. doi: 10.1016/j.meatsci.2017.03.015.

77) Osaili TM, Al-Nabulsi AA, Shaker RR. *et al*. Prevalence of *Salmonella serovars, Listeria monocytogenes*, and *Escherichia coli O157:H7* in Mediterranean ready-to-eat meat products in Jordan. *Journal of Food Protection*. 2014; 7(1): 106-111.

78) Christison C, Lindsay D, Von Holy A.Microbiological survey of ready-to eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. *Food Control*. 2008: 19(7); 727-733.

79)Zarei M, Maktabi S, Ghorbanpour M. Prevalence of *Listeria monocytogenes*, *Vibrio parahaemolyticus, Staphylococcus aureus*, and *Salmonella spp*. in seafood products using multiplex polymerase chain reaction. *Foodborne Pathogens and Disease*, 2012;9(2): 108-112.

80) Cabedo L, Picart i Barrot L, Teixido i Canelles A. Prevalence of *Listeria monocytogenes and Salmonella* in ready-to-eat food in Catalonia, Spain. *Journal of Food Protection*. 2008; 71(4): 855-859.

81) Linam WM, Gerber MA. Changing epidemiology and prevention of *Salmonella* infections. *Pediatr Infect Dis J*. 2007;26 (8): 747-748.

82) Bagley ST, Seidler RJ, Talbot HWJ. *et al*. Isolation of *Klebsiellae* from within living wood. *Appl Environ Microbiol*. 1978; 36: 178-185.

83) Brown C, Seidler R J. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. Appl Microbiol. 1973; 25: 900-904

84) Edberg SC, Piscitelli V, Cartter M. Phenotypic characteristics of coliform and noncoliform bacteria from a public water supply compared with regional and national clinical species. *Appl Environ Microbiol*. 1986; 52: 474-478.

85) Matsen JM, Spindler JA, Blosser RO. Characterization of *Klebsiella* isolates from natural receiving waters and comparison with human isolates. Appl Microbiol. 1974;28: 672-678.

86) Seidler R J, Knittel M D, Brown C. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. Appl Microbiol. 1975; 29: 819-825.

87) Podschun R, Ullmann U. *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews*. 1998;11(4): 589-603.

88) Puspanadan S, Afsah-Hejri L, Loo YY. Detection of *Klebsiella pneumoniae* in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR) *International Food Research Journal.* 2012*;* 19(4): 1757-1762.

89) Munir EH, Khalifa KA, Mohammed AM. status of food safety due to bacterial contaminants of poultry meat and poultry products in Khartoum State. *Journal of Scientific Research and Reports.* 2014; 3(14): 1897-1904.

90) Cagneya C, Crowleya H, Duffya G. *et al.* Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology*. 2004; 21: 203–212.

91) Vazgeçer B, Ulu H, Öztan A. Microbiological and chemical qualities of chicken döner kabab retailed on the Turkish restaurants. *Food Control*. 2004; 15: 261-264. doi: 10.1016/S0956-7135(03)00065-3.

92) Yang S, Pei X, Wang, G. Prevalence of food-borne pathogens in ready-to-eat meat products in seven different Chinese regions. *Food Control.* 2016; 65: 92–98.

93) Göktan, D. *Gıdaların Mikrobiyal Ekolojisi: Et Mikrobiyolojisi*. Ege Üniv. Müh. Fak. Yayınları, İzmir. 1990; 1:292.

94) Vanderzant, C, Splittstoesser, DF. *Compendium of Methods for the Microbiological Examination of Food*. Washington DC: American Public Health Association; 1992.

95) Jöckel J, Stengel G. Döner kebab-Unterschung und beurteilung einer türkischen spezialitat (Döner kebap manufacturing and assessment of Turkish specialities) *Fleischwirtschaft*. 1984;64(5):527–540.

96) Aran N. İstanbul Piyasasında Tüketilen Bazı Hazır Gıdaların Tüketici Sağlığı Yönünden Değerlendirilmesi, *Gıda Sanayii*. 1988; 2: 36-42.

97) Krämer J. Lebensmittel Mikrobilogie. Eugen Ulmer GmbH and Co. Wollgrasweg 41, Stuttgart 70 (Hohenheim). Germany, 1987.

98) Prändl O, Fischer A, Schmidhofer T. et al. FleischTechnologie und Hygiene der Gewinnung und Verarbetiung. Verlag, Eugen Ulmer, Stuttgart, Germany, 1988.

