Investigating Contamination of Edible seeds by *E.coli* and *Pseudomonas aeruginosa* and Genotype Profiling of the Outcome Obtained from Antibacterial Effectiveness Test in Bangladesh.

by

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of
Bachelor of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Investigating Contamination of Edible seeds by *E.coli* and *Pseudomonas aeruginosa* and Genotype Profiling of the Outcome Obtained from Antibacterial Effectiveness Test in Bangladesh" submitted by
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of Spring,2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor in Biotechnology on 02/01/25

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Ethics Statement

This study was conducted to maintain proper safety during the collection of samples. They were carried in a cleaned decontaminated bag and separate bags were carried for each different sample. Also all the procedure which was operated by maintaining proper lab precautions and no human or animal was injured during this process.

Abstract

Contamination is a major problem for food particles worldwide, especially for developing countries. This study was conducted to detect the occurrence of two common pathogens *E. coli* and *Pseudomonas aeruginosa,* in edible seeds. For analyzing, six categories of seeds were known for causing the outbreak. The purpose of this study was detecting any contamination occurring inside the seed sample and later the presence of ESBL producing gene was examined based on results. All these seeds are known for their high nutritional quality and they enhance the immune system by protecting the body from different diseases. This test procedure was initiated by collecting samples from five different places of Dhaka city and later they were washed with distilled water and soaked properly. For Culturing spreading method was utilized for bacteria detection and two agar media was used which are UTI agar. After performing DNA extraction and preparing PCR products they were examined on 2 % of agarose gel electrophoresis methods. For *E. coli,* 13.7% of the samples were positive, and the most positive sample category was flaxseed, and for *Pseudomonas aeruginosa,* 42% sample showed positive result and most positive category was sunflower seed by percentage most positive result obtained from sesame seed by quantity. AST analysis done for *E. coli* showed that the most resistant group was kanamycin, streptomycin, and ampicillin, which showed full resistance, and erythromycin, and the most sensitive group was ciprofloxacin (13.7%). For *Pseudomonas aeruginosa, the* most resistant discs were tazobactam, meropenem, and amikacin, and the most sensitive group was clavulanic acid, tigecycline, and nitrofurantoin. Finally, genotyping was done to find ESBL-producing genes, which were the bla *TEM1, bla NDM1,* and *bla* IMP_1 Gene .For *genes. ,* 7.8% (n=4) showed positive for *bla TEM1,* and all of them were flaxseed samples. For *Pseudomonas aeruginosa,* 2% (n=1) were positive for *bla* IMP_1 of sesame seed. Three of the isolates were positive for *bla NDM1,* and among them were sesame seeds, and two of them were sunflower seeds.

Keywords: Food. ,Nutrition ,Seeds, Agriculture , Microbiology , Bacteria, Outbreak .*E.coli* ,*Pseudomonas* , Gram Negative , Agar media , Fluorescence, Biofilm ,PCR Detection, Gel Run ,Antibiotic Susceptibility Test ,ESBL Gene ,

Dedication

Dedicating this to my parents

Rezaul Karim

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List of Acronyms

PCR- Polymerase chain reaction

E. coli *-Escherichia coli*

MHA*-Mueller–Hinton agar*

Bp- Base pair

NA *– Nutrient Agar*

DNA- Deoxyribonucleic acid

EPEC*-Enteropathogenic Escherichia coli*

EIEC*-Enteroinvasive Escherichia coli*

ETEC*- Enterotoxigenic Escherichia coli*

EHEC*- Enterohemorrhagic Escherichia coli*

bla– *Beta Lactatamase*

ESBL –External Spectrum Beta Lactamase

TEM *–Temoniera*

IMP *– Imipenem*

NDM *–New Delhi Metallo*

Min- Minute

Sec -Second

TAE- Tris-acetate-EDTA

Glossary

Multidrug-resistant (MDR) is acquired resistance to at least

one antimicrobial agent from three or more antimicrobial

groups.

PCR: Polymerase chain reaction is a type of laboratory technique for amplifying

millions to billions of copies of a attained section of DNA in a short

period.

Isolation: Bacterial isolation is amethod used for isolation of one species of

bacteria from a mixed culture of bacteria using various

plating methods such as pouring, spreading, streaking, and

serial dilution.

