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Investigation of Bacterial Diversity Associated with Kitchen Sponges

by

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Dedicated to ALLAH Almighty, Hazrat Muhammad (PBUH), my parents and my respected teachers for their encouragement, guidance, motivation during my research work and supporting me spiritually throughout my life.

CERTIFICATE OF APPROVAL

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"And we had certainly brought them a book which we detailed by knowledge- as guidance and mercy to a people who believe."(Al-Qur'an)".

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Abstract

Different surfaces in kitchen are rich in germs. Mostly dish wash cloth, brushes, rags, sponge and chopping boards are the places where bacteria colonize. Pathogens associated with kitchen utensils play important role in foodborne diseases. Foodborne diseases are a major concern for the general people and their health. Raw food and food handlers can be direct source of infection. Samples were prepared to isolate the bacteria which can be a cause of cross contamination. For this purpose 100 samples of sponges taken from the kitchens of Islamabad. After preservation of samples, bacteria were grown on nutrient agar. The inoculation of almost all samples on nutrient agar showed the variety of bacterial growth. Bacterial growth was also found on differential media i;e MacConkey agar where gram negative bacteria were mostly observed, MSA characterize the *Staphylococcus spp* and EMB characterized the *Pseudomonas spp.* Then biochemical characterization of isolated bacteria was done. For this purpose four types of tests were performed. The citrate test indole test, coagulase test and oxidase test. Citrate test was negative for *E.coli spp* and *Staphylococcus spp* while positive for *Pseudomonas spp.* oxidase test was negative for *E.coli* and *Staphylococcus spp.* while positive for *Pseudomonas spp.* Indole test was positive for *E.coli* and Negative for *Pseudumonas* and *Staphylococcus spp*, whereas the coagulase test was shown negative for all of the isolated strains. Gram staining was also performed. In this *E.coli spp* and *Pseudomonas spp* were gram negative bacteria while *Staphylococcus spp* was gram positive bacteria. After that 16SRNA sequencing was performed on 2 prevalent strains and it was referred as *E.coli* and *Staphylococcus.* Antibiotic sensitivity of these two strains was checked against seven antibiotics Ciprofloxacin, Ampicillin, Amikacin, Augmentin, Azithromycin, Cefatzidime, Imipenem. *E.coli* and *Staphylococcus* were found highly resistant pathogens which can lead to a food contamination and food borne diseases. These pathogens can be threat of our skin microbiota which ultimate lead to illness.

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Abbreviations

BLAST: Basic Local Alignment Search Tool

B.cereus : *Bacillus cereus*

C.coli: *Campylobacter coli*

C.jejuni: *Campylobacter jejuni*

CoNS: *Coagulase-negative staphylococci*

CopS: *Coagulase-positive staphylococci*

E.coli: *Escherichia coli*

EMB: Eosin Methylene Blue Agar

LGRM: Low Grade Regulating Material

MCC: MaCconkey Agar

MSA: Mannitol Salt Agar

NCBI: National Center for Biotechnology Information

S. aureus: *Staphylococcus aureus*

S.hominis : *Staphylococcus hominis*

Chapter 1

Introduction

The home's role in a number of public health and hygiene concerns has been under increasing scrutiny during the past ten years. Homes play as a key factor in spread of food borne diseases. Globally, the prevalence of foodborne illness is rising. In spite of the way that information gathering strategies for foodborne illness ordinarily overlook the extraordinary greater part of locally established paces of irregular disease, it is currently that many examples of foodborne disorder are brought about by customers overseeing and preparing food inadequately in their own kitchens. Global insights on contaminations with *Salmonella species* and *Campylobacter species* give the absolute most persuading proof [1].

By plan, the house is a multifunctional space, which straightforwardly affects the requirement for more elevated levels of sanitation there. One urgent element to consider is the rising number of older and other resistant compromised individuals residing at home, who are probably going to be more helpless with the impacts of foodborne disease. Millions of patients are being cared for at home as a result of a radical shift in healthcare delivery occurring in several affluent countries. Some homegrown food handling issues for the twenty-first century incorporate the continuous globalization of the food supply, the results of unfamiliar travel and the travel industry, and the impacts of foodborne infection on unfortunate countries [2]. Foodborne diseases are a major concern for the general people and their health. There are many incidents of medical concerns that have not

been reported to the WHO. Cases of foodborne illness are also on the rise. Cross contamination of food is the most dangerous risk in the kitchen. The kitchen may be a source of contamination [3]. Kitchen cloths, hands, and utensils are examples of secondary contamination transmission. Raw food is a direct source of infection. Bacteria are abundant in most locations. Research is now being conducted out to screen for microbial contamination on moist surfaces. According to the CDC, kitchens are the most common sites for foodborne illness. The way food is prepared reveals more about the sickness that it causes. Pathogens can be acquired via uncooked foods, the surroundings, or any individual [4]. Any individual who eats debased food can turn out to be sick from a foodborne illness, however a few gatherings are more inclined to ending up being infected and having a more serious disorder. Highly vulnerable population include young children, the elderly, women who are expecting and those who are immunological weakened (e.g., cancer patients, diabetics).The three following categories best describe the causes:

1. Biologic dangers include those posed by bacteria, viruses, and parasites. Bacteria and viruses are responsible for the majority of foodborne illnesses. The greatest danger to food safety is posed by biological risks. They may be a result of improper treatment (such as using excessive time or temperature) or inherent in the product.

2. Chemical pollutants and natural poisons are examples of chemical dangers. Certain mushrooms and PSP in molluscan shellfish are examples of natural toxins that are linked to the food itself. When food is incorrectly cooked or kept, bacteria in the food release additional natural poisons (i.e., histamine development in certain seafood species). For instance, certain people may be at danger from sulfates.

    - Chemical risks are posed by dietary allergens. Food proteins can cause reactions in certain persons. Every meal is unique. Milk, eggs, fish, prawn, shellfish, lobster, crab, and shrimp), wheat, soy, peanuts, and tree nuts are the eight most common food allergies.

3. Physical risks might include glass fragments, plastic particles, and metal shavings from cans. Various foodborne illnesses can be brought on by different pathogens. The bacteria themselves can result in disease if a contaminated product has been consumed [6].

A key contributing element in such an incidence is a lack of sufficient cleanliness in a food handling areas [6]. Maintaining good cleanliness in every feasible aspect in a food handling environment is critical, as it can lower the likelihood of foodborne disease significantly. This is true for domestic kitchens or eating areas such as, office/ school/ college/ university cafeterias, clinic food stalls, and hotel lunchrooms [7].

The appropriate hygienic standards differed across world in different researches, with some suggesting that the level of hygiene in one state may be okay while the same state may not be. But at the other hand, most people seem to believe that a decent food handling area must have a clear indicator of sanitation and cleanliness [8]. Disease causing bacteria can enter food handling surfaces directly through raw or undercooked meals, water, and people's unwashed hands, packing materials, infected or inadequately washed household goods, cleaning wipes, and other sources [9].

Numerous signs suggest that the prevalence of foodborne illness is rising internationally and is a significant source of morbidity and death on a global scale. According to reports, affluent countries often reported to one-third of their population suffer from a foodborne illness each year. Foodborne illnesses are thought to be the cause of 76 million sickness episodes per year in the US. Even while the vast majority of instances are mild, a significant number of fatalities do occur, and the high rates of acute infections and chronic diseases result in billions of pounds' worth of healthcare costs and lost productivity [10]. It is conceivable that the prevalence of foodborne illness is much higher in the developing world than it is now; however it is challenging to get the data necessary to confirm this hypothesis. It has for some time been accepted that most of looseness of the bowels cases in non-industrial nations are waterborne, not with standing Kaferstein's new case

that it is a grave slip-up to disregard the job of defiled food and that there is a pressing need to coordinate food handling alongside water and sterilization programs as a fundamental system to stop the loose motion [11]. Reviewing data from North America and Europe reveals that many cases of foodborne illness are caused by people mishandling and cooking food improperly in their own kitchens. This is now commonly acknowledged [12]. Furthermore, a report of *Escherichia coli O157* flare-ups in the US viewed that as 80% of the suspect cheeseburgers were ready and eaten at home [18]. 90% of Salmonella species diseases in Australia are for the most part credited to non-produced food sources and homegrown conditions. According to statistics from Canada for the years 1996 and 1997, the house is the most well-known openness region for instances of *Salmonella species, Campylobacter species* , and pathogenic *E coli* disease [13].

Foodborne illness outbreaks in the home are likely to be caused by a variety of factors, for example a regularly contaminated raw supply of food, a lack of public awareness, careless home handling and cooking of food, and the intentional consumption of uncooked and raw animal products, that is frequently said to as "risky eating behavior" [12], [13]. Raw foods should be regarded as possible entry points for foodborne pathogens into the household, including eggs, fish,meat and shellfish, as well as foods grown from the ground. *Salmonella, Campylobacter, E. coli O157* and *Listeria sp* are among the pathogenic organisms that entered homes through food [14]. Foodborne infections can also come from the people and animals that live there. Both humans and animals have the capacity to act as post symptomatic excreters as well as symptomatic and no symptomatic carriers. Pathogens can be temporarily transferred on hands from a number of sources to living or inert contact areas in the home as well as straight to other foods or inhabitants. Humans have been responsible for bringing *Rotavirus, Staphylococcus aureus, Salmonella, Shigella sonnei* and hepatitis A virus into homes [15].

The four most frequent errors made when cooking and handling food at home are the improper food storage, any activities that lead to cross-contamination, inadequate refrigeration, and failing to reach the necessary cooking and/or reheating temperature. Inadequate storage of food and cross-infection were found to be the

utmost frequent errors, rationalizing for 50% and 28% of described causal variables, respectively, in a report of 101 home-based outbursts [16]. Among all of them, the most effective and simplest route of moving microbes into and around areas is through inadequately cleansed hands [17]. When harmful bacteria from unsanitary hands enter food, they can cause foodborne sickness, neurological issues, renal and hepatic ailments, and other illnesses in people [18]. The most common toxins delivering wellbeing concerns incorporate pathogenic types of *E.coli, Salmonella sp., Serratia sp., Aeromonas sp. Shigella sp, S.aureus, Campylobacter sp.,* parasites, and even viruses [19], [20].

Microorganisms can adhere to food handling surfaces and produce biofilm if organic matter is present that has not been thoroughly cleaned away with detergents. Biofilm is considerably harder to eliminate. This biofilm is responsible for the continued transmission of pathogenic bacteria such as *E. coli, Proteus spp., Salmonella spp., Pseudomonas spp.*, and *Klebsiella spp.* to humans [21]. Appropriate sanitizing and disinfection sanitization of food handling surfaces are critical for reducing the spread of harmful microbes into the food chain, resulting in fewer foodborne infections [22]. This unsanitary situation exists mostly owing to a lack of basic hygiene expertise, a lack of seriousness and commitment to work, insufficient investments in cleanliness programs, and so on [23].

Foodborne Sickness Effect and The study of disease transmission Reference Gathering distinguished around 582 million foodborne ailments and 351,000 passings overall in a 2015 WHO report [24], [25].

The kitchen is one of the germiest areas in the house because bacteria are drawn to warm, damp surroundings. The home items with the greatest germ counts were identified in a 2011 research by NSF International. They discovered that kitchen sinks, toothbrush holders, pet bowls, coffee reservoirs, faucet handles, worktops, stove knobs, and cutting boards were the dirtiest home goods, followed by sponges and dish rags. Contrary to common assumption, the bathroom is not the dirtiest room in the house. The research found that the kitchen had more Coliform bacteria than the bathroom, which is a sign of possible faecal contamination. Coliform was

really discovered in 75% of dish wash cloth/sponges, 45% of kitchen bowls, 32% of worktops, and 18% of chopping boards.

1. According to Dr. Chuck Gerba, a lecturer of microbiology at the Arizona University, Sponge or towel is usually often the grimiest item in your home; he reported how illnesses spread from the surroundings. His research has revealed that, in comparison to the typical toilet seat, which has around 50 germs per square inch (or 6.5 square cm), a sponge and a dishcloth contain roughly 10 million and one million bacteria, respectively, per square inch. Individuals frequently utilize a similar wipe to clean their kitchen surfaces, dishes, and table, supplanting it like clockwork, ought to know that, as per a report gave in Logical Reports on July 19, 2017, these wipes are vigorously tainted with *Acinetobacter* species of *Moraxella* and *Chryseobacterium*. Due to their porous structure and propensity to hold water, kitchen sponges are excellent habitat for microbes.

2. Towel:- Towels are used for a variety of tasks, including drying hands, cleaning surfaces, utensils, etc.; there are more bacteria on them. A safer choice is to keep toilet paper in hand for wiping down worktops and to air-dry them in a crockery basket or dry clean utensils with special towels.

3. Despite having a beneficial effect on the environment, the typical metal ventilated shield at the end of a kitchen tap may also serve as a breeding ground for bacteria, some of which may even develop into harmful bacteria that adhere to the screens.

4. Stove buttons are among the top 10 places for germs to lurk, despite not being a location that many of us consider. While people regularly clean their stove tops, don't often pay as much attention to the handles.

5. An essential environmental health strategy for preventing illness is the removal of domestic wastewater. Wastewater that is not properly evacuated creates stagnant pools that serve as breeding grounds for disease vectors. As a result, several illnesses are more prevalent during the wet season than

they are during the dry season. Important building maintenance procedures include drain cleaning and the application of appropriate disinfectants [26], [27].

6. We can identify potential sources of biohazards (such as Salmonella and Campylobacter in raw meat and poultry), synthetic dander (such as cleaning specialists and greasing up oil from blender processors), and physical danger in our kitchen on the off chance that we put on our sanitation proficient glasses (e.g., glass, rubber, plastic, wires, staples from grocery bags, etc.).

   When any bacteria or other microbes are accidentally moved from one location to another, i.e. from one food dish to another, this is known as cross-contamination of food. In the kitchen, cross-contamination can occur in three different ways:

- Food-to-food contact, such as when raw meat and cooked meat are stored together.

- People-to-food contact, such as when a food worker handles raw meat then touches cooked meat with the same hands without properly washing them first.

- Equipment-to-food, The USFDA exhorts subbing utilized hacking loads up as they become utilized over the long run since discouragements made by sharp edges cutting into the board are colonizing locales for microorganisms.

  One illustration of instruments to-food is the point at which a cook utilizes similar slicing board to cut both crude meat and prepared to-eat new plate of mixed greens vegetables [28], [29]. This all comes under the potential risks and protective measures.

The goal of this research was to look into the bacterial variety linked with household goods and locations, as well as the recognition of microbes and their antibiotic resistant in the bacterial population.

1.1 Problem Statement

In future foodborne diseases will continue to be matter of consideration in all over the world and ultimate source include unhygienic practices starting from kitchen. Food preparing areas and utensils plays critical role in such cases. It is of great concern to identify the microbes associated with these objects.

1.2 Proposed Solution

The isolation and identification of bacterial pathogens from kitchens at home can aid in the identification of bacterial species linked to various diseases, as well as the investigation of the function of washing utensils in disease transmission.

1.3 Significance

Finding the association between disease and pathogen can be useful to identify the sources/routes of transmission of microbes.

1.4 Aim and Objectives

The aim of this study is to explore the antibiotic resistant pathogens associated with the sponges that are mainly involved in cleaning or washing kitchen utensils and can be source of illness or foodborne disease.