99) Bryan FL, Standley SR, Henderson WC, Time-temperature conditions of Gyros. *Journal of Food Protection*, 1980; 43(5): 346-353.

100) Stolle A, Eisgruber H, Kerschhofer D. *et al*. The gyros-study on variations of Doner-Kebab and microbiological and hygienic aspects in Munich. *Fleischwirtschaft*. 1993; 73(8): 78-81.

101) Lambertz ST, Nilsson C, Bradenmark. *et al*. Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010. *International Journal of Food Microbiology*. 2012: 160, 24e31.

102) Bohaychuk VM, Gensler GE, King R. *et al.* Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail market place in Edmonton, Alberta, Canada. *Journal of Food Protection*. 2006; 69(9): 2176-2182.

103) Leong D, Alvarez-Ordonez A, Jordan K. Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland. *Frontiers in Microbiology*. 2014; 5: 436.

104) Gıda, Tarım ve Hayvancılık Bakanlığı: Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği, Resmi Gazete. Sayı: 28157 (3. Mükerrer) 2011. Available from: http://www.resmigazete.gov.tr/eskiler/2011/12/20111229M3-6.htm Access: 28.03.2017

105) Grazia LP. Bagni RA. *et al*. The role of moulds in the ripening process of salami. Food Microbiol. 1986; 3**:** 19-25.

106) Huerta T, Sanchís V, Hernández J. *et al***.** 1987. Enzymatic activities and antimicrobial effects of *Aspergillus* and *Penicillium* strains isolated from Spanish dry cured hams: quantitative and qualitative aspects. *Microbiol. Alim. Nutr.* 1987; 5**:** 289-294.

107) Samson RA, Hoekstra ES, Frisvad JC. *Introduction to food and airborne fungi.* Utrect: Centraalbureau voor Schimmelcultures; 2004.

108) Brian F, Samson RA, Miller DJ. *et al.* Microorganisms in home and indoor work environments: Diversity, Health Impacts, Investigation and control 2001. In: Hoog GS, Guarro J, Gene J, ed. Atlas of Clinical Fungi, 2nd ed, Centraalbureau voor Schimmelcultures, 2000.

109) Yiannikouris A, Jouany JP. Mycotoxins in feeds and their fate in animals: a review. *Anim Res. 2002;* 51:81–99.
In: Hogg GS, Guarro J, Gene J, ed. Atlas of Clinical Fungi, ‘nd ed, Central albureu voor Schimmelcultures, 2000.

110) Steyn PS. Mycotoxins, general view, chemistry and structure. *Toxicol Lett.* 1995; 82(8): 843–51.

111) Sheikh-Ali SI, Ahmad A, Mohd-Setapar SH. *et al*. The potential hazards of *Aspergillus spp*. in foods and feeds, and the role of biological treatment: a review *J Microbiol*. 2014; 52: 807. doi:10.1007/s12275-014-4294-7.

112) Diener UL, Cole RJ, Sanders TH. *et al* Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annual Review of Phytopathology* .2013; 25(1):249-270.

113) Bouakline A, Lacroix C, Roux N. *et al.* Fungal contamination of food in hematology units. *Journal of Clinical Microbiology*. 2000; 38(11): 4272-4273.

114) Xess L, Mohanty S, Jain N *et al*. Prevalence of *Aspergillus* species in clinical samples isolated in an Indian tertiary care hospital *Indian J Med Sci* 2004 ;58(12):513-9.

115) Kirk PM, Cannon PF, Minter DW, *et al. Dictionary of the Fungi.*10th ed. Wallingford, UK: CABI. 2008.

116) Zheng RY, Chen GQ, Huang H. *et al.* A monograph of *Rhizopus. Sydowia*. 2007; 59 (2): 273–372.

117)Abe A, Asano K, Sone A. Molecular phylogeny-based taxonomy of the genus *Rhizopus*. *Bioscience, Biotechnology and Biochemistry. 2010;*74 *(7): 1325–1331.*

118) Baumann U, Bisping B. Proteolysis during tempe fermentation. *Food Microbiol*.1995; 12(0):39–47.

119) Wiesel I, Rehm HJ, Bisping B. Improvement of tempe fermentations by application of mixed cultures consisting of *Rhizopus* spp. and bacterial strains *Appl. Microbiol. Biotechnol.* 1997;47(3):218–225.