Pathogenicity: It is a rate of being pathogenic, or the propensity to cause illness, whereas virulence is the capacity of an organism to cause disease,or its degree of pathogenicity

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1. Introduction

Plants are an essential part for the survival of human, wildlife and environment and seeds are the main component of it. They are responsible for plant production through germination. Basically seeds are ovular parts which contain embryo covering with a protective layer.They are constructed by the ovule of the plant and remain dormant until they get proper condition for germination. Beside their crucial part in producing plants, some edible seeds are useful for gaining better nutrition and boosting the immune system in the human body. The consumption of edible seeds is very widespread due to its high fiber protein and mineral content which ensures a better digestive and metabolic system and reduces several diseases such as diabetic, ulcer ,high blood pressure etc. It also played a vital role in weight loss and controlling blood pressure by protecting cells against several oxidative damage (Link, 2024). The use of edible seeds in humans eating plan is not a recent thing. It has been used for ayurvedic purposes for many decades. The oil extracted from seeds is believed to fasten healing processes from any kind of wounds. Nevertheless, Every single particle consumed by human beings can be contaminated with different types of harmful microorganisms that may cause several serious death-causing diseases. Here, From poultry products to plant based food, the presence of hazardous bacteria is increasing day by day. The chances of contamination of edible seeds is high since they directly come in contact with soil and urban areas. It is no surprise that different type of aerobic and anaerobic bacteria remained in seed particle due to lack of awareness in bio-safety during harvesting and less amount of quality control measurement taken by farmers..Here, aerobic bacteria are those which can grow in the presence oxygen such as *E.coli, Actinobacter, Pseudomonas aeruginosa* which often found in soil, water and sewage line (Vorvick, 2020). Among anaerobic bacteria, *Salmonella, Staphylococcus, Clostridium, Botulism* are found most. (Bush &Pertejo,, 2023). This paper is focused on six basic edible seeds that have a huge impact on human health but recently contamination reports have been reported by both aerobic and anaerobic bacteria. The seeds which are covered in this paper are Flax seed, Pumpkin seed , Sesame seed, sunflower seed, Fennel seed and Chickpea seed and inspection was done to access whether they were contaminated with two most profound bacteria E.coli and Pseudomonas aeruginosa via various detection process. After these the susceptibility and resistance ability of seed were checked by AST analysis by which it can be stated that which antibiotic can be used for treatment of infection appeared from these two microorganisms. The gene detection was done further for portraying a better analysis of that resistant result. Two microorganisms that were selected which were both aerobic and they are well known for spreading various severe diseases. This bacteria was commonly found on soil among plants. it is essential to know their characteristics and shape to access better understanding about the bacteria.

1.1:E.coli

E.coli is a gram negative facultative anaerobe bacteria which can survive in the presence or absence of the oxygen. It was fist invented by Theodor *Escherich* isolated from the feces of a

baby. The structure of *E.coli* is mainly rod shaped and non speculating coli form which is normally found in the environment. It has superoxide dismutase that can turn superoxide anions into oxygen and H_2O_2 .Some of them contain catalase which has the ability to break down oxygen and water. Typically, the size of *E.coli* was 1-3 μm × 0.7 μm and its optimal condition to grow is 37°C indicates that they can grow at the normal temperature. They are non spore forming and can exist as both single and pairs (Basavaraju&Gunashree, 2022). Moreover, It can be discovered among warm blooded animals and specific portions of the environment such as soil, root and plant. Here ,around 150 -200 type *E.coli* serotypes could be found in an environment which depends on three antigen such as flagella antigen, capsular K antigen and somatic antigen structure. Between these three structure somatic antigens are most common and has virulence factor rather than other K and H structure.Typically it has lipo-polysaccharide on its surface which constructed by the O antigenic structure that can attack both innate and adaptive immune system According to the recent epidemiological study, several outbreaks from O antigenic structure *E.coli* has been recorded which cause gastrointestinal illness and several other infections (DebRoy et al., 2011). Different types of *E.coli* strains are found in the environment and most of them are harmless, Few of the *E.coli* pathotypes are harmful which can be found among soil and seeds and they are described below:

(a) ETEC

Enterotoxigenic Escherichia is capable of harming organs such as intestine and stomach through the passage of different strain of bacteria when individuals consume contaminated food. It causes diarrhea to underage children amid endemic areas and is also responsible for 100,000 deaths in 2015. Basically *ETEC* can produce enterotoxin and colonization factors which bind with specific receptors upon epithelial cells. They enhance the permeability of intestinal cell membrane to chloride ion that eventually cause leakage of chloride to intestine followed by sodium and water to move to well and diarrhea (Zhang et al., 2022).