1. Isolation of bacterial microbes from the sponges of kitchens located in Rawalpindi / Islamabad city.

2. Biochemical characterization of isolated bacterial microbes collected from sponge sample.

3. Molecular characterization of isolated bacterial microbes.

4. Evaluation of antibiotic sensitivity of identified bacterial strain.

Chapter 2

Review of Literature

2.1 Home Source is of Foodborne Diseases

Unhygienic behavior in food handling at home led to the food borne diseases. Diseases linked to foodstuff and *Salmonella* outbreaks in households. Food-related illnesses were moderate, unconfirmed, and under reported. Food-borne infections, as per specialists, were more prevalent as compared to other infections [30]. Many incidences of food borne outbreaks, as well as the related financial expenses, might be the outcome of easily avoidable cooking errors at kitchens [31].

Infections with *Norvirus* and *Salmonella* accounted for significantly more than a third of all food borne illness infections in the United States between 1998 and 2008, accounting for 9 to 15% of all infections. One out of every five sickness outbreaks is caused by birds and green vegetables. More than half of all *Salmonella* poisoning in Europe had a domestic connection, accounting for nearly one-third of all cases of foodborne illness. Although specialists believe that most incidents of food contamination are infrequent, modest, unconfirmed, and unreported, they contend that cases resulting from improper food delivery at home are significantly more common, with some numbers approaching 95% [32].

While many individuals did not consider their homes to be a risky site for food-borne disease, experts think that homes are one of the principal sites where the

majority of food-borne illness cases occur. Nearly two-thirds of individuals never explore the possibility that a family member exhibiting "flu-like" symptoms (fever, chills, and vomiting) may actually be afflicted with a foodborne illness brought on by foods prepared at home. Only 8% of individuals, less than 10% during 2011, thought the house would be a cause of a food borne illness [33].

2.2 Foodborne Pathogens

Foodborne microorganisms are natural agents that might result in a food borne illness incident. Examples include diseases, bacteria, and parasites. Food borne disease happens when a microorganism is ingested with food and lays down a good foundation for itself (and normally duplicates) in the human host, or when a toxigenic microbe secures itself in a food item and produces a poison, which is then ingested by the human host [34].

In this manner, foodborne disease is by and large ordered into:

(a) foodborne contamination and

(b) foodborne inebriation.

In contrast to foodborne intoxications, foodborne contaminations generally entail a brooding phase, which lengthens the time between intake and the onset of symptoms. There have been identified more than 200 unique food-borne illnesses. The most outrageous situations will often affect people who are very young, very elderly, have limited safe structure ability, and strong people who have been exposed to an extremely high piece of an animal.

For the year 2015, the European Union (EU), 26 section states uncovered an amount of 4,362 food-borne eruptions, including waterborne episodes. As a general rule, these eruptions caused 45,874 occurrences of infection, 3,892 hospitalizations and 17 deaths [35]. The general revealing pace of food-borne flare-ups in the EU

was 0.95 per 100,000 populace, which addresses a slight reduction contrasted and information accommodated 2014 . The majority of the flare-ups detailed in 2015 were achieved by bacterial trained professionals (33.7% of all episodes), explicitly *Salmonella spp.* (21.8% of all eruptions) and *Campylobacter spp.* (8.9% of all episodes), regardless of the way that the declaring of eruptions including these experts has been declining over the new year.

Bacterial toxins came in second place among those who could determine the cause of food- and water-borne outbreaks and made up 19.5% of cases of the hard and fast eruptions while contaminations, which were the specialists most often announced in 2014, represented 9.2% of absolute episodes in 2015. Parasites and other causative specialists, specifically receptor, were accounted for in fewer than 3% of the flare-ups. Moreover, for 33% of the detailed flare-ups (34%) the causative specialist stayed obscure [34], [35].

The majority of the trapped food items were made of animal origin, particularly eggs and egg products, pork, grill meat, and cheddar. These foods accounted for 10% of all strong confirmation episodes, respectively, and were followed by endlessly fish products (7%), milk and milk-based products (5%), meat that resembles an ox (4%), and scavengers (3%). The Salmonella spp.in egg was one of the top 5 food-microorganism combinations and was associated with the greatest number of specific foodborne incidents. One of the top 5 food-microorganism combinations in 2015 was *Salmonella spp* [36].

2.3 Foodborne Bacteria

Microorganisms are the most well-known reason for foodborne sicknesses exist in a variety of sizes, varieties, and characteristics. A few harmful microscopic organisms are fit for spore development and subsequently, exceptionally heat-safe (for example *Bacillus cereus, Clostridium botulinum, Bacillus subtilus, C. perfringens,*). Some are equipped for delivering heat-safe poisons (for example *Staphylococcus aureus, Clostridium botulinum.* The majority of microorganisms are mesophilic, with

optimal growth temperatures between 20 and 45 C. Be that as it may, certain food-borne microbes (for example psychrotrophs, for example, *Yersinia enterocolitica* and *Listeria monocytogenes* may both grow in the refrigerator or at temperatures below 10 °C [37].

2.3.1 *Bacillus cereus*

Bacillus cereus are individuals from the family Gram-positive, motile poles belonging to the family Bacillaceae that may also frame spores. The majority of Bacillus species are found worldwide, including soils, new and marine water conditions *B. cereus* spores are more hydrophobic than some other Bacillus spores and contain pili and extremities. Due to these characteristics, the spores are able to adhere to diverse surfaces and resist evaporation during cleaning and disinfection. Depending on the strain, *B. cereus's* vegetative cells may grow at temperatures ranging from 4 to 15 to 35 to 55 °C [36].

The pH range where the organic entity grows is 4.9 to 9.3, however the pH's inhibitory effect on food kinds is lessened as evidenced by the limited growth of meat at pH 4.35. The basic law for development has been set at 0.93, however it has been advised to use 0.912 as the base anticipated for development since seared rice often has aw values ranging from 0.912 to 0.961 and supports *B. cereus* growth right away. *B. cereus* generates two different toxins, one that causes diarrhea and the other that causes emesis (heaving). The emetic poison that tiny organisms provide when the meal is being developed progressively causes the emetic condition. Diarrheal toxins given during the growth of the bacteria in the tiny digestive tract are what cause the diarrheal illness. The emetic type's rapid onset is characterized by sickness and retching, whereas the diarrheal types delayed onset is characterized by loose stools and stomach pain [37], [38].

The two illnesses (diarrhea and emesis) are brought on by *B. cereus* endospores surviving the digestive tract, which leads to germination and further proliferation of vegetative cells later on during capacity. Meat, soups, vegetables, puddings,

sauces, and various milk products are among the food types that frequently become contaminated with *B. cereus* diarrhoea. Side effects are described by stomach agony, sickness, and looseness of the bowels after a brooding time of roughly 8-16 h. Diarrheal condition side effects by and large persevere no longer than 12-24 h. After a 1-5 h brooding period, emetic disorder side effects incorporate fundamentally sickness and spewing and persevere for 6-24 h. Broiled and cooked rice, pasta, noodles, and baked goods are among the food types contaminated with B. cereus emetic food [39].

While the emetic condition type of food contamination also contains the action of a thermostable poison, the diarrheal disease type is caused by a thermolabile enterotoxic compound *B. cereus* is often present in food formation settings and then spreads to a variety of food sources due to the placement of endospores. They generate a variety of dangerous substances that, when present in food or the gastrointestinal tract, may cause individuals to suffer from dreadful illnesses. They are one of the main foodborne harmful bacteria yet, most illnesses are mild and short-lived [38], [39].

2.3.2 *Campylobacter jejuni*

Campylobacter jejuni is among the most widely recognised causes of diarrheal disease. One of the members of the family Campylobacteriaceae. Around 850,000 illnesses, 8,500 hospitalizations, and 76 fatalities are caused by *C. jejuni* each year in the US . According to the World Wellbeing Association (WHO), just 1% or less of Western Europe's population will infect with *Campylobacter* each year. *C. jejuni* is widely distributed throughout nature and has the ability to colonize the digestive systems of both warm-blooded transmission to birds, animals, and people happens through tainted food items. This microbe can pass through the epithelial layer by adhering to the cells that make up the epithelium and then migrating inside of them. Loose stools result from epithelial cell damage. More severe illnesses can also be caused by basic contaminations [39]. The information retrieved from NCBI indicates that 932 genomes have been completed to date. The

genome's average overall length is 1.686 Mb *Campylobacter spp.*, which include 18 species, six subspecies, and two biovars, are tiny (0.2-0.9 m broad and 0.2-5.0 m long), twisting-framed, Gram-negative bacteria. *Campylobacter* genomes are often unstable; a few factors, such as bacteriophage activity, DNA recombination and modification, have been identified as potential causes for this hereditary instability. They are fundamentally microaerophilic, filling optimally in an environment holding back around 10% CO2 and approximately 5% O2, which sets them apart from other microbes linked to foodborne illness. The species that are harmful to humans also have a very narrow temperature range for growth, with a maximum extreme temperature of 46 °C and a minimum temperature of 30 °C [40].

Thermophilic *Campylobacters* are what they are known as since roughly 2005, *Campylobacter* has consistently ranked as the most common gastrointestinal bacterial pathogen in humans in the EU. A total of 229,213 confirmed cases of human campylobacteriosis were reported, a 5.8% decrease from the incidence in 2014. A variety of sound-producing domesticated and wild animals, such as farm animals, swine, hens, waterfowl, and swans, as well as canines, felines, rodents, and marine vertebrates, depend on *Campylobacter spp.* for their normal digestive health. These organisms are frequently associated with bodies of water like ponds and streams. Unpasteurized milk, tainted water, and raw or undercooked chicken meat are the main causes of campylobacteriosis or from these items infecting several food sources at once [41].

2.3.3 *Clostridium botulinum*

The family Bacillaceae includes the spore-framing microorganisms *Clostridium spp.*, which include obligately anaerobic or aerotolerant sporeforming shafts that don't make spores there of air and, in the beginning phases of life, are much of the time Gram-positive. Growing cells noticeable as straight or bent poles in a variety of species, ranging from small coccid bars to long filamentous constructions with adjusted, tightened, or harsh ends, which appear alone, in pairs, or in different length chains [40]. Although *Clostridia* occur everywhere in the earth, they are

most prevalent in soil and in animal digestive tracts. Endospores, which grow in situations difficult for cell growth and increase single terminal or sub-terminal cells are credited with giving clostridia their distinctive state. Endospores of many species are exceedingly tough and can resist prolonged bubbling in water and exposed to air spores thrive in conditions conducive to vegetative development, including as anaerobiosis and the presence of natural substrates. *Botulinum neurotoxins*, the most lethal toxin known, are produced by *Cl. Botulinum*, which are motile due to peritrichous flagella. There are seven main forms of poison based on the epitope specificity of the poison generated by each strain of *botulinum neurotoxin*, designated From A through G Botulism is caused by types C and D in avian and vertebrate, types A, B, E, and F in people, and type G does not appear to be definitely engaged in a botulism case right now. By suppressing *Cl. Botulinum* spores, warm handling is the most well-known method for delivering stable, low-corrosive, wet food sources [41].

According to the developmental point of view, *Clostridia* are viewed as the most antiquated microscopic organisms. It is thought that current *Mollicutes* (Eubacteria) developed backward from gram-positive, clostridia-like ancestors with low GC content in DNA. (i.e.,through genome reduction). There are a few different kinds of *Clostridia*, such as *Cl. perfringens, Cl. botulinum,* and *Cl. Tetani* deft poison delivering microbes in creatures and people. A few animal species are capable of transporting sub-atomic hydrogen, natural solvents (such as CH3)2CO, ethanol, and other beneficial combinations. There are other creatures that can fix atomic nitrogen, which makes them major contributors to nature's organic nitrogen cycle [42].

The most well-known and often seen *Cl. Botulinum* strains and *Serovars* are those that generate type A toxin. This toxin is used in cosmetology and other applications needing neuroparalitic treatment. As of now, 177 genomes have been completed, according to data obtained from NCBI. The genome's average absolute length is 3.898 Mb. *Botulinum neurotoxin* side effects develop 12-36 hours after consuming contaminated food and may include nausea and regurgitation at first. However, these side effects are followed by more distinct neurological symptoms,

such as visual impairment and severe limp weakness, which first affects the muscles of the body, face, and respiratory tract before spreading to the breast and limits and potentially leading to death from respiratory distress caused by upper aviation route or stomach lost motion [41].

The basic toxic fraction of *Cl. botulinum neurotoxin* is not totally fixed, yet from a human wellbeing and sanitation stance. There shouldn't be any resistance to the real poison or to the environment that allow animals to grow into food species. Initiated by proteolytic cleavage, botulinum neurotoxin is given after cell lysis after being organized throughout cell formation. There are four different types of botulism, including the classic form that results from ingesting preformed poison found in food sources, wound botulism caused by the development of poison after life forms have formed in a tainted injury, and baby botulism caused by the development of poison in the digestive system of newborn children and botulism because of gastrointestinal colonization in more seasoned kids and grown-ups with digestive issues or confusions bringing about an absence of microbial contest. *Botulinum neurotoxin* that has been administered in either of these classes travels via the circulatory system to neuromuscular junctions, where the poison forms an irreversible bond with receptors on edge sensitive areas and then absorbs into the nerve cell [42].

2.3.4 *Cl. perfringens*

Clostridium perfringens, formerly known as *Clostridium welchii*, is a member of the Bacillaceae family and it is a major contributor to foodborne illness. They are non-moving, characterized Pole-shaped cells that produce protein poisons and produce spores that are resistant to a number of environmental conditions such as sunlight, withering out, and warmth. Bacterial spores may thrive at temperatures as low as 6°C and as high as 50 °C, although they prefer an optimal temperature between 43 and 47 °CA basic average of 0.93, a sodium chloride concentration of less than 5-8% depending on the strain, and a pH of 5.0-9.0 are all desirable are necessary for development, however 6.0–7.2 is preferred. With the exception of excrement, *Cl.*

Perfringens has been found to be the most common type of *Clostridium* in human clinical examples. It has been associated to minor injury contaminations such as myonecrosis, clostridial cellulitis, intra-stomach sepsis, gangrenous cholecystitis, post-abortion contamination, intravascular hemolysis, and bacteremia, as well as asthma, pulmonary and pleural effusion acute interstitial, and bloodstream infections. Due to their ubiquity across the climate, spores and live cells are frequently linked to dust tainting on a variety of surfaces, including food types like meat and shellfish. With 1,000,000 infections caused by it each year, *Cl. Perfringens* is the second most prevalent microbial cause of foodborne disease in the United States [43].