120) Chinn RY, Diamond RD. Generation of chemotactic factors by *Rhizopus oryzae* in the presence and absence of serum: relationship to hyphal damage mediated by human neutrophils and effects of hyperglycemia and ketoacidosis. *Infection and Immunity. 1982;* 38*(3): 1123–29.*

121) Bottone EJ, Weitzman I, Hanna BA. *Rhizopus rhizopodiformis*: emerging etiological agent of mucormycosis. *J Clin Microbiol.* 1979; 9(4):530-7.

122) Larone DH. *Medically Important Fungi*. A Guide to Identification. American Society for Microbiology, Washington, DC, USA, 3rd edition, 1995.

123) Fleet GH, Balia R. The public health and probiotic significance of yeasts in foods and beverages In: Querol A, Fleet GH, ed. Yeast in food and beverages. Berlin, Heidelberg, Springer; 2006:381-398.

124) Fleet GH. Yeasts in fruit and fruit products. In: Boekhout T, Robert V, ed. Yeasts in Food. Beneficial and Detrimental Aspects. Behr, Hamburg, Germany; 2003:267.

125) Wirth F, Goldani LZ. Epidemiology of *Rhodotorula*: An Emerging Pathogen. *Interdiscip Perspect Infect Dis*, 2012;7. Article ID 465717. doi:10.1155/2012/465717.

126) Strausbaugh LJ, Sewell DL, Tjoelker RC. *et al*. Comparison of three methods for recovery of yeasts from hands of health-care workers. *Journal of Clinical Microbiology*. 1996; 34(2): 471- 472.

127) Alexander B, Pfaller M. Contemporary tools for the diagnosis and management of invasive mycoses. *Clin Infect Dis*. 2006; 43:15–27.

128) Gıda, Tarım ve Hayvancılık Bakanlığı: Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği, Resmi Gazete. Sayı: 28157 (3. Mükerrer) 2011. Available from: http://www.resmigazete.gov.tr/eskiler/2011/12/20111229M3-6.htm Access: 28.03.2017

129) Rydell SA, Harnack LJ, Oakes JM. *et al*. Why Eat at Fast-Food Restaurants: Reported Reasons among Frequent Consumers. *Journal of the American Dietetic Association* 2008; 108(12): 2066-2070.

130) Brown MH. *Meat Microbiology*. Applied Science Publishers Ltd. London and New York. 1982.

131) Vanderzant C, Splittstoesser DF. *Compendium of Methods for the Microbiological Examination of Food*. American Public Health Association: Washington DC; 1992.

132) Todd CDE, Szabo R, Spiring F. Donairs (Gyros)-Potential hazards and control. *Journal of Food Protection*. 1986; 49(5): 69-377.

133) Berzins A, Terentjeva M, Korkeala H. Prevalence and genetic diversity of *Listeria monocytogenes* in vacuum-packaged ready-to-eat meat products at retail markets in Latvia. *Journal of Food Protection*. 2009; 72(6): 1283-1287.

134) Bryan FL, Standley SR, Henderson WC, Time-temperature conditions of Gyros. *Journal of Food Protection*, 1980; 43(5): 346–353.

135) KÄFERSTEIN F. Foodborne diseases in developing countries: aetiology, epidemiology and strategies for prevention, *International Journal of Environmental Health Research*. 2003; 13: 161-168.

8 CIRRICULUM VIRTAE

1. Adı Soyadı **:** BUSE SARIKAYA

2. Doğum Tarihi **:** 07.01.1990

3. Öğrenim Durumu **:**

Derece	**Lise/Üniversite**	**Alanı**	**Yılı**
Lise	Samsun Atatürk Anadolu Lisesi	Fen	2004-2008
Lisans	Yeditepe Üniversitesi	Beslenme ve Diyetetik	2009-2013
Yüksek Lisans	Yeditepe Üniversitesi	Beslenme ve Diyetetik	2013-

5.Bildiği Diller / İngilizce

5.1. İngilizce sınav notu: 2015 YDS Sonbahar: 78.7

6. İş Deneyimleri

6.1. 2013-2014 Süreyya Paşa Göğüs Hastalıkları Hastanesi
6.2. 2014-2015 Özel Çağıner Hastanesi
6.3. 2017-**….** Amasya Üniversitesi Sağlık Yüksekokulu