(b)EPEC

Entero-pathogenic E.coli is a well known agent of diarrhea and it was also responsible for some of the early outbreak in the 1900s. It is capable of causing secretory diarrhea, low fever and anorexia etc. This organism proceeds by attaching to the intestinal mucosa and resulted termination of brush border by influencing the vesiculation of the microvilli. These process referred as attaching –effacement and cause leakage of water in intestine (Kaur & Dudeja, 2023) .

(c) *EHEC*

This pathotype is mainly known as shiga toxin producing bacteria which has the ability of damaging and lining the intestinal wall. The outbreak spread through *EHEC* from eating raw undercooked meat along with plants such as spinach, lettuce, sprout, apple etc.

In this subcontinent, intestinal diseases are most common which are caused by the contamination from different types of unwashed food. These mostly occurred from the *E.coli* bacteria but not all the strain of the *E.coli* is effective. The most harmful strain is known as *E.coli 0157:H7* which is shiga toxin producing bacteria and is responsible for diarrhea, hemolytic uretic syndrome and gastrointestinal infection among human beings. This strain of the bacteria was first isolated in 1982 and it caused several incidents of diarrhea, colitis among several patients. *E.coli 0157:H7* damages the alimentary tract and constructed abdominal cramps that cause hemorrhagic diarrhea. Firstly cattle was thought to be the main reservoir of this strain and water was considered the second reservoir for this strain as various incidents of waterborne infection were reported earlier through drinking water , lake ,pool etc (Fatima, 2023).

1.2 Virulence Rate and pathogenicity

Earlier, this particular strain of *E.coli* caused 2.8 million infection cases per year .It is also responsible for causing 63,000 colitis yearly in the USA. Over two decades *E.coli 0157:H7* is accountable for causing renal failure and diarrhea among children which is one of the main deaths causing infections around south asia. For these, this paper is focused on this strain of *E.coli*. A condition named vacuities which is impairment of blood vessels through inflammation caused by *E.coli 0157* which may result in organ damage, blockage off arteries if not treated well. It may also start an inflammatory cascade that results in aggregation of blood components.

Mechanism

This strain resulted from shiga toxin that causes damage to intestine and disposes of intestine mucosa which ultimately result in hemorrhagic diarrhea. At first, they bind with microvilli of the intestinal epithelial cell. The attachment of the bacteria is believed to cause effacing lesions and flatten the microvilli. Furthermore, re-shaping of cytoskeleton actin occurred under epithelial cell that ultimately cause disrupted cell function. Along with these, large hemorrhage and edema type function of the intestinal inflammation this strain attached with the intestine mucosa after consuming food containing *E.coli* which released harmful shiga toxin. When shiga toxin got released, they initiated the disarrangement of the protein synthesis among the epithelial cell line of intestine mucosa and finally resulted in cell death. Later they dispose of mucosa which ultimately causes diarrhea. Furthermore, a study found that after three days of ingestion and came in contact with the contaminated food, 90% of the patients showed bloody diarrhea and hemorrhagic diarrhea appeared after seven days. In most of the cases fatality would not occur, but if the right antibiotic did not apply on the right time then the patient might die. In the lab this bacteria was detected using culture methods in agar media plates. Also they can be detected using liquid broth technique (Ameer, 2023).