Through the Foodborne Illness Flare-up Observation Framework, nearby, state, and regional health offices knowingly report *Cl. Perfringens* incidents. 289 complete cases of the *Cl. Perfringens* illness were linked to 15,208 diseases, 83 hospital admitted cases, and 8 fatalities between 1998 and 2010. The quantity of flare-ups revealed every year went from 16 to 31 with no obvious pattern after some time. The average number of disorders associated with episodes each year increased from 359 to 2,173. The most often identified location for food preparation was a restaurant (43%); other locations included a kitchen office (19%), a private residence (16%), a jail or prison (11%), and others (10%). Meat was the most popular food (46% of the 144 (half) flare-ups) attributed to a single food item, with 66 incidents trailed by poultry (43 flare-ups, 30%), and pork (23 episodes, 16%). Flare-ups brought about by *Cl. Perfringens* happen consistently, are many times huge, and can cause significant horribleness yet are preventable in the event that tainting of crude. At the ranch or slaughterhouse, or after defilement, provided that the meat and chicken items are appropriately cared for and arranged, notably in cafés and cookery offices. Foodborne illness is frequently caused by improper use of heat, and in many cases the food carrier was inappropriately prepared meat or a meat product that had been ready to prepared, potentially cool, or have undergone insufficient warming, allowing persistent spores to sprout and causing vegetative cell growth [42], [43]. The most typical adverse effects were squeezing and stomach pain after consumption and a brooding period of 7–30 hours, however

nausea and regurgitating may also occur and last for 24-48 hours. There are five distinct types of *Cl. Perfringens* that may create poison (A through E), and each of them produces an alpha-poison (phospholipase) that contributes to myonecrosis Type B strains generate beta- and epsilon-poisons, type D strains also generate epsilon-poisons, and type E strains generate some poison [44]. Food sources with more than 106–107 live vegetative cells that sporulate in the gastrointestinal tract and produce toxins are the cause of type A illness, which results in almost unquestionably many cases of foodborne gastroenteritis. During cell lysis, the enterotoxin created during inoculation is transferred along with the spores. Once released, the enterotoxin attaches to epithelial cells, causing cytotoxic cell layer damage and subsequent porousness adjustment, resulting in bowel movements and abdominal cramping [43], [44].

2.3.5 *E. coli*

Because some of the rods are linked and some are not, Escherichia coli may be adaptive. The organic organism is a facultative anaerobe that develops basic carbohydrates like glucose into lactic, acidic, and formic acids; the optimal pH range for growth is 6.0 to 8.0; however, development may also occur at pH values as low as 4.3 and as high as 9 to 10. A huge and diverse collection of bacteria make up *E. coli*. The majority of *E. coli* strains are harmless, but certain strains have developed traits, such the ability to produce toxins, that make them hazardous to humans. Currently, 5351 genomes have been completed, according to data obtained from NCBI. The genome's 5.171 Mb midway full length. Because of their low irresistible quantities and ubiquitous transmission via food and water, a significant number of these pathotypes constitute a major public health problem. Pathogenic *E. coli* variations (pathovars or pathotypes) cause widespread suffering and mortality [45].

When food or water contaminated with the faeces of diseased people or animals is consumed, *E. coli* is transmitted. The handling and butchering of creatures frequently results in the contamination of creature objects. Produce and water

system water can get contaminated when compost from steers or other animals is used as manure for horticultural harvests [44]. *E. coli* may be prevelent in the environment for longer time period and grow in various foods, including vegetables

2.3.6 *L. monocytogenes*

Listeria monocytogenes can withstand limits of both temperatures (1-45 ℃) and salt concentration, making it a very risky food-conceived microorganism, especially on food that isn't warmed and is transmitted asymptomatically by various creature species. It is identified in decomposing vegetable debris, sewage, water, and soil. The bacterium has been discovered in a wide range of uncooked food sources, including raw meat and vegetables, as well as food types that get infected after frying or preparation. The fetal-placental unit, the focused sensory system, and the digestive tracts can all become contaminated. Septicemia (the foundational spread of microorganisms and toxins in the blood) can result from contamination, as can meningitis (inflammation of the layer encompassing the vertebral column and cerebrum), abdominal (aggravation of slimy films of the stomach and digestive tract), and infection [45], [46]. The information retrieved from NCBI indicates that 1243 genomes have been completed to date. Listeriosis is a dangerous illness typically occur by consuming *L. monocytogenes*-tainted food. Despite being one of the worst food-borne hazards, the disease has a high fatality rate (20–30%) and is rather uncommon. Listeria grows more quickly in cold conditions, such freezers, in contrast to many other foodborne bacteria. In moist settings, it may spread swiftly by dropping onto meals from pipes or ceilings. After entering a facility that processes food, Listeria bacteria can survive there for years and occasionally contaminate the food items [46].

2.3.7 *Salmonella spp*

Among the most likely reasons of enteric infections (gastroenteritis) worldwide, this genus of Enterobactericiae has pathogenic traits. They have name *Salmonella*

choleraesuis in honour of Dr. Daniel Salmon, a doctor who discovered the first such bacteria in a pig's gut. Salmonella enterica and Salmonella bongori are the two species of the genus that may infect people. In accordance with the Kaufmann-White composition model, which was first reported in 1934 and divides *Salmonella* strains according to their external and flagellar immunogenic features, *Salmonella* is also divided into serotypes. Serotype designations are often used to refer to *Salmonella spp. Salmonella enterica subsp. Enterica*, for instance, is further divided into a number of serotypes, such as *S. Enteritidis* and *S. Typhimurium*. *Salmonella enterica* serovars are responsible for other severe diseases including typhoid fever. Salmonella spp. may colonize and contaminate have live things due to the presence of a few pathogenicity islands (PAIs) that encode various destructiveness characteristics [47].

Salmonella pathogenicity island 1 and 2 (SPI-1 and SPI-2) are two important PAIs that encode two distinct kind III discharge frameworks for the delivery of effector particles into the host cell, resulting in assimilation of the microbes, which subsequently leads to fundamental dissemination. As of today, 5323 *Salmonella enterica* genomes have been completed, according to data retrieved from NCBI. The primary bacterial causes of food-borne illness in the US are *Salmonella spp.* According to the CDC, more than 1 million Americans develop Salmonella annually, with a typical 19,000 hospitalizations and 380 fatalities. Most farmed animals' digestive tracts and those of many wild animals are home to *Salmonella spp.* The most common cause of *salmonella spp.* sickness is eating food tainted with the excrement of animals or people who are carrying the germs. *Salmonella* infections are typically linked to eggs, pork, and ducks, but these microbes can also taint other food types, such as those derived from the soil. More recently, the CDC has identified 258 individuals from 24 states and the District of Columbia who have contracted Salmonella Bareilly (247 individuals) or *Salmonella Nchanga* (11 individuals) episodes. No deaths have been reported despite the hospitalization of 32 unwell patients. The plausible source of this incident is a frozen crude yellow blade fish product from Moon Marine USA Enterprise known as Nakaochi Scratch, according to cooperative investigation efforts of state, local, and government general

health authorities [48].

2.3.8 *Shigella spp*

Shigella are facultative anaerobic, Gram-negative, non-spore-forming, non-motile poles. They may develop at temperatures between 6 and 48 °C although they grow mainly at 37 °C, and *S. sonnei* seems to be more equipped than the other serogroups to withstand lower temperatures, according to all indications. Although development has been accounted for between pH 4.8 to 9.3, ideal development occurs between pH 6.0 and 8.0 [49]. *Shigella spp.* and *E. coli* have similar DNA sequences, and they also exhibit some of the same metabolic traits and antibody reactivity. Despite these similarities, it is important to distinguish between the two because of variations in the adverse effects that infected people have experienced. Shigellosis can develop after consuming water or food that has been polluted centrally, despite the fact that the main method of transmission is from one person to the next contact. *Shigella species* are more prevalent in environments with poor sanitation and sterilization [50]. Milk, mixed greens, poultry, seafood, and other fresh vegetables have been linked to instances of shigellosis and have been provided at a variety of locations, including restaurants, residences, schools, sorority houses, business carriers, travel boats, and military crash corridors.

There is no one sort of food that *Shigella spp.* has been explicitly related to. Shigellosis is an infection that affects around 20% of people in the US who have travelled abroad (for example, travelers who have diarrhoea). High mortality rates have been brought on by these pestilences, especially in the elderly, immunocompromised individuals, and malnourished children [49].

2.3.9 *Staphylococcus aureus*

Staphylococcus aureus are gram-positive, non-motile cocci that can appear alone, in pairs, in quadruplicates, in short chains, or in distinctive "grape like" groupings.

Except for *Staph. aureus subsp anaerobius* and *Staph. saccharolyticus, Staphylococci* are facultative anaerobes that grow more rapidly in high-impact environments. *Staphylococcus spp.* are widely distributed in nature and may be found on a variety of organs, including the blood stream, oral cavity, glands, and the gastrointestinal, urinary tract, and upper respiratory systems of sick people. One of the safest non-spore-shaping microbes is *Staph. Aureus* because it can survive outside the body for longer time periods in a dry condition and has been kept away from air, residue, dirt, and water. Along with natural causes of contamination, some specific food items that have been shown to include *Staph. Aureus* include salmon steaks, shellfish and shrimp etc [50].

S. aureus prefers a temperature between 40 and 45 °C for optimal growth. It may grow between 7 and 47.8 °C and produce enterotoxins between 10 and 46 °C, depending on the strain. The bacterium can thrive in a pH range of 4.5 to 9.3, with an ideal range between 7.0 and 7.5, and it is particularly tolerant of high salt concentrations (>10% sodium chloride). Although base aw of 0.86 is required for the generation of enterotoxins, growth has been observed at a w of 0.83. Skin infections such boils, cellulitis, impetigo, and surgical injury contamination are frequently brought on by *S. aureus*. However, it can also be linked to more serious illnesses such as bacteremia, pneumonia, osteomyelitis, cerebritis, meningitis, and abscesses of the muscle, urogenital plot, focal sensory system, and various stomach organs [51].

S. aureus contamination has been linked to harmful shock disorder, a syndrome that resembles septic shock and developed as a result of poisonous shock disorder toxin 1. People are the main source of *S. aureus*, and contamination of food can occur directly, indirectly through skin sections, or directly through respiratory plot beads. The majority of staphylococcal food contamination cases are linked to food contamination during planning as a result of inadequate refrigeration, inadequate cooking or warming, or poor personal hygiene. The side effects of the enterotoxin may include retching, nausea, stomach cramps, migraine, dazedness, chills, sweating, general weakness, strong squeezing as well as surrender, and looseness of the bowels that may contain blood after ingestion and a brooding time of less

than 6 and up to 10 hours. According to the CDC's estimation, staphylococcal food contamination causes around 241,188 illnesses, 1,064 hospitalizations, and 6 fatalities per year in the US [52].

2.3.10 *Vibrio spp*

Nearly half of the more than 35 species in the class Vibrio, which belongs to the family Vibrionaceae, have been identified in the past 20 years, and 33% of them are harmful to humans. This family of organisms includes non-spore-framing, mostly motile, facultatively anaerobic, and straight or bent pole Gram-negative organisms. They are primarily found in harsh or marine environments in tropical or calm regions since their rate completely declines when water temperature falls below 20 ℃. A single polar-sheathed flagellum allows *V. cholerae* to move about; these bent bars grow in their biological habitat as a part of the micro flora discovered in estuaries. Despite having a marine or bitter water source as its primary natural source, *V. cholerae* has been identified from areas without these resources, including freshwater lakes and streams, birds, and herbivores. *El Pinnacle*, the predominate biogroup of the current epidemic, and the example biogroup, which has been dissociated throughout previous pandemics, make up *Vibrio cholerae O1* [53].

Eating infected food, such as raw, undercooked, or even polluted after cooking mollusks, or scavenging are common ways for V. cholerae to enter the human body. Another common route is via coming into contact with contaminated water. The severity of the conditions brought on by *V. cholerae O1* illness may range from moderate to the most severe type known as "cholera gravis," as 75% of the El Peak biogroup and 60%) of the example biogroup produce asymptomatic contaminations. In addition, the El Pinnacle biogroup affects 2% of infected people with severe sickness and 23% of those with moderate or direct infection, whereas the outstanding biogroup affects 11% of people with severe illness and 30% of people with light or direct infection. Depending on the size of the inoculum and the quantity of food ingested, after a brooding period of a few hours to five days,

typical side effects include muscle cramps brought on by severe dehydration (liquid loss up to 500–1000 ml/h) caused by vomiting, increased peristalsis followed by diarrheas leading to watery stools, and bodily fluid seen in the runs, which is typical for cholera [54].

2.3.11 *Yersinia enterocolitica*

There are 10 listed species in the Yersinia class, which belongs to the Enterobacteriaceae family, albeit only three are considered to be harmful to humans or other animals. The Yersinia pestis bacteria that causes plague, the *Yersinia pseudotuberculosis* bacteria that primarily affects animals but can contaminate humans after ingesting contaminated food or water, and the Yersinia enterocolitica bacteria that has been implicated in the development of foodborne gastroenteritis in humans. Although *Yersinia spp.* are non-spore-forming, Gram-negative or gram-variable bars that can grow in both anaerobic and aerobic environments, they are thought of as facultative anaerobes. All *Yersinia spp.*, with the exception of *Y. pestis*, have peritrichous flagella and are motile between 22 and 30 °C, but not at 37 °C. In 2015, 26 component states reported 7,202 confirmed cases of yersiniosis, ranking it as the third most often reported zoonosis in the EU [55].

The most well-known species identified as unrelated to human cases was *Y. enterocolitica*; there was a demonstrably crucial declining 8-year pattern in 2008–2015. The O:3 serotype was the most well-known, followed by O:9 and O:5,27. The 4,304 confirmed instances of yersiniosis for which this data was accounted for in 2015 do not include any fatalities. *Y. enterocolitica* are frequently found in the environment and have been found in fish, people, raw milk, sewage-contaminated water, soil, and various warm-blooded animals, namely chickens and pigs. Even while *Y. enterocolitica* frequently outgrows competing psychrotrophs in the presence of refrigeration, the psychrotroph may still be harmful in tainted sources of chilled food. *Y. enterocolitica* are frequently found in the environment and have been found in fish, people, raw milk, sewage-contaminated water, soil, and various warm-blooded animals, namely chickens and pigs. Even while *Y. enterocolitica*

frequently outgrows competing psychrotrophs in the presence of refrigeration, the psychrotroph may still be harmful in tainted sources of chilled food. Y. enterocolitica may grow at temperatures between 0 and 45 °C, however it prefers an optimal temperature between 25 to 30 °C. This psychrotroph can withstand soluble environments just like other gram-negative bacteria, but it doesn't fare well in acidic ones since growth occurs best between pH 7.6 and 10.0, with pH 4.0 being the optimal range. Additionally, *Y. enterocolitica* may grow at fixations of up to 5% in the presence of NaCl [56].

2.4 Contaminated Areas in Kitchens

High-risk contamination was dispersed by sponges, clothes, hands, and equipment. E.coli had a high success rate in clothing for up to 48 hours. 92 % of those who wash utensils with their clothing don't change them. The remaining 9% alter them on a weekly basis, 44% on a monthly basis, and 5% until they were no longer useful. Kitchen utensils, for example, had a high level of contamination, according to study, 14 percent of illnesses from diseases were caused by chopping boards The majority of users did not clean their cutting board before using tools. Disposable supermarket bags can potentially transmit contamination. Raw meat and foods are usually carried in the same bag. It has been reported that bacteria were abundant in reusable bags and absent in fresh bags. Only 3% of people who used reusable supermarket bags washed them, indicating that reusable bags were filthy and a major source of foodborne disease [57].

Campylobacter spp, Cryptosporidium spp, Salmonella spp, Shiga toxin-producing Escherichia coli O157, Shigella spp, and Yersinia spp had the highest incidence rates among children under the age of four. As pathogens increase in baby feeding bottles, contamination by microbes was a common concern, certain species had been verified in laboratory, many species were involved in the transmission of illnesses. In both residential and commercial kitchens, bacterial contamination is highest. In the United States, one kid out of every five gets infected with this.

Scientists had discovered that pollution occurs when items were carried in the kitchen for a short period of time, such as handbags Pets, trash, soiled clothing and plant pots were all common sights [58].

2.5 Cross Contamination in Home Kitchens

Diseases were spread through microorganisms being transmitted via meal, substrate, touch, cooking utensils, and hands all transmit germs. Microbes readily circulate in the kitchen as a result of that hands must be washed before beginning to prepare food to avoid infection. Hand washing should be required after handling food. It had been found among 100 persons touched raw meat, such as chicken, 73 of them had washed their hands. On their hands, *Campylobacter* was still prevalent. This suggested that their hands were not thoroughly cleansed, resulting in the presence of *Campylobacter jejuni* on them. The group of individuals who wash their hands before making meals was unclear and the handles of the pots and pans are also unknown. Microbes were present on dish clothes and sponges, contact surfaces, kitchen equipment and hands that cause contamination. For 48 hours *E. coli* was still present. It has been reported out of all the people who use dish clothes and sponges, 9 out of 92 said they replace their cleaning equipment and weekly percentage of changing them is 44. The remaining 5 say they did not do it until and unless the cloth was left of no use . A contaminated reusable grocery bag was linked to a Nor virus outbreak in a female soccer team [59]. Mostly all users state that these things are cleaned once they are used, according to their reports. As per the information accumulated, crude meat clients don't wash their cutting sheets and other equipment completely enough to keep away from disease. As per an examination; clients ought to utilize 3% hydrogen peroxide arrangement, which is promptly open in pharmacy, to clean melons before to cutting [60]. Only 3% of customers reported to wash their supermarket bags on a routine basis. A Nor virus epidemic in a female soccer team was connected to a tainted reusable shopping bag [61]. In 2007, Hussaii and colleagues published a study in Pakistan that found Campylobacter in various meats and dairy.

Accordingly, crude chicken meat had the best frequency of *Campylobacter* (48%) among meat tests, trailed by crude hamburger (10.9%) and crude lamb (5.1 %). In such manner, veggie plates of mixed greens (40.9 %), sandwiches (32%), cheeses (11%), and crude stock milk tests (10.2%) were the food sources with the most elevated risk rates. Similarly, Fowoyo observed contamination of ready-to-eat food by air microorganisms. *Bacillus subtilis, Micrococcus spp., Staphylococcus aureus, Salmonella spp., Shigella spp.,* and *Escherichia coli* were among the microorganisms found in food. *Aspergillus spp., Penicillium spp.*, and Mucor spp. were the fungal species recovered from vended food samples [62].

2.6 Water

It has been observed that the utilization of water is critical during meal preparation. However, in the event of infection, using water for food preparation and cooking, drinking, and utensil washing might increase the risk of foodborne disease. Enteropathogens such as *E. coli, Salmonella spp.*, and *Campylobacter spp.*, among others, have been shown to spread through water. The shortage of potable water for cooking and serving food has been repeatedly found in studies from Asia, Africa, and South America. Because of the shortage of clean drinkable water, numerous dealers like to reuse it, especially for washing utensils, and the proof showed that the pre-owned water was of high bacteriological quality and had been tainted a few times by different coliforms was taken note [63].

2.7 Utilization of LGRM

Some vendors and restaurant management utilize inexpensive and contaminated goods including unapproved chemicals additions from unlicensed sources to get a financial advantage, which may increase the dangers connected with prepared cuisine. Moreover, crude meat, chicken, and veggies are regularly defiled with a scope of microbes, including food-borne sicknesses like *B. cereus, C. perfringens,*

C. jejuni, E. coli, L. monocytogenes, Salmonella, and *S. aureus* . Spices are thought to include a large number of *Bacillus* species, anaerobic spore formers, *Enterococci,* and *Enterobacteriaceae,* as well as moulds, yeast, and other harmful microbe such as coagulase-positive *Staphylococci.* Spices have been discovered to act as spore carriers and, as a result, may induce food poisoning [64].

These spore formers in spices survive heat exposure and they replicate later if suitable circumstances are available. Pathogens such as *B. cereus, S. aureus, C. perfringens, V. metschnikovii,* and *E. coli* have likewise been found in crude meat and different vegetables. They might be available before to buyer buy or following cross pollution during food taking care of and readiness. Poultry meat has recently been discovered to be nutritionally rich and widely used in Pakistan. However, the risk of food-borne pathogenic bacteria contaminating chicken meat is always present throughout slaughtering, processing, and marketing. The safety of accessible food products is frequently dependent on the use of proper tools for cooking and storing prepared meals. Toxin production, pathogen development, and recontamination may occur as a result of poor food quality caused by improper handling. Food safety is influenced by the form, texture, and sanitary status of equipment and utensils. Unhygienic pots can lead to the accumulation of victuals residues, microbial development, and, eventually, pollution.

As a result, proper utensil selection can reduce cross contamination from raw materials [63]. Furthermore, serving utensils at the retailing site have been shown to be often infected with *Micrococcus spp.* and *Staphylococcus* spp. Because of early openness of the sellers' tainted hands, areas, and dishcloths to dishwashing water and cross defilement locales between dishwater, food readiness surfaces, and the actual food is the source. It is claimed that used dishwashing water and other sources might cling bacteria on utensil surfaces, posing a risk in the food industry. *Salmonella* and Shigella were found in kitchenware surfaces and cutlery after microbiological examination. Another study found that during food preparation, when raw materials are sliced and chopped frequently by a same tool without being cleaned, they act as a vector for contamination by insects' feces, dust, and other microorganisms [65].

2.8 Food Safety in Home Kitchen

Foodborne illnesses and the cost of treating them motivate people to handle food safely at home. *Salmonella* was the cause of the incidents that were reported from the US between 1998 and 2008 they were connected to food. *Salmonella*-related illnesses are on the rise in households. Food-related diseases are generally minor, unreported, and unconfirmed. Experts claim that ailments caused by eating are more common [66].

2.9 Food at Work and School

Most people spend the bulk of their time away from their homes at work or school. These businesses are renowned for offering freshly prepared meals and snacks. A lot of workers eat their lunches at their desks. There is a risk of illnesses brought on by contaminated food since just 38% of employees frequently clean their workspaces and a similar number only seldom or never do so. Just half of laborers report cleaning up when lunch like clockwork, while just 25% of office coolers are cleaned one time per week. The "chill" culture at work has improved a little, with 66% of workers expressing they store their meal in the fridge, though half case they forget about transient food at room temperature for over two hours [67]. Youngsters snacks might introduce "chill" food handling issues since daytime dinners are every now and again put away for a few hours at room temperature and many schools ask students to discard leftovers and packaging while present at home [68]. Almost always (88%) preschoolers' meals were found to be warmer than room temperature. Only approximately 2% of daily meals served at temperatures over the danger zone contain items that have degraded. Even though there were several packets of ice used, the majority of the day meal items were dangerously warm. Sandwiches entered the risk zone of temperature range very quickly after school began and stayed there until the end of the day, as per research on the "temperature venture" of stuffed feasts brought to school in Ireland [69]. Since reusable lunch packs and boxes are routinely cleaned with a dishcloth, one of

the most significant vehicles for cross pollution in home kitchens, they represent a "perfect" food handling danger. Instead of eliminating microorganisms, this cleaning procedure may introduce them. The highest microbial count was reported in zipped and insulated day meal boxes, washed with moist towel, with positive testing for *S. aureus* [70].

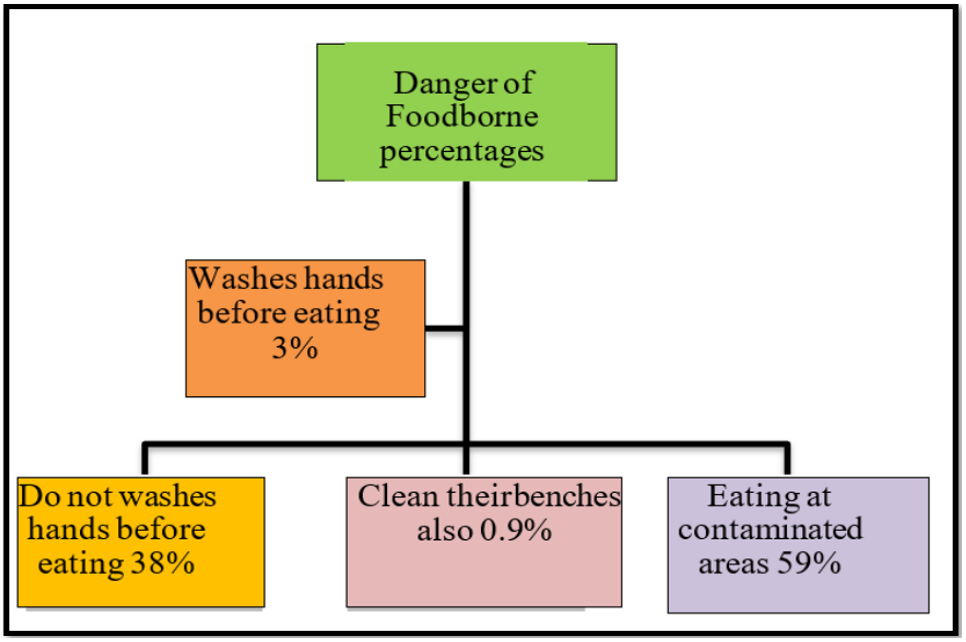


FIGURE 2.1: Danger of food borne diseases related to contamination level [71].

2.10 Handling Errors During Food Making

The most likely groups to handle food improperly are men, those under thirty and over sixty-four, and those with a minimal degree of post-secondary education. The majority of those at risk are those who often prepare food [72]. Consumers of all ages, especially those who are more vulnerable to illnesses brought on by foodborne pathogens; make significant errors while handling food properly. Additionally, they assert that they don't reheat some foods, such as soft cheeses, etc. Prior to making meals, doing diaper changes, or feeding their young children. Some moms of young kids claim they don't wash their hands with soap and water [73].

It to cultural variations in food consumption habits when compared to other groups. The predominance of Listeriosis is more noteworthy among Hispanic ladies

because of the prominence of Mexican-style cheeses. Yersiniosis is frequently reported in African and American infants and babies due to diverse dietary cultures, which include fresh chitterlings (cleaned and prepared intestines make it evident that some illnesses caused by foodborne pathogens are more common in specific ethnic groups due To pig) [74]. Due to their preference for eating raw and undercooked foods, Caucasians are more likely than minority groups to become infected with *E. coli 0157:H7* [75].

2.11 Safe Food Handling Practices

Because the meal was handled improperly, there are several different diffusions. The following are some of the most frequent barriers to handling food safely.

FIGURE 2.2: Safe Food Handling [76].

2.12 Optimistic Bias

Almost six out of ten customers think their chance of getting a foodborne disease is minimal. A few buyers think they have low risk of getting a foodborne sickness

contrasted with others. This ideal inclination is associated with hazardous ways of behaving and an inability to carry out safeguard measures, the two of which are related with a higher gamble of mishaps and foodborne sickness [77]. 90% of respondents believe their own risk of becoming sick from eating food they prepared is minimal [78]. Just 41% of respondents who were asked about the likelihood that people in their social circle would become ill from eating food they had prepared disagreed [79]. Just 41% of respondents who were asked about the likelihood that members of their social circle would become ill from eating meals they had prepared agreed [80]. Rating one's own gamble rather than others in one's gathering (those with whom one looks at oneself) uncovers a low inspiration to change careful propensities [81].

2.12.1 Illusions of Control

Two-thirds of respondents think they have a lot of control over handling food properly [82]. At the point when asked what grade a sanitation expert might give them for their dinner readiness, supplier, and capacity at home, everything except 2% of respondents granted themselves good grades. At the point when purchasers utilized a home-adjusted offered food agenda to rate the food, their evaluations were a lot of lower. Scores were much lower and, on average, home kitchens received failing grades when professional auditors assessed them [[83], [84].]. The members of those domestic study groups were aware that researchers might visit their home to look at and assess their culinary practices, which only adds to the depressing nature of the results [85].

2.12.2 Consumers Handle Food Safely

As a few thoughts are introduced, for example, the Wellbeing Conviction Model, Social Mental Hypothesis, and Hypothesis of Arranged Conduct, speculations connecting with wellbeing conduct are continuously being changed. Hardly any food handling mediations have been hypothesis based, notwithstanding the way that

many examinations show that these thoughts might be utilized to construct powerful developing for various wellbeing conducts, including sanitation [86].

TABLE 2.1: Percentages of bacterial strains isolated from different surfaces of kitchen [[87], [88], [89].]

Bacteria	**Percentage of bacteria strain**	**Isolates**
S. aureus	34-43%	Isolated from domestic refrigerators and multiple surfaces [96].
Fecal coliforms	48 to 67%	Isolated from dishcloths sponges and kitchen sinks [97].
E. coli	3-15%	Isolated from dishcloths sponges and kitchen sinks [98]

It has been reported that 14 % bacteria (*S. aureus*) was found in sample collected from domestic refrigerators and 2 % was isolated in from different surfaces. *S. aureus* presence in homegrown refrigerators address a particular sanitation problem because of the living being capacity to develop and deliver poison when represented to gentle misuse of temperature [90]. Clashing reports have been distributed with respect to this tainting. Another studies shows secluded presence of *S. aureus* rarely (1 to 6%) from refrigerators [91], [92]. While other tracking down such a high commonness of population (33 to 41%) of contamination from these surfaces [93]. In this study the microorganisms waste coliforms, *E. coli, and S. aureus* were completely associated with individual neatness moved from hand contact materials. A shortfall of cleaning things in the home including the texture or paper towels was solidly associated with a couple of kinds of contamination, including microorganisms, for example, coliforms, waste coliforms, *E. coli, and S. aureus*. These reports recommend that gigantic trade of these microbes occur in unattractive kitchens helped a couple of instances of failure to wash hands precisely [94].

In different cases, this exchange might happen through the cleaning utensils for example wipes and dish fabric since it has been observed a huge relationship

between population on kitchen wipes and dishcloth and tainting in different pieces of kitchen of same home. This example proposes that cleaning surfaces as they planned to do these things may really do the inverse in the case they are not cleaned as expected and consistently. Due to the reality and high death rate (19.5%) related with Listeriosis, the power of *L. monocytogenes* in kitchens may be a justification for concern [95].

During a study, five kinds of Listeria were isolated from 15% of the residences; in which 3 % of *L. monocytogenes* are present in every kitchen of homes. Refrigerators sample contain 9% population of Listeria and 1 percent of *L. monocytogenes* was harbored by one refrigerator [96]. A few separate investigations support the low level of surface contamination in the refrigerators with Listeria spp. (1 to 9%) and *L. monocytogenes*(1 to 6%). 60% of the refrigerator shelves in Mexico reportedly tested positive for *L. monocytogene*.

Listeria has never seen such high recurrence of contamination, which may be linked to the population's typical increased intake of unpasteurized milk and cheese. The findings show that Listeria sp. infection in refrigerators running above the recommended temperature was inevitable (4.48C, 408F). Consumers need to be reminded to practise proper refrigeration techniques in order to lower the danger of listeriosis.

The successful isolation and culturing of notoriously fragile *Campylobacter* in environment have been reported from kitchen cloths Regardless of this delicacy, it can separate reasonable C. jejuni from three kitchens. One more review announced about presence of 2% Campylobacter on kitchen surfaces exceptionally sink during standard activity. C. jejuni detachment from kitchens in which food was not arranged effectively shows that kitchen could become supply for C. jejuni that might ruin or pollute other food [97]. Albeit not commonly viewed as delicate as *Campylobacter, Salmonella* has likewise not been much of the time identified in purchaser homes. Most investigations announced Salmonella as being available on under 5% of tests from dishcloths and two other studies revealed a lot noteworthy recurrence (somewhere in the range of 10 and 33%) of positive samples [98].