Outbreak of *E.coli*

In recent years shiga toxin producing *E.coli* has been a major source of outbreak in many countries . It is the origin of an outbreak among various foods such as seeds, sprouts, vegetables,

fruit etc. The contamination took place due to several reasons. Firstly, food might be polluted by coming in contact with the fecal oral pathway which was carrying *STEC*. Secondly, several foods might be contaminated during the handling of crops and production of the food by an individual infected with *STEC*. Several outbreaks have been reported which were caused by contaminated irrigation systems. In 2005, *STEC 0157-H7* cases were recorded in Sweden in lettuce vegetable which got contaminated by the water of irrigation by the cattle feces. (Soderstrom et a1., 2005) .In 2006 a study showed that contamination of spinach occurred due to the polluted irrigated water(Gelting et aI., 2011). During the infection, individuals might face gastrointestinal sickness, and diarrhea. An outbreak was reported in 2001 in Germany due to *STEC* infection and total 10 persons were infected among EU member states from 2004 – 2009 .Another outbreak of Germany showed that the main source of the outbreak were raw vegetable such as tomato, cucumber and leafy salads. The strain which was responsible for outbreaks was *0157-H7* belongs to the group D and group other group such as B1 and A was associated with the non *0157-H7.*The outbreak which happened around 2011 cause gastroenteritis and HUS that originated by serotype *0104-H4* and *MLST ST 678* belongs to the clonal group of B1. The outbreak of Germany which was responsible by *O104* : *H4* serotype and their gene characterize as :

Shiga Toxin 1 negative

Shiga -toxin 1 Positive

Intimin Negative

aatApositive andaggR positive

Infection of *E.coli* from plant based food has become more common since the 2000s and more outbreaks were reported due to the seed, sprout and vegetable consumption. Around 2004-2009 around 14 members were infected with *STEC* in fruit vegetable type products. Another research paper revealed that 5910 samples of different categories were tested. Among them, most of them were fruit and vegetables and 8 samples were found positive of *STEC 0157-H7*. Another paper revealed that sprouts and seed products were likely to be contaminated with seeds (Cc et al., 2022).

According to the FDA, several outbreak causes have occurred among them, In Missouri Iowa Texas and Illinois in USA, 14 individuals have been infected by the *E.coli 0103* via contaminated clover sprouts. In 2020, several incidents of infection by *E.coli* were reported and this occurred by the consumption of sunflower seed in the five states of the USA. Four of the infected person were hospitalized and one individual has kidney failure . Between 2004-2006 , a report showed that Denmark had an Outbreak of *E.coli* in herb which caused infection of 250 people that occurred in school. (*2019 E. Coli Outbreak Linked to Fresh Express Sunflower Crisp*

Chopped Salad Kits | E. Coli Infections Linked to Fresh Express Sunflower Crisp Chopped Salad Kits | December 2019 | E. Coli | CDC,).

1.3 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a species of bacteria that belongs to the family of *pseudomonadaceae* that usually grow in the presence of oxygen. These bacteria are gram negative and rod shaped which has endo-spore formation and exhibit positive result in catalase test. The length of this organism is about 1.5 -3 µl and around 0.8 µl long. They can exist both in extreme cold temperature and warm temperature which is why it also known as mesophile. Throughout the history, this species has several different name . At first Charle Gessard observed that this microorganism was responsible for several infection and referred as *Bacillus pyocyaneus*.Later in 1894, Migula suggested that it was a species named *Pseudomonas aeruginosa.* The adaptive ability to grow in both harsh and warm temperature makes it more prone to contaminate a wide variety of products such as , fresh water, salt water , soil and by this components, it can contaminate several food products such as fruits, vegetable, seed, dairy product. The species *Pseudomonas aeruginosa* is motile that indicated that it is capable of moving along with a single polar flagella. Basically the cells of *Pseudomonas aeruginosa* contain single cell with short chains. Usually they appear as blue in front of fluorescence due to its pigment which glows in the presence of fluorescent light. The culture of this organism turns brown when they age. They do not have the ability to ferment but they can oxidize glucose rapidly. In addition, it synthesizes several types of vitamins which are related with the infection to humans. *Pseudomonas aeruginosa* is mostly known as obligate aerobes as they can live without oxygen due to its ability to make electron acceptor and nitrate. These organisms can grow rapidly within the pH of 5.6 -8 as they do not require any organic growth factor. They could be isolated in the culture media where they exhibit various types of colonies. For clinical samples, they showed 2-3 mm of colonies and for sample from natural source colonies usually appeared as small and fluffy. To exhibit blue green colonies in the presence of fluorescence, *Pseudomonas aeruginosa* have pigments named pyomelanin and pyorubin which control the formation of pyocyanin. The formation pyoverdine is required to form the fluorescence.Like many other bacteria, they have their own defense mechanism which is done by the antimicrobial agent named pyocins which is responsible for both attack of the bacteria and defense of the bacteria from the ecological niche. Several factors responsible for the virulent ability of *pseudomonas* described below:

Virulence Factor

The potential of causing damage to the host cell operated by some virulence factor and it determined the ability of this organism to be pathogenic. Here this organism proceeds by a different method for invasion. Firstly, virulence factors released and increased in logarithmic phase and later their cell density increased, After the release of this factors, first step was

colonization and later local invasion occurred by multiplication of bacteria that ultimately resulted infection. Among them it has several type of virulence factor such as flagella, pili that attached to cells and cause adhesion which later multiply instantly. In some cases, Outer membrane vesicles produce virulence factor that causes colonization of bacteria and attacking of immune system and runoff from host immune cells. In other cases, they produce toxins that have the ability to stop protein synthesis among animal and human beings (Urgancı et al., 2022).

Pathogenicity

Pseudomonas aeruginosa is also known as an opportunistic pathogen as it normally does not harm individuals with adequate immune systems but it can be fatal for the people with immunosuppressive diseases. It is accountable for causing infection like folliculitis that leads to severe disease such as pneumonia, otitis, osteomyelitis. The infection from these organism occurred from invasion to the individual with immunosuppressive diseases such as cystic fibrosis cancer ,AIDS, bronchiectasis, organ transplant etc. These bacteria has the ability to form unique type of biofilm that is very hard for detection (Wilson, 2023). The formation of hazardous biofilm is responsible for the 65%-80% of the nosocomial infection. They may cause critical acute and chronic infection in blood stream, soft tissue ,respiratory tract and ultimately cause life threatening disease like meningitis, otitis media and urinary tract infection. This formation of biofilm structure consist of extracellular polymeric substance act as a scaffold for wrapping upon the surface of the bacteria that shield them from the outer pressure. It consist of biofilm, they became less likely to be susceptible to the antibiotics.The organism with this biofilm formation proceeds by attaching the suitable host and then initiated the formation of the micro colonies . Along with these, *Pseudomonas aeruginosa* can be present in different types of food products such as poultry, processed food, fruit , vegetable, edible seed etc. Raw vegetables such as lettuce, spinach, tomatoes, and carrots contain this organism. Several types of edible seeds such as sesame seed , pumpkin seed, tomato seed carry this organism (Li et al., 2022) .

This bacteria might appear as wound, pruritic rash, fever and folliculitis as it is very prone to cause damage to the patient with the cysticfibrosis. It has been prevailed that chronic infection occurred among 60 % of patients who already have cystic fibrosis by this organism. Along with these, this could be the reason for puncture wounds in the foot which might lead to bigger infections. Moreover, It is very known that patients with diabetic have weaker immune systems which may also lead to the infection by this bacteria. Among diabetic patients, foot ulcer is a common infection which leads to hospitalization. This can be treated using several antibiotics .Mostly ciprofloxacin and tazobactam and gentamicin used to treat this bacteria (Shree et al., 2023).

Outbreak Related to *Pseudomonas*

Pseudomonas aeruginosa is one of the main reason for contaminating food source such as vegetables, water rice and many others food particle. *Pseudomonas aeruginosa* has unique

characteristics such as high metabolic versatility, high amount of reproductive and adaptive capacity along with frequent growth ability in the low temperature which makes it very easy for these microorganism to grow in the harsh condition. According to the CDC this bacteria can be the number one source for osteochondritis and second most causative agent for nosocomial pneumonia. Along with these, several other infections such as surgical site infection, fungal isolate infection could appear due to this microorganism. It can be fatal for other infections such as sepsis ,burn wound infection are related with *Pseudomonas aeruginos* infection and cause up to 50% of the fatality when individuals are infected with the melioidosis blood stream infection and untreated glanders. Furthermore, they are responsible for ventilation associated with pneumonia and it can be really dangerous for the infected patient as it can cause up to 68 % of the fatality rate due to VAP. The disease occurred due to *Pseudomonas aeruginosa* which can infect both adult and younger children. For example, endocarditis infects the black man and infants who are younger than 1 year might have osteomyelitis (Mph, n.d.).