TABLE 2.2: Contamination rate of pathogen in kitchen [99].

Bacteria	**Presence**	**Percentage**
Listeria spp	Refrigerators	2%
L. monocytogene	Refrigerators	1-6%
Campylobacter	Refrigerators	50%
C. jejuni	Refrigerators	33-44%
Salmonella	Slabs	1-9%

Built environment are the places where humans spend 90% if their life. There is a large amount of different micro habitats enriched with microbial species. They have adapted to available niches the built environment micro biome is built by these organisms [100]. The BE environment has heterogeneous behavior and variations. They can vary from villages to ICUs of hospitals micro biome effects humans and cause infections and food related disease [101].

Microbial indicators include our kitchens and bathrooms. The success of forming of colonies by these microbes occurs by contact to body and surfaces. Microbes form colony based on their environment and nutrients. There are more microbes in kitchen than in bathrooms. Kitchen sponges have widely contributed to it. Kitchen sponges are said to be the largest contribution of bacteria [102].

Sanitation of kitchen sponges is important. Providing heat for example boiling etc. reduces bacteria [103]. It has been shown that bacteria can only be reduced up to 60%. Kitchen sponges do not only store microorganisms but also are a source of spread of them that causes contaminations and diseases and causes food related illnesses. Sponges have been studied and Cultivated to target microbes. But less studies have been done on micro biome of sponge on molecular level. Deducing in a solitary report result were coming from only a solitary wipe test, broke down among 82 other kitchen surfaces [104]. The nature of sponges is porous and water soaking ability that provides a favorable condition for microorganism survival. It can be viewed by some pie charts of composition of micro biome and their taxonomy of kitchen sponge, as determined by pyro sequencing

of 28 sponge samples' 16S rRNA gene libraries (upper and lower samples of 14 sponges, respectively). Just the 20 most common orders plus families are listed for easier reading. Bacterial micro biome of home kitchen sponges that were used were analyzed by throughput of 16rRNA sequencing of gene to understand that real taxonomic diversity and assemblage and also to examine the effect of selected intrinsic and extrinsic factors on structure of micro biome also pathogenic capacity of sponge micro biota was estimated to look out, by complementing and validating the sequencing data, 3D-microscopy using fluorescence in situ hybridization in blend with confocal laser-filtering microscopy (FISH-CLSM) was utilized to examine the dissemination example of microbes which is spatial in the kitchen wipe tissue [105].

2.13 Gap Analysis

Home kitchens are associated with significant food borne diseases. The diversity of bacteria in Kitchen remains unknown, as most of the study for microbes has been focused on pathogen detection. In Pakistan, the major ignorant areas are the kitchen and no such investigation has yet been reported till date in the foods for foodborne diseases. The source of theses contaminations is particularly kitchen where food is being washed and cooked. There are few reported studies which heed to investigate these sources.

2.14 Research Question

Major research question are:

1. What type of bacterial pathogens are associated with washing utensils?
2. What pathogenic bacteria is most frequent present in home kitchen?

Chapter 3

Research Methodology

3.1 Methodology Chart

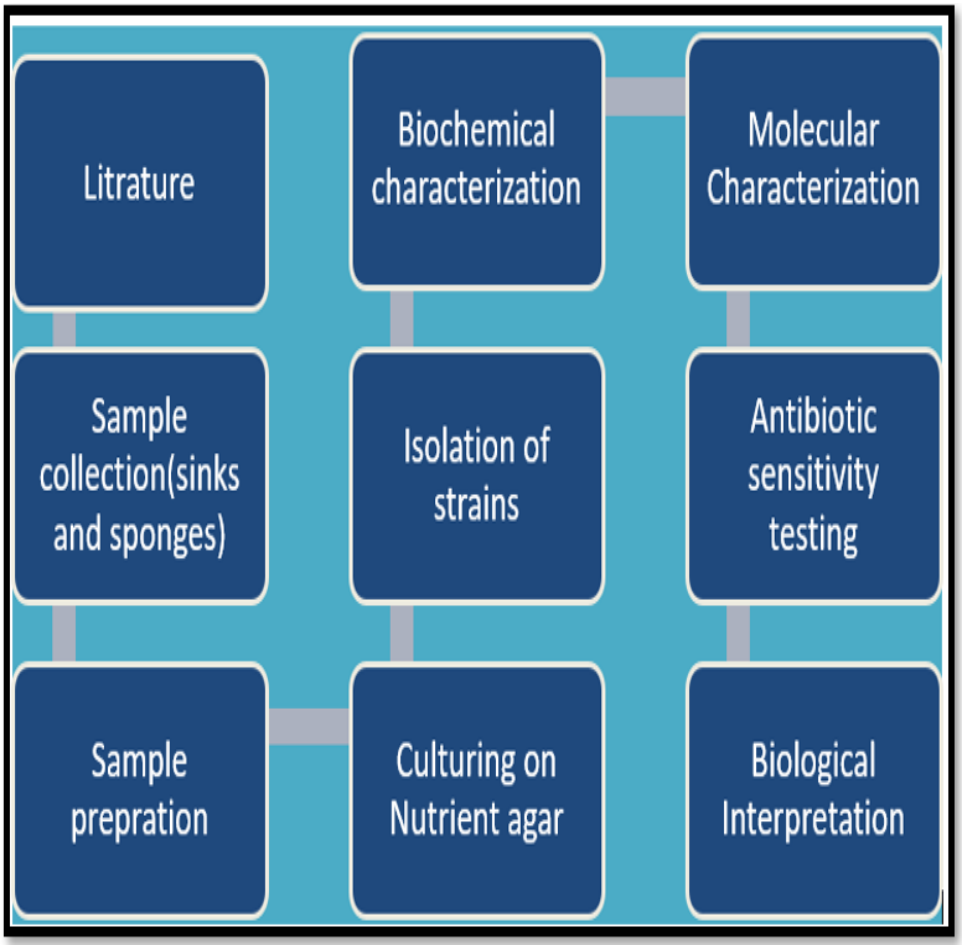


FIGURE 3.1: Flow chart of reserach methodology

3.2 List of Equipment

Autoclave, Magnetic Stirrer, Weighing Balance, Laminar Flow, Incubator, Vortex, Microscope, Shaker, pH Meter, Micro Centrifuge, Centrifuge, Microwave Oven, Refrigerator, Beaker (500ml), Spatula, Conical Flasks (500ml), Eppendorf Tube, Micropipette, Petri Dishes (10cm), Spirit Lamp, Plastic bottles.

3.3 List of Materials

Inoculation Loop, Dropper, Parafilm, Graduated Cylinders (100ml), Glass slides, Falcon tubes, Cotton bud, Aluminum foil.

3.4 List of Chemicals

Nutrient Agar (OXOID) MOO3 500g, MacConkeys Agar (OXOID) CM0007 500g , Mannitol Salt Agar (HIMEDIA) REF M118-500g, Simmon Citrate(BIOLAB) REF ECIT20500 500g, EMB (HIMEDIA) REFMO22-500g, Kovac reagent, Gram Iodine Solution, Safranin Solution, Crystal Violet Solution and Decolorizing Solution, Phosphate Buffer Saline (PBS), Distilled Water and Glycerol.

3.4.1 Selection Criteria

Samples were collected based on the criteria as mentioned in literature [63]. It includes the nature of material being used for washing kitchen utensils. Only sponges were selected as they have water and moisture retention ability that plays critical role in microorganism's growth.

Only sponge used for duration of 2-4 weeks were used during this study. Nature of detergent used for washing. Number of persons in family. Samples were collected from family with the adult members of 7-10; with condition of frequent washings.

3.4.2 Sample Collection

A sample of 100 sponges were used for washing of utensils in kitchen were collected. The material of the kitchen wipes was polyester (delicate yellow side) and polyurethane (grating side). Each wipe was coded and cut into three sections utilizing a sterile blade. The typical elements of the wipe parts was 6.6 cm × 3.2 cm × 3.3 cm. The sponge samples were placed in glass containers with lid and samples were brought to lab and kept in refrigerator at temperature of 4C.

FIGURE 3.2: Sample collected in falcon tubes.

3.4.3 Sample Preparation

PBS (Phosphate Buffer Saline) was made by adding 23g of NaCl, 62.8g of $Na_2(HPO)_4$ in 400 ml distilled water. After that pH was adjusted to 7.4 by adding HCl in it PBS was transferred to falcon tubes. Samples were collected by cotton swab and 2ml of PBS were added to plastic bottles and then transferred to the laboratory. Samples were vortexed for 5-7 minutes and tubes were labeled according to location.

3.4.4 Preparation of Nutrient Agar

The material was cultivated on Nutrient agar to confirm the presence or interaction of bacterial pathogens with the kitchen sponges. Bacterial pathogens were grown on nutrient agar. By using a measuring balance, 5.6g of nutrient agar was measured out and 200 ml of distilled water were added. For 15 to 20 minutes, the mixture was autoclaved at 121 degrees. A consistent 20ml stream of autoclaved media was laminarly poured into sterile petri dishes. Through the use of micropipette tips that had been autoclaved and centrifuge tubes, petri dishes were filled. Using a spreader, the sample was evenly distributed on the Petri plate. 10 plates containing Nutrient Agar were distributed with 5ml of the produced sample. Each location was replicated 5 times. Plates incubated for 48 hours on 37 . Plates were incubated in upside down direction to avoid the moisture. Each place was reproduced 5 times. 37 plates underwent 48 hours of incubation. To avoid moisture, plates were incubator in an upside-down direction.

3.4.5 Gram Staining

3.4.5.1 Crystal Violet Preparation

2g of crystal violet was dissolved in 10ml of ethanol to create gram-staining crystal violet. In eppendorf tubes, solution was kept for later use.

3.4.5.2 Preparation of Gram Iodine Solution

Iodine solution was made by dissolving 0.03g of iodine pearl, 0.667g of potassium iodide, and 0.1g of sodium bicarbonate in 10ml of distilled water.

3.4.5.3 Preparation of Safranin Solution

To make the stock solution, 0.1g of safranin was dissolved in 4ml of 95 percent concentrated ethanol.

3.4.5.4 Preparation of Distaining Solution

The destaining solution was created by adding 5ml of 95 percent ethanol and 5ml of acetone. To further preserve it for Gram staining, it was placed in an eppendorf tube.

3.4.5.5 Procedure of Gram Staining

Hans Christian Gram invented the Gram staining technique in 1844. It distinguishes between gram-positive and gram-negative bacteria as a differential staining technique. The dilutions were made by adding a loop full of pure bacterial culture to 2 ml of sterilized water in the beaker after cleaning the glass slide with 75% ethyl alcohol. Slide was filled with a drop of bacterial suspension, and then allowed to air dry. After that, heat was applied for 60 seconds using a spirit lamp to fix. A drop of crystal violet was applied to the heat-fixed bacterial stain and kept there for 30 seconds before being washed with sterile water and dried with blotting paper. The slide was then treated with 3–4 drops of Gram iodine and left for a minute. Once more, sterile water was used to rinse the slide for a minute. The slide was once again cleaned with sterile water after the decolorizer, which contains 95% ethanol, was run through the stained region to remove the colour and decolorize the stain. Three to four drops of safranin were then added, and after one minute, washed. Three to four drops of safranin were then added, and after one minute, washed. The slide was covered with a cover slip, which blotted the moisture from the sides. The slide was then examined at a magnification of 40X. Gram-positive bacteria display a purple tint, whereas gram-negative bacteria display a pink colour.

3.5 Growth on Differential Media

Differential media were used for identification of bacteria including MaCconkey Agar [Macc], Mannitol Salt Agar [MSA], and Eosin Methylene Blue Agar [EMB].

3.5.1 Growth on MacConkey Agar [MCC]

13.75g of dry MaCconkey powder was mixed continuously by a magnetic stirrer with 250 ml of distilled water before being autoclaved at 121 for 15 to 20 minutes. Petri dishes were filled with the finished media. A total of 10 plates were made and allowed to harden at room temperature inside the laminar flow hood.

3.5.2 Growth on Eosin Methylene Blue [EMB]

9.375g of Eosin methylene blue agar was combined with 250ml of distilled water. Media were properly mixed and stirred using a magnetic stirrer. The prepared medium was autoclaved for 15–20 minutes at 121 degrees. Media was poured into petri plates and allowed to solidify at room temperature.

3.5.3 Growth on Manitol Salt Agar [MSA]

To a conical flask holding 250ml of distilled water, 27.75g of powdered MSA was added. On 121, the media were combined and autoclaved for 15 to 20 minutes. After pouring, the media has been allowed to solidify at room temperature.

3.5.4 Streaking of Culture Media

The differential medium was streaked with the bacterial colonies produced on Nutrient agar. The selection of bacteria was based on morphology, colour, and form.

3.6 Biochemical Characterization

Murray carried out a variety of biochemical assays to characterize the biochemistry of bacteria.

3.6.1 Citrate Utilization Test

Bacterial strains that utilize citrate are referred to as citrate positive, while those that do not are referred to as citrate negative. A 100ml batch of the Simmons citrate solution was made in preparation for this test. In a conical flask, 100ml of distilled water was used to dissolve 2.424g of Simmons Citrate. It was then autoclaved at 121 degrees for 15 to 20 minutes. The petri dish was filled with media. Six plates in all were ready for the biochemical test. By removing a loop full of bacteria from each plate, the isolated bacterial strain was injected into the Simmons citrate media plates. In the wake of being appropriately wrapped, the plates were incubated for 48-72 hours at 37 in the incubator. Citrate positive media is defined as turning green into blue; citrate negative media does not change colour.

3.6.2 Indole Test

This analysis uncovers the limit of specific microbes to change over the medium-amassing amino corrosive tryptophan to indole.

The test for indole synthesis is crucial for identifying enterobacteria; the majority of *E. coli, P. rettgeri, P. vulgaris,* and *M. Tryptophan* is broken down by *Morgani* and *Providencia* species, which results in the production of indole 4 ml of tryptophan broth was poured a sterilized test tubes and inoculation was inoculated 24 hours grown culture. Immunized test tubes incubated at 37°C for 24 hours. After that 0.5 ml of Kovac's reagent was added to the way of life and presence or nonappearance of ring was noticed.

3.6.3 Oxidase Test

A cotton swab was dip into the hydrogen per oxide and it was directly touched on the isolated colony which was streak on the plates and change in color was observed.

3.6.4 Coagulase Test

Staphylococcus aureus (positive), which produces the coagulase enzyme, is distinguished from *S. epidermis* and *S. saprophyticus* (negative), which do not produce coagulase; that is Coagulase Negative *Staphylococcus*. A drop of physiological saline was placed on two separate slides and the loop isolated colony in each drop was added.

After that drop of rabbit plasma was gently mixed in one of the slide containing suspensions. To differentiate granular appearance of the organism from true coagulase clumping, the second slide was kept without plasma. Both slides were observed clumping.

3.7 16s rRNA Sequencing

The use of the 16s rRNA sequence, which appears to be the most preserved one, is the earliest and highest throughput method to investigate microbial ecology. It is an economical strategy for a community's bacterial survey. The samples were analyzed by Microgen Korea using 16s sequencing to identify the micro biota associated with houseflies. Preserved strains were sent for this purpose. Then the analysis was done when the raw sequence will obtained.