1.4 :Pathway of Contamination for *E.coli* and *Pseudomonas aeruginosa*

Bacterial contamination in seeds and vegetables might occur in both surface and internal way . They invade plants by the method of attachment, adhesion and colonization process. In another way they invade food products by the internalization method which mostly happens to the leaf and roots of the vegetables and seedlings. Nevertheless *E.coli* damages seeds by the adhesion and biofilm formation. It can survive very long which makes it more harmful bacteria . In a literature, it has been found that *STEC 0157: H7* can stay for 177 days upon the plants when sprayed directly upon the parsley(Solomon et aI., 2003). In south Asia, contamination during pre harvesting time is one of the main problems. Most of the time, no attempts were taken to eliminate either infectious agent from or carrier animal. Also the practice of using disinfecting agents in the field is not very common which is why the inoculation of harmful microorganisms in the field resulted in *STEC* formation in the seeds. Most of the time, the roots and other harmful waste remaining in the field could result in *E.coli* formation in the food when cultivation is initiated. Furthermore, the retailer who sold the product often kept them openly without covering the seeds properly with hood and often they were sold by the bare hands which is another reason contamination by *E.coli*. During harvesting seeds might get contaminated if the fields are not properly maintained. Irrigation is not properly handled in this region and most of the time cattle farms are very close to the field which make it more likely to be contaminated via cattle fecal when they come in contact with the soil during sowing seeds. During processing, *E.coli* can survive and grow well in the normal temperature and they proliferate without any problem . It is already known that quality control is a major issue in developing countries and the rate of contamination has increased a lot recently for poor handling of the food product. Throughout the

time of cutting or packaging, many of the stores are situated in a place with higher humidity which is also another issue for high number contamination among seeds and sprout.

Like other organism, *Pseudomonas aeruginosa* could also occurred during the process of cultivation. It is very common to use organic manure such as abattoir, waste ,excreta, slurries during cultivation which might be a leading reason for spreading *Pseudomonas aeruginosa* among seeds. Different type of pathogens may reach to the edible seeds by manure during cultivation. For these, minimum manure should be used which will prevent the seeds from exposing to huge quantity of bacteria. Furthermore, Many of the cultivators use plastic pipe for transporting the water through the field which might act as a route for contamination. *Pseudomonas aeruginosa* has unique quality of forming biofilm that can attach to the wall and colonized in both warm and cold temperature. So the pipe might be contaminated if not cleaned properly and later the seeds and other type of crops will be contaminated easily during the time of the cultivation by irrigation water. Several other factor can also played a crucial such as the temperature and rate of natural disaster moisture, air quality etc. During the time of drought contaminated water might spread several harmful microorganisms to the field. The groundwater is relatively good but when it comes in contact with surface runoff, there is a chance of contamination which is harmful for the seeds. Additionally, Many of the cultivation fields are near river and ponds, that can be a major source for the contamination. Sometimes, many ponds were used for shower purposes, and throwing wastes which can be mixed with the irrigation system of fields. In some developing countries sewage line are often connected with the irrigation system of the field by which the cultivated product can be contaminated. For reducing the contamination level it is necessary to avoid all the fact that cause exposure of unwanted bacteria in the field. For these, the system of cultivation, irrigation and condition of soil should be checked and maintained. The following diagram showed how different sources of field can contaminate the food product (Alegbeleye et al., 2018).