3.8 Antibiotic Sensitivity Test

Identifying the pattern of bacterial antibiotic resistance to various antibiotics is the most crucial step in illness therapy. The disc diffusion method was invented by Kirby and his collaborators A. W. Bauer as an alternative to earlier broth dilution techniques. The test was developed to determine if isolated and sequenced bacteria were sensitive to antibiotics or antibiotic-resistant less zone of inhibition indicates resistance to that particular antibiotic, whereas larger zone of inhibition indicates susceptibility. In light of this, nutritional broth was first made, and 3g of TSB

were added to a flask filled with 100ml of distilled water to make 100ml of TSB. After a thorough shaking, the flask was covered in aluminum foil and autoclaved for 15 minutes at 121 degrees with six cleanly wrapped test tubes. In order for the bacteria to grow into the broth, it was injected with bacteria and incubated overnight at 37 degrees in the incubator.

3.8.1 Kirby Bauer Method Procedure

- Muller-Hinton agar mediums were established with standardized ingredients.
- Muller-Hinton agar medium was injected 4 mm deep into 150 mm petri plates
- The agar media was maintained at pH range of 7.2 to 7.4 and broth culture was used for inoculation.
- A sterile swab that had been through a broth culture of bacteria was used to inoculate the culture plates.
- The bacteria-inoculated agar medium plates were permitted to dry for about five minutes.
- Utilizing sterile needles, the antibiotic discs were applied to the inoculated.
- To make sure that each disc is properly in touch with the surface of the agar media, the discs were gently pressed with flame-sterilized forceps.
- For the whole night, the plates were incubated at a temperature of 37°C.
- To assess if an antibiotic was effective against bacteria, the zone of inhibition for each antibiotic disc was measured using a scale or screw gauge.

Chapter 4

Results and Discussions

4.1 Growth on Nutrient Agar

Kitchen sponges that are being used to wash the kitchen utensils were used to isolate the bacterial microbes. For this purpose, general purpose nutrient agar media was used. This media is being used for the growth of assortment of bacteria and fungi [106]. The compound creation of supplement agar incorporates peptone, meat concentrate and agar. This basic piece gives the adequate supplements to gram-positive as well as gram-negative microscopic organisms which are essentially required for the growth and replication [107]. The inoculation of almost all the sample on nutrient agar showed the growth of variety of bacteria (Fig 4.1). Variety of bacterial pathogens have been reported to be associated with sponges, dishwashing cloths and surfaces of the Kitchen [108].

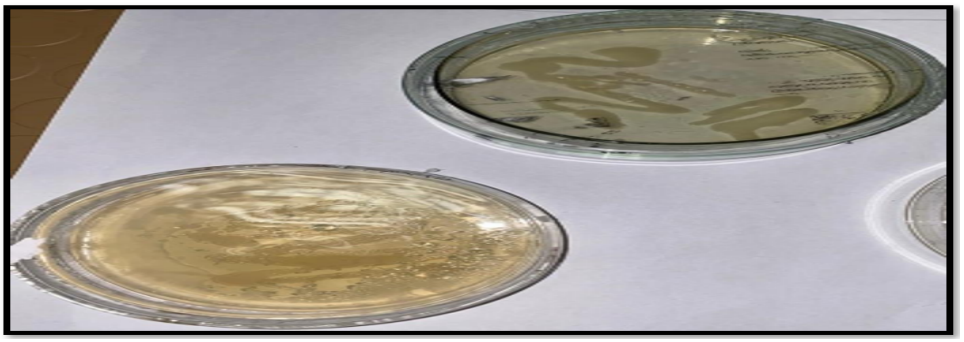


FIGURE 4.1: Growth of bacteria on nutrient agar isolated from sample

4.2 Growth on MacConkey Agar

MacConkey agar is utilized to separate the maturing gram negative microbes from lactose non-maturing gram negative microorganisms. It is composed of gelatin and peptones which is obtained from an extraction of meat and casein that act as source of nutrients and vitamins for the growth of microorganisms. MacConkey agar contain Bile salts which inhibit most of gram-positive organisms to grow. Neutral red and crystal violet present in this medium are very lethal to bacteria. It is additionally used to confine coliforms and digestive microbes in water, dairy items and living specimens. The bacterial pathogens which grow on MacConkey agar i.e. includes *E. coli, Enterococcus, Aerobacter pseudomonas*. MacConkey media only allows the growth of gram-negative bacteria hence it inhibits the growth of gram positive bacteria [109].

Gram-negative bacteria are more resistant to the dyes present in this medium than gram-positive bacteria. The presence of Bile salts likewise diminishes poisonousness for gram-negative microorganisms and increment the harmfulness for gram-positive microscopic organisms. That is why gram negative bacteria usually shows more significant growth on such medium and these bacteria can also be differentiated due to their lactose fermenting ability.

The lactose aging bacterial strains shows red or pink shaded settlements and which might be encircled by a zone of acid precipitated bile. The red colored pattern is just due to the releasing of acid from lactose, when pH of medium drops below 6.8 in the result.It is additionally used to confine coliforms and digestive microbes in water.

The results showed that all the four locations specimens showed the bacterial growth indicating the presence of gram-negative bacteria. Some samples showed a shiny pink color colony indicating the presence of *E. coli*, some cultured samples showed colorless round appearance indicating non-lactose fermenters and small red orange colonies showing the presence of *Enterococci* as shown in figure 4.2 and figure 4.3.

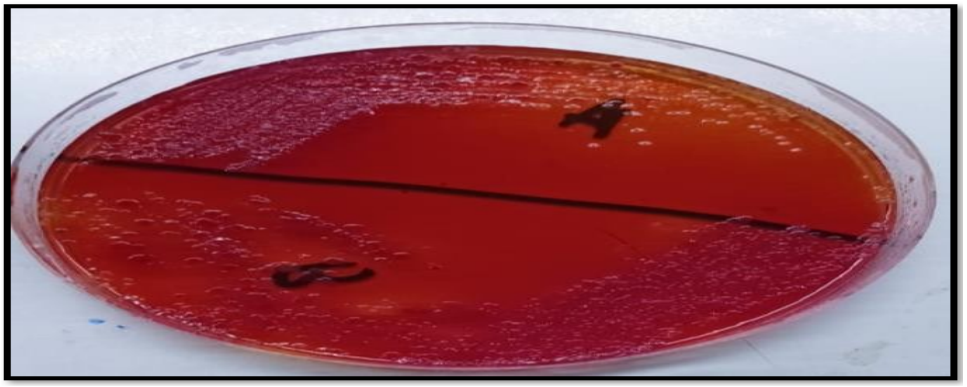


FIGURE 4.2: Growth on MaConkey

FIGURE 4.3: Growth on MacConkey agar

4.3 Growth on Eosin Methylene Blue Media (EMB)

Commercially available powder is in rehydrated form consist of the following components (g/L): peptone (Bacto-peptone or Gelysate) 10.0, lactose 5.0, sucrose 5.0, dipotassium phosphate 2.0, agar 13.5, eosin 0.4, and methylene blue 0.065. Last pH is 7.2 ± 0.2. Media with these parts permits the development of gram negative microscopic organisms and restrains the development of Gram positive microorganisms. It helps in the identification of E. coli, from nonpathogenic lactose-fermenting gram negative rod shaped bacteria [110]. Samples showed green metallic sheen color which depicts the presence of *E. coli* as shown in fig 4.4.

A large portion of the types of *E. coli* states have trademark green sheen on EMB agar. Fast decrease in the pH of the EMB agar is the basic calculate the

arrangement of the green metallic sheen saw with *E.coli*, quick maturation of lactose and development of strong acids.

Microbes without lactose maturation are either lackluster or light lavender. The essential part of EMB are enzymatic condensation of gelatin, lactose sugar that significantly help to separate lactose fermenter from non-lactose fermenter, it likewise contains dipotassium phosphate, eosin Y: pointer, agar, and methylene blue. Therefore, the primary purpose for which we use this media is to separate lactose fermenter bacteria from non -lactose fermenter bacteria. Green sheen color colony indicate the presence of *E.coli* as shown in fig 4.4.

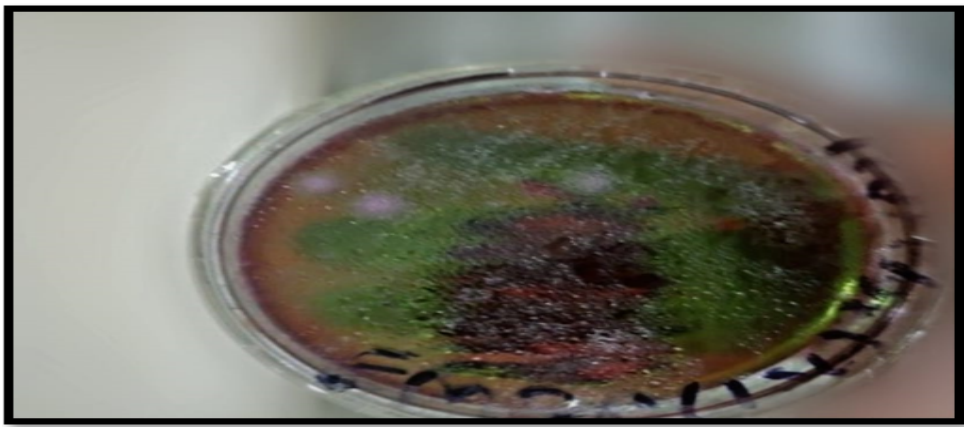


FIGURE 4.4: Growth on EMB.

4.4 Growth on Manitol Salt Agar

This agar consists of 7.5% sodium chloride. It is being utilized for those microbes which can endure high salt fixations. Sugar mannitol is the main carbohydrate in the MSA which is used to distinguish bacteria on the basis of fermentation. Mannitol fermentation is indicate by change of media color, not only by colony color.

This process is predominantly visible as several micrococci are pigmented [111]. All the samples collected showed the presence of growth but media remains light pink in color, colonies are colorless indicating the probability of *Staphylococci* in the isolatesas shown in fig 4.5.

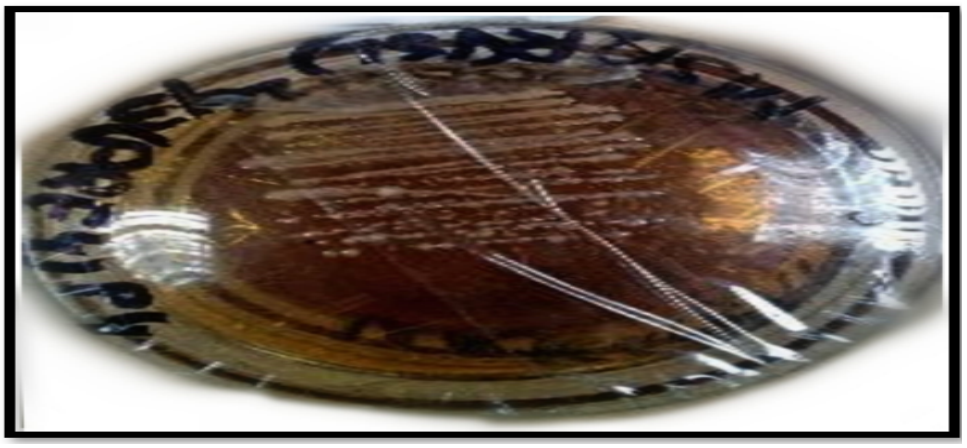


FIGURE 4.5: Growth on MSA.

4.5 Isolation of Bacteria Strain

Bacterial species or genus was categorized based on the color characteristics and morphology on differential media. Different types of bacteria were obtained with different morphology, different color characteristics, and different colony characteristics.

4.5.1 Gram Staining

The staining is also called differential staining and it was performed for isolated cultures of bacteria obtained on differential media. This system is utilized to separate from Gram negative and Gram-positive microorganisms. Due to differences in chemical composition of bacterial cell wall, Gram staining produces two different colors. Contrasted with Gram negative microbes, Gram positive microorganisms have a thicker peptidoglycan covering in their phone walls with lipid-containing external layer. High lipid concentrations in Gram-negative bacteria lead to the formation of wide holes that allow crystal violet to escape, leading the decolonization of the bacteria and afterwards apply a counterstain. The peptides are thick and cross-linked dehydration and hole closure in gram-positive cell wall, preserving is the main stain. When stained using Gram's technique, the bacteria that keep the primary stain look dark blue or violet and are not decolorized, but those that lose the crystal violet employed the counterstain, safranin. Gram negatives are

classified as appearing red. The various reagents for the Gram stain are used in the following order: crystal violet, iodine solution, alcohol, and safranin. The findings were noteworthy since they showed that the bacteria collected on MacConkey are stained pink, indicating that the species cultivated there are Gram negative. Furthermore, a microscopic analysis of them reveals that they are circles.

The Mannitol Salt agar strains show purple in stain, which denotes that they are Gram Positive bacteria as in fig 4.6. The pink colour of the spots seen on the EMB indicates that they are Gram negative as in fig 4.6.

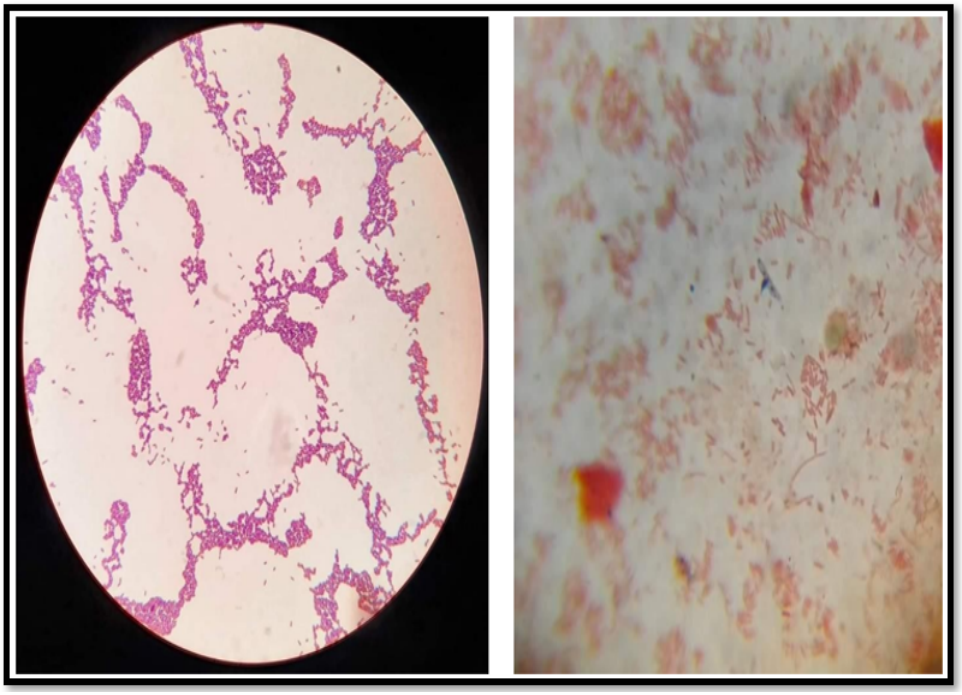


FIGURE 4.6: a). Gram Positive Bacteria and b) Gram negative Bacteria

4.6 Citrate Utilization Test

In this experiment, Simmons Citrate Agar serves as the only supply of carbon. When the pH rises over 7.6, bromothymol blue acts as an indication, changing from green to blue. It creates alkaline products if it employs citrate. The data demonstrate that the strain produced favorable media coverage and became blue after 4 days. That suggests that this particular strain is using citrate for metabolic processes.

4.7 Indole Test

This experiment shows that certain bacteria can break down the medium- accumulating amino acid tryptophane into indole. The test for indole synthesis is crucial for identifying enterobacteria. The majority of *E. coli, P. rettgeri, P. vulgaris,* and *M. Tryptophan* is broken down by Morgani and Providencia species, which results in the production of indole. Bacteria that express the tryptophanase enzyme may deaminate and hydrolyze the amino acid tryptophan. By using the intermediary molecule indolepyruvic acid, tryptophan is converted to indole by reductive deamination. The deamination cycle, in which the amine (- NH2) gathering of the tryptophan atom is removed, is catalyzed by tryptophanase. The response's finished results incorporate energy, pyruvic acid, ammonium (NH4+), and indole. As a coenzyme, pyridoxal phosphate is important. The arrangement changes from yellow to cherry red when indole is added to Kovac's Reagent, which incorporates hydrochloric acid and p-dimethylaminobenzaldehyde in amyl liquor. Amyl liquor isn't water solvent, accordingly the red variety will amass at the highest point of the soup as a slick covering.

In the spot test, indole and p-Dimethylaminocinnamaldehyde (DMACA) blend in the channel paper network at a acidic pH to form a blue to blue-green atom. Table results are displayed.

4.8 Oxidase Test

Creatures that have cytochromes produce the intracellular oxidase compound. The oxidation of cytochrome c is catalyzed by this oxidase protein. Oxidase-positive organic entities variety the reagent blue or purple since they have cytochrome C as a part of their respiratory chain, Oxidase-negative life forms don't oxidize the reagent, leaving it colorless inside the boundaries of the test, and are accordingly cytochrome c lacking. Oxidase test was negative for *E.coli sp.* and *Staphylococcus sp* while positive for *Pseudomonas sp.* Results are shown in a table 4.1.

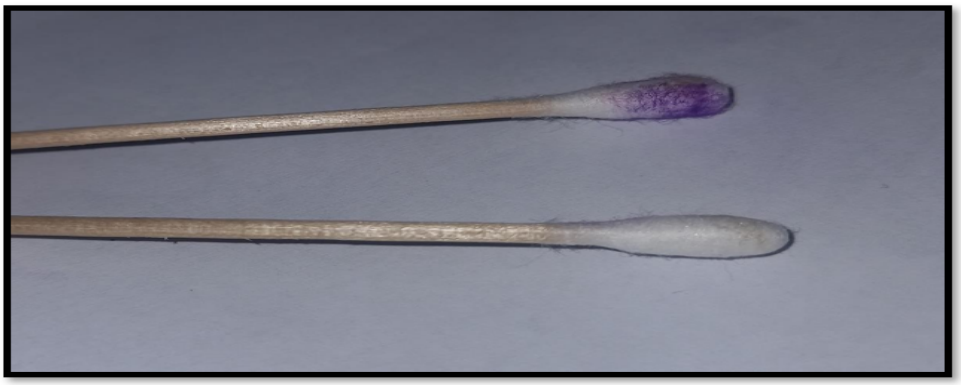


FIGURE 4.7: Oxidase test positive results for *Pseudomonas*.

4.9 Coagulase Test

Plasma clusters because of coagulase, a catalyst like protein that changes fibrinogen into fibrin. *Staphylococcus aureus* generates bound and free coagulase in contrast to *S. epidermis* and *S. saprophyticus*, which are coagulase-negative because they do not make it. Results are shown in a table 4.1.

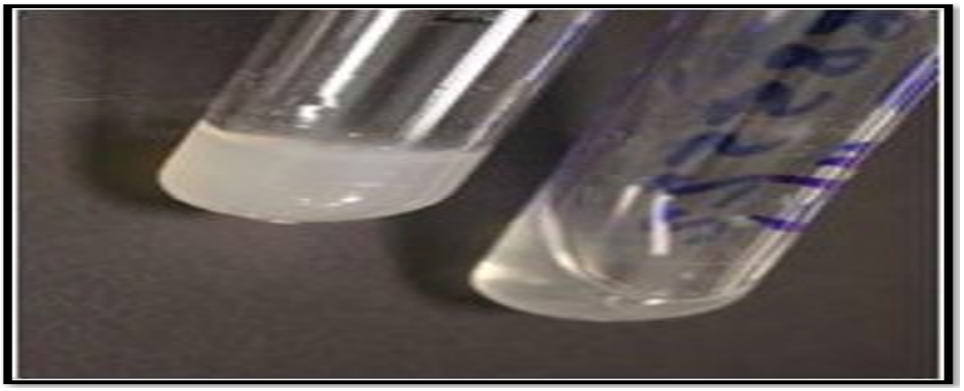


FIGURE 4.8: Coagulase Test

TABLE 4.1: Biochemical characterization of isolated bacterial stains

Name of Bacterial Stains	**Microscopic examination**	**Gram Staining**	**Simon Citrate Test**	**Oxidase Test**	**Indole Test**	**Coagulase Test**
E.coli sp.	Rod	-tive	-tive	-tive	+tive	-tive
Pseudomonas sp.	Cocci	-tive	+tive	+tive	-tive	-tive

TABLE 4.1: Biochemical characterization of isolated bacterial stains

Name of Bacterial Stains	**Microscopic examination**	**Gram Staining**	**Simon Citrate Test**	**Oxid-ase Test**	**Indole Test**	**Coagu-lase Test**
Staphlococcus sp.	Capsulated	+tive	-tive	-tive	-tive	-tive

4.10 Antibiotic Sensitivity Test

The antibiotic sensitivity test was performed for the samples which were collected from the kitchens of Islamabad. The isolates of *E.coli* were checked against seven antibiotics. Disk diffusion method was used for this purpose [112]. The disk diffusion susceptibility method is simple and practical and standardized method which is mostly used in clinical labs. In this test the bacterial inoculums introduced to the surface of large (150mm diameter) Muller-Hinton agar culture plate. The disc diffusion method is mostly preferred due to their simplicity because the test does not require any specialized equipment and the final results can easily interpret by clinicians. The drug resistance of all the antibiotics in the form of zone of inhibition against *E.coli* was given in the appendix 5.1.

The percentage ratio of sensitivity was 100% in Amikacin, 93.3% in Imipenem and 98.3% in Azithromycin 98.3%., Ceftriaxone high resistance ratio i.e 100%, Ceftazidime resistance ratio was 46.6%, Augmentin resistance ratio was 53.3% and Ciprofloxacin resistance was 51.6%. Whereas intermediate values of above antibiotics were also calculated as shown in table 4.2 and 4.3.

TABLE 4.2: percentage ratios of antibiotic drugs against E.coli

	Amikacen	**Ceftazidime**	**Cesftriaxon**
Resistant	0	28	60
Intermediate	0	19	0
Sensitivity	60	13	0

TABLE 4.2: percentage ratios of antibiotic drugs against E.coli

	Amikacen	**Ceftazidime**	**Cesftriaxon**
R%	0	46.6	100
I%	0	31.6	0
S%	100	21.6	0

TABLE 4.3: Percentage ratios of antibiotic drugs against E.coli

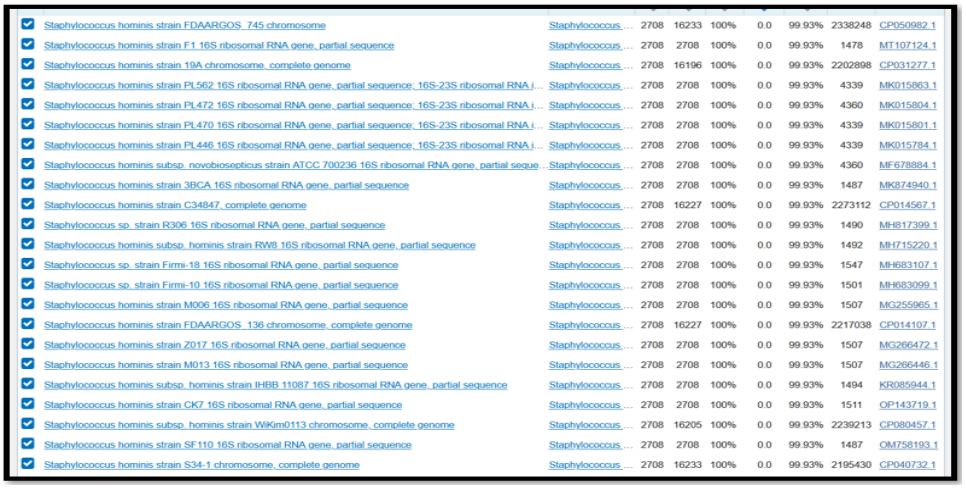
	Imipenem	**Augmentin**	**Ciprofloxin**	**Azithromycin**
Resistant	4	32	31	0
Intermediate	0	19	21	1
Sensitivity	56	9	8	59
R%	6.6	53.3	51.6	0
I%	0	31.6	35	1.6
S%	93.3	15	13.3	98.3

Consumers frequently use kitchen sponges for dishwashing and scouring pans and casseroles, but they can also be used to clean other kitchen surfaces including sinks, refrigerators, and stovetops. In Norway, brushes predominate among the tools used for manual dishwashing. According to an observational study conducted in the UK, 29%, 50%, and 77% of customers utilized brushes, sponges, and cloths, respectively, when doing the dishes.

4.11 Results of 16sRNA Sequencing

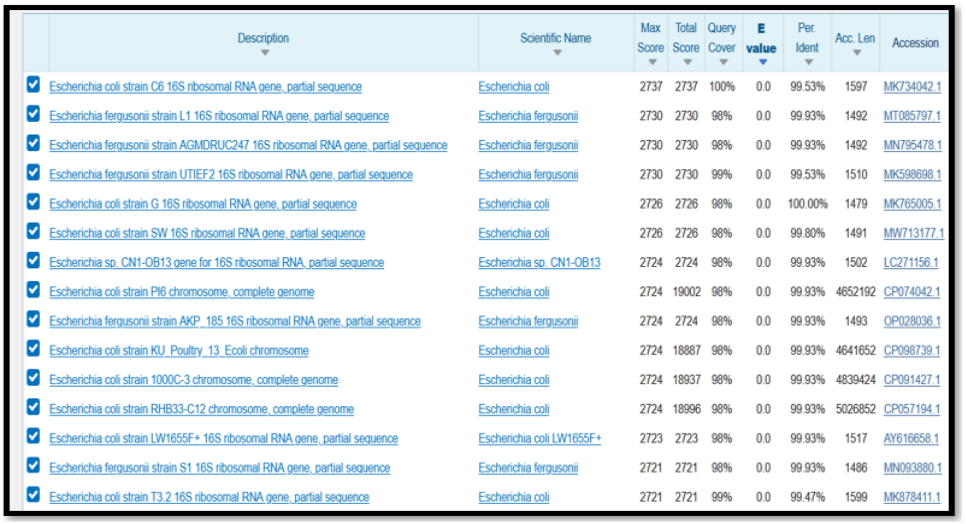
Sequence similarity was considered as to check the similarity of the sequences with the other reported sequences. For this purpose, the NCBI (National Center for Biotechnology Information) offers a Blast research engine which will use nucleotide sequences for the alignment and also provided the sequence similarity. This can be used for many analyses in the biological research field such as in phylogenetic

analysis, mutational analysis as well as in identification of the novel genes. In our case we provided the two sequences of bacterial species such as *E-coli* and *Staphylococcus hominis*. The result shows that *Staphylococcus hominis* has 100 % similarity whereas *E-coli* has 100 to 98% similarity with the other sequences that was reported in the NCBI and it was realized by seen the query coverage in figure 4.9 and 4.10.



Staphylococcus hominis strain FDAARGOS_745 chromosome	Staphylococcus ...	2708	16233	100%	0.0	99.93%	2338248	CP050982.1
Staphylococcus hominis strain F1 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1478	MT107124.1
Staphylococcus hominis strain 19A chromosome, complete genome	Staphylococcus ...	2708	16196	100%	0.0	99.93%	2202898	CP031277.1
Staphylococcus hominis strain PL562 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA i...	Staphylococcus ...	2708	2708	100%	0.0	99.93%	4339	MK015863.1
Staphylococcus hominis strain PL472 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA i...	Staphylococcus ...	2708	2708	100%	0.0	99.93%	4360	MK015804.1
Staphylococcus hominis strain PL470 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA i...	Staphylococcus ...	2708	2708	100%	0.0	99.93%	4339	MK015801.1
Staphylococcus hominis strain PL446 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA i...	Staphylococcus ...	2708	2708	100%	0.0	99.93%	4339	MK015784.1
Staphylococcus hominis subsp. novobiosepticus strain ATCC 700236 16S ribosomal RNA gene, partial seque...	Staphylococcus ...	2708	2708	100%	0.0	99.93%	4360	MF678884.1
Staphylococcus hominis strain 3BCA 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1487	MK874940.1
Staphylococcus hominis strain C34847, complete genome	Staphylococcus ...	2708	16227	100%	0.0	99.93%	2273112	CP014567.1
Staphylococcus sp. strain R306 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1490	MH817399.1
Staphylococcus hominis subsp. hominis strain RW8 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1492	MH715220.1
Staphylococcus sp. strain Firmi-18 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1547	MH683107.1
Staphylococcus sp. strain Firmi-10 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1501	MH683099.1
Staphylococcus hominis strain M006 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1507	MG255965.1
Staphylococcus hominis strain FDAARGOS_136 chromosome, complete genome	Staphylococcus ...	2708	16227	100%	0.0	99.93%	2217038	CP014107.1
Staphylococcus hominis strain Z017 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1507	MG266472.1
Staphylococcus hominis strain M013 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1507	MG266446.1
Staphylococcus hominis subsp. hominis strain IHBB 11087 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1494	KR085944.1
Staphylococcus hominis strain CK7 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1511	OP143719.1
Staphylococcus hominis subsp. hominis strain WiKim0113 chromosome, complete genome	Staphylococcus ...	2708	16205	100%	0.0	99.93%	2239213	CP080457.1
Staphylococcus hominis strain SF110 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2708	2708	100%	0.0	99.93%	1487	OM758193.1
Staphylococcus hominis strain S34-1 chromosome, complete genome	Staphylococcus ...	2708	16233	100%	0.0	99.93%	2195430	CP040732.1

FIGURE 4.9: *Staphylococcus hominis* sequence similarity.



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Escherichia coli strain C6 16S ribosomal RNA gene, partial sequence	Escherichia coli	2737	2737	100%	0.0	99.53%	1597	MK734042.1
Escherichia fergusonii strain L1 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	2730	2730	98%	0.0	99.93%	1492	MT085797.1
Escherichia fergusonii strain AGMDRUC247 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	2730	2730	98%	0.0	99.93%	1492	MN795478.1
Escherichia fergusonii strain UTIEF2 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	2730	2730	99%	0.0	99.53%	1510	MK598698.1
Escherichia coli strain G 16S ribosomal RNA gene, partial sequence	Escherichia coli	2726	2726	98%	0.0	100.00%	1479	MK765005.1
Escherichia coli strain SW 16S ribosomal RNA gene, partial sequence	Escherichia coli	2726	2726	98%	0.0	99.80%	1491	MW713177.1
Escherichia sp. CN1-OB13 gene for 16S ribosomal RNA, partial sequence	Escherichia sp. CN1-OB13	2724	2724	98%	0.0	99.93%	1502	LC271156.1
Escherichia coli strain PI6 chromosome, complete genome	Escherichia coli	2724	19002	98%	0.0	99.93%	4652192	CP074042.1
Escherichia fergusonii strain AKP_185 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	2724	2724	98%	0.0	99.93%	1493	OP028036.1
Escherichia coli strain KU_Poultry_13_Ecoli chromosome	Escherichia coli	2724	18887	98%	0.0	99.93%	4641652	CP098739.1
Escherichia coli strain 1000C-3 chromosome, complete genome	Escherichia coli	2724	18937	98%	0.0	99.93%	4839424	CP091427.1
Escherichia coli strain RHB33-C12 chromosome, complete genome	Escherichia coli	2724	18996	98%	0.0	99.93%	5026852	CP057194.1
Escherichia coli strain LW1655F+ 16S ribosomal RNA gene, partial sequence	Escherichia coli LW1655F+	2723	2723	98%	0.0	99.93%	1517	AY616658.1
Escherichia fergusonii strain S1 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	2721	2721	98%	0.0	99.93%	1486	MN093880.1
Escherichia coli strain T3.2 16S ribosomal RNA gene, partial sequence	Escherichia coli	2721	2721	99%	0.0	99.47%	1599	MK878411.1

FIGURE 4.10: *E.coli* sequence similarity.

So, from the above results it was identified that these two species of Pakistan have no novelty in them they were the same with the other reported species in NCBI.

Sponges can soak up a lot of liquids. After usage, sponges frequently remain moist, which can lead to the formation of germs. Rapid drying can prevent this growth

or even kill the bacteria. Salmonella is known to disappear in dry sponges but may reappear in moist kitchen towels. There isn't much evidence in the scientific literature connecting factors like sponge drying propensity and water intake to the development and survival of harmful bacteria. Brushes will probably absorb less water than sponges, but additional research is needed to determine how this difference impacts bacterial survival and development [79]. 14 utilized wipes recuperated in Germany were fundamentally pervaded by *Acinetobacter, Moraxella,* and *Chryseobacterium*, according to two previous investigations, while 20 sponges in another German research were dominated by *Acinetobacter, Enhydrobacter, Agrobacterium, Pseudomonas,* and *Chryseobacterium*.

We are not aware of any information on the variety of bacteria found in old kitchen brushes. It was discovered that *Serratia* and *Pseudomonas* were predominate in a prior laboratory examination utilizing new brushes and wipes, different microbes segregated from kitchen surfaces and kitchen materials, as well as *Salmonella* and *Campylobacter and* a food soil blend. High level of coliforms, Enterobacteriaceae, and oxygen consuming mesophilic microorganisms were found in kitchen wipe tests, which was proof of the second rate sterile nature of the apparatuses utilized for dealing with food. Food contamination episode regularly happen because of ill-advised food arrangement, some of the time including cross-defilement notwithstanding insufficient cooking or stockpiling. Since they can spread microorganisms to surfaces where they can make due for hours or days and taint food staying in these sickness vehicles, kitchen wipes and dishcloths might cause cross-pollution in kitchens Wipe defilement can result from food extras, unfortunate cleanliness procedures utilized during food arrangement, cross-defilement brought about by sullied surfaces, and capacity in specific areas.

These findings suggest that while raw food is most likely the primary source of contamination in the kitchen, the sink, garbage trap, and surroundings can also serve as reservoirs for a variety of microorganisms that host and support colonies of free-living bacteria and fungus. *Klebsiella, Enterobacter, Citrobacter, Proteus,* and *E. coli* were among the *Enterobacteriacae spp.* identified for the study. Other research also noted a tendency that was comparable. Even while these species

are often not harmful to an adult who is in good health, they must be taken into account as signs of uncleanliness. *Pseudomonas aeruginosa* and *S. aureus* were among the other isolated species. Approximately 47.4% of the 213 houses surveyed had *Listeria spp.*, which were recovered from moist areas such kitchen sinks, dishcloths, and cleaning brushes [112].

The refrigerator and the toothbrush separated *L. monocytogenes* from fridge surfaces in 2.2% of the homes. Additionally, 4.2% of households had *Yersinia enterocolitica* and 10.9% had Bacillus cereus isolated from the sink area. The availability and popularity of household cleaning solutions with and without antibacterial chemicals are both widespread. Although product marketers tout the goods' health advantages, there isn't any proof connecting the usage of antibacterial products to positive health results. Since kitchen sponges were shown to be possible carriers of germs in residential kitchens and since viruses were able to live in kitchen sponges for at least weeks, the danger of cross-contamination during routine household cleaning is crucial.

As per research done in ten kitchens in the US of America, 33 and 67% of the wipes tried positive for waste and Escherichia coliforms respectively. Further research revealed that 15.4% of sponge samples collected from homes had Salmonella spp. More exploration has uncovered that the microorganisms *Campylobacter spp., Enterobacter cloacae, E. coli, Klebsiella spp., Proteus spp., Salmonella spp., Acinetobacter, Moraxella,* and *Staphylococcus spp.* were available in kitchen wipes that were taken from home kitchens. Similar investigations have shown that due to inadequate kitchen sponge sanitization procedures, kitchen sponges harbor significant levels of aerobic mesophilic bacteria, coliforms, enterobacteriaceae, yeasts, and moulds.

The *Enterobacteriaceae* family of bacteria, collectively known as *Coliforms*, has been employed as an indicator group of microorganisms for hygienic surveillance.

The presence of coliforms in the kitchen is caused by improper cleaning techniques, poor sanitation, hygiene standards, and contamination from raw materials, and cross-contamination from tainted food. In a research on domestic cleanliness, drain

traps had the greatest coliform burden, while kitchen sponges came in second. Notwithstanding the significant pessimistic impacts that microbial sicknesses have on individuals' wellbeing, prosperity, and economies, the ascent of anti-microbial safe microorganisms represents a significant put to individuals' lives in danger in both developed and emerging countries.

Antibiotic-resistant Enterobacteriaceae have the potential to cause severe illnesses. The creation of ESBLs by Enterobacteriaceae, which results in multidrug resistance and the emergence of pan-resistant bacterial strains, is a significant cause for worry. Although they are increasingly common in the population, ESBL-encoding Enterobacteriaceae are often identified in healthcare facilities.

Restricted treatment choices would result from the far and wide recuperation of multidrug resistant Enterobacteriaceae from chicken in case of ailments [113].

The skin is an unfavourable environment for many bacteria because of its acidic pH, fatty acids, antimicrobial peptides, dryness, ongoing exposure to ultraviolet radiation, and other issues. On healthy skin, however, a wide variety of bacteria, archaea, fungi, and viruses survive and flourish. Coagulase-negative staphylococci (CoNS) are the most frequent bacterial skin colonisers and active participants in the cutaneous microenvironment.

Colonization resistance processes prevent opportunistic infections from infecting healthy skin by protecting skin appendages including hair follicles, glands, and epidermal and dermal tissues. To promote barrier function and stability, CoNS can modify how the epidermal barrier develops train or fine-tune the cutaneous immune response , and create a range of antimicrobial compounds . However, it is still unknown how complex and wide-ranging CoNS colonisation resistance mechanisms are [114]. Other commensal CoNS may protect the skin more than previously believed, according to recent research. Staphylococcus hominis is the second most typical isolation of CoNS from healthy human skin unlike *S. aureus, S. epidermidis, S. hominis* does not develop in AD lesions or skin colonisation. As a result of multiple research demonstrating the bactericidal substances generated by *S. hominis and aureus* strains may both actively support skin defence [115].

A member of the coagulase-negative staphylococci is *Staphylococcus hominis* (Co NS). CoNS include S. hominis is a frequent species found in clinical samples, often isolated from the axillae and glabrous skin of human arms, legs, and trunk . Similar to other CoNS, Although S.hominis does not often cause disease in humans, it is becoming more widely acknowledged as a potentially opportunistic and nosocomial bacteria that can occasionally infect individuals with abnormally weakened immune systems. Infective endocarditis and other potentially fatal illnesses have been linked to it in certain cases. There have also been reports of cases of *S. homoinis* endophthalmitis and capsular hypopyon [116].

As a frequent blood culture contaminant and a normal component of skin flora, *Staphylococcus hominis (S.hominis)* is also occasionally known to produce native valve endocarditis (NVE) with embolic events. Its dominance as a contaminant and less virulent characteristics compared to other, more typical infectious causes of endocarditis make *S.hominis* diagnostically problematic hominis infection. Damage to the heart's endocardium and subsequent colonization by an organism that attaches to the tissue, most frequently on damaged valves, results in infectious endocarditis [117].

Harmfulness factors divided between *E. coli* disconnects. *E. coli* diseases in creatures are exposed to different drug medicines including antimicrobials. For example, ampicillin, streptomycin, sulfonamides,or oxytetracyclines are usually used to treat ox-like mastitis, yet wide range cephalosporins and fluoroquinolones additionally have signs through fundamental or nearby organization relying upon the seriousness of the clinical side effects and the obstruction properties of the causative *E. coli* secludes. Regardless, the job of antimicrobials in the treatment of coliform mastitis is turning out to be increasingly more open to discuss [118]. Suggestions accommodated veterinarians allude to the ideal utilization of first-line antimicrobial specialists and evasion of antimicrobial treatment during the get dry time of dairy steers.

Worldwide information and patterns on the counter microbial obstruction of *E.coli* in mastitis have been featured in a few public reports and differ among nations

despite the fact that pertinent examinations are difficult. Until this point in time, the worldwide picture shows that antimicrobial vulnerability of *E. coli* in mastitis stays high. Specifically, broadened range β-lactamases (ESBLs) or overexpressed cephalosporinases (AmpCs) delivered by *E. coli* and presenting protection from expansive range cephalosporins have been irregularly segregated from milk samples [119]. Those families of antimicrobial agentsmay additionally be recommended in babies impacted by looseness of the bowels. Once more, activity plans against antimicrobial opposition in the creature area continually encourage veterinarians to utilize antimicrobial prudently and emphasize then consider any remaining preventive and restorative choices and restrict the use of antimicrobial agents to those situations where it is fundamental. For example, systems to forestall and treat neonatal the runs ought to incorporate not only the prescription of antimicrobial subtle so good colostrum the board practices to guarantee sufficient detached insusceptibility and proper oral or intravenous fluid treatment to make up for lack of hydration, acidosis, and electrolyte awkwardness [120].

Chapter 5

Conclusions and Recommendations

Kitchen pathogen plays significant role in people's health. These pathogens contribute in different foodborne diseases. There are many surfaces in kitchens which facilitate bacterial growth. Sponges, dish wash clothes, brushes and towels colonize bacterial strains which are involved in different foodborne diseases. Now it has become the matter of great concern that kitchen utensils might be a cause of food borne diseases. Many cases had been reported that suggest the poor hygienic conditions of the food handlers lead to food borne diseases. The first objective of this study was to isolate the microbes from the washing utensils of kitchen. For this purpose 100 samples of kitchen sponges were collected from the areas of Islamabad and grown on the nutrient agar. Bacterial growth was also found on differential media i;e MACConkey agar where gram negative bacteria were mostly observed, MSA characterize the *Staphylococcus spp* and EMB characterized the *Pseudomonas spp.* The second objective of my research was to biochemical characterization of isolated bacteria. For this four types of tests were performed i:e citrate test indole test, coagulase test and oxidase test. Citrate test was negative for *E.coli spp* and *Staphylococcus spp* while positive for *Pseudomonas spp.* Oxidase test was negative for *E.coli* and *Staphylococcus spp* while positive for *Pseudomonas spp* which was important.

Indole test was positive for *E.coli* and Negative for *Psedumonas* and *Staphylococcus spp*, whereas the coagulase test was shown negative for all of the isolated strains. Gram staining was also performed. In this *E.coli spp* and *Pseudomonas spp* were gram negative bacteria while Staphylococcus spp was gram positive bacteria.

As per third objective of this study 16S RNA sequencing of two strains was revealed i.e *E.coli* and *Staphylococcus hominis*. *E.coli* is a pathogenic bacteria that is responsible for the transmission of multiple infections as urinary tract infections, diarrhea, neonatal meningitis and Pneumonia.

S.hominis is the microbial fauna of the skin. It can be speculated that dishwashing with strong detergents can be harmful for the skin microbiota. On other hand it is also referred as opportunistic pathogen that can cause.

The fourth objective was to check the sensitivity of pathogens against drugs i.e Amikacin,Ceftazidime, Ceftriaxone.Imipenem,Azithromycin, Augmentin and Ciprofloxacin. These seven drugs were tested against *E.coli*. *E.coli* was highly sensitive against Amikacin, Imipenem and Azithromycin and resistant against Ceftriaxon.

From above mentioned study it can be concluded that kitchen sponges are highly contaminated and can be the risk of food borne diseases. Based on the significant use of sponge in kitchen and source of contamination, further study must be planned with the larger sample size. Same studies must also be planned for food cafeteria or restaurants as they are involved in transmission of food borne illness. Effect of dish washing detergent on skin and gut micro biome must be evaluated.

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Table 5.1: E.coli against antibiotics

E.coli						
Amikacin	**Ceftazidime**	**Ceftriaxone**	**Imipenem**	**Azithromycin**	**Augmentin**	**Ciprofloxacin**
24	16	9	25	27	7	17
21	9	11	26	23	11	13
25	14	14	24	28	17	13
18	7	9	24	23	8	21
18	16	16	31	18	11	11
24	24	6	25	25	13	22
25	10	11	26	30	9	17
20	19	6	27	18	13	15
19	15	10	24	22	7	9
24	17	15	30	21	11	19
20	19	15	34	27	17	15
28	14	18	32	28	15	14
26	17	18	29	23	18	15
20	17	21	24	30	7	11
32	16	23	28	31	13	17
19	9	19	27	22	15	15

Table 5.1: E.coli against antibiotics

E.coli						
29	22	6	24	29	11	13
21	13	11	28	27	15	17
30	17	15	33	30	17	9
23	9	10	24	26	19	11
25	10	10	24	35	9	18
27	15	17	28	27	13	21
25	15	22	25	23	19	23
22	13	11	27	25	11	17
30	17	19	13	28	15	11
19	22	9	32	19	13	15
23	15	17	33	20	17	19
19	9	11	24	17	14	21
34	13	11	27	32	18	13
23	17	15	27	21	13	17
21	13	17	26	21	15	19
21	17	13	12	25	12	14
24	17	19	25	21	15	15

Table 5.1: E.coli against antibiotics

E.coli						
22	18	9	23	22	21	18
17	24	14	25	22	12	19
19	22	17	31	19	12	16
26	19	10	24	25	11	17
19	13	13	27	22	18	11
19	8	17	26	24	17	13
20	18	13	12	19	10	20
26	18	9	26	24	6	19
22	13	22	27	27	0	16
26	14	18	31	22	14	15
22	9	12	25	24	18	9
17	9	7	24	22	10	9
23	13	9	25	23	19	13
29	7	15	24	29	16	16
22	10	19	26	23	13	12
21	13	9	23	22	16	12
28	17	7	25	27	15	15

Table 5.1: E.coli against antibiotics

E.coli						
21	8	21	28	24	15	23
28	25	14	26	21	9	13
18	17	19	26	30	14	17
26	10	17	29	22	11	19
30	13	22	32	28	11	21
24	25	18	10	24	18	15
30	10	13	26	21	7	21
19	17	25	25	27	13	19
21	17	25	26	29	17	13
26	11	13	28	32	11	9
Amikacin	Ceftazidime	Ceftriaxone	Imipenem	Azithromycin	Augmentin	Ciprofloxacin