1.5 : ESBL gene

ESBL is the short term for Extended Spectrum Beta lactamase that exhibited resistance to large groups of antibiotics including beta lactamase. Beta lactamaseare is widely known enzyme which cause disruption of the function of antibiotics and make it ineffective. This enzyme immobilized the activity of antibiotics by hydrolyzing the peptide bond of beta lactamase ring (Majiduddin et al., 2002). Here, Beta lactam played a crucial role in the structure of antibiotics. It is a four member structure containing three carbon atoms and one nitrogen atom along with a carbonyl group. This carbonyl group in the ring gives a better attacking site for the hydrolysis and the structural region of the beta lactam provides high reactivity to the antibiotics for disabling the penicillin binding protein (Kim et al., 2023). Basically this ring helped to inhibit the last stage of peptidoglycan synthesis by acylating the trans-peptidase and ultimately disrupted the formation of peptidoglycan. The resistance caused by the ESBL genes are prone to cause morbidity and longer time of sickness among patients. Due to the overuse of the antibiotic in recent days , the effectiveness of antibiotic has been decreased and occurrence the drug resistant gene increased rapidly. ESBL producing genes showed resistant against some specific popular antibiotics, among them most gene expressed resistant towards penicillin group of antibiotic such as ampicillin and amoxicillin. Carbopenem is one of the most known drugs used for treating infection from enterobacteriaceae for a long time. But the prevalence of Carbopenem resistant gene hindered the choice of considering these as an effective antibiotic. Several resistant genes disrupt and lower the effectiveness of antibiotics are basically transmitted through the horizontal gene transfer method via plasmid. Usually ESBL are known as hydrolyzed enzymes produced from the gram negative bacteria of the *Enterobacteriaceae* and genes which encode these often found upon insertion sequence of plasmid and transposon. For these, they spread more rapidly and showed resistance towards multiple antibiotics. ESBL resistant gene mainly spread from gram negative bacteria such as *E.coli, Pseudomonas aeruginosa Klebsiella ,Enterobacter* Spp etc. These are known bacteria that cause several deadly infection resistant genes and are also spreading very fast among developing countries. It has been estimated that over 1.5million people has been colonized with ESBL producing enterobacteriaceae in developing countries and resistant gene might increase the difficulty to treat the patients. The ESBL encoding genes are very diverse and they can be classified as several family along with different characteristics such as *bla-TEM*, *bla IMP*, *bla NDM, bla SHV* and each of these resistant gene resist specific group of antibiotic. In this paper three of the genes has been used and those are *bla TEM*, *bla IMP* , *bla NDM*. Among these, *bla TEM* is resistant for penicillin group*, bla IMP* is resistant for Imipenem and *bla NDM* is resistant gene for Carbopenem group and many other group (Kim et al., 2023) (Pandey, 2023).

bla TEM 1

Among different classes of ESBL gene, *bla TEM* is one of the most resistant and earliest discovered genes. It was discovered in 1960 in a hospital and capable of hydrolyzing the penicillin and cephalosporin antibiotic group. Different classes of *blaTEM* are available among them, *TEM* was the first discovered ESBL gene that resisted cephalosporin along with third generation antibiotic such as clavulanic acid .Till now, there are 243 variants of *TEM* and 228 variants of *bla SHV* has been discovered. This *blaTEM* can be diverse based on their characteristics although some of the classes differ from each other based on the mutation. They showed point mutation variants of both *TEM1* and *TEM 2* and there are few classes of ESBL which have been found and carry common biochemical property which is *Beta lactam*. It discovered originally from *E.coli* from a blood sample in Greece. Both *TEM 1* and *TEM 2* have single amino acid substitution Gln39Lys from the original *TEM* beta lactamase. *TEM 2* acts as a progenitor for the *TEM* type ESBL. The substitution of the *TEM* gene occurred for a limited number of positions. The most frequently used amino acid residue are Gly238 and GLy240 which situated on the beta-pleated sheet. The role of two substitute named Gly238Ser and Gly240 Lys is very important as they played a crucial role for the production of ESBL phenotype. Furthermore, most of them showed similar resistance towards antibiotic group of penicillin and cephalosporin but few of the new variants showed resistance to several other group. They displayed better resistance to ceftazidime and cefotaxime than the aztreonam group To discover new variants, WGS are mostly used but sometimes they were also discovered by phenotypic character. Different variants of *TEM* found among different countries. For example *TEM3* was founded in France, the variants of TEM_{10} were found in the USA and TEM_{26} found all over the world.

ESBL producing genes operated by some group of components named mobile genetic element which includes transposon, insertion sequence integrons and they dissemination of several ESBL genes. This process helped to move genes from one place to another with the cells horizontally via conjugation or transmission process .Various types of transposon proceeds to carry the genes Among them Tn_1 Tn_2 Tn_3 helped to move *TEM1* and *TEM2* gene. It showed mobile genetic elements carrying *bla TEM* encoding ESBL genes. All of them were surrounded by plasmid . The *bla TEM* genes are mostly found on *Enterobacteriaceae* and they could also found in *Pseudomonas aeruginosa* , In a study in Dutch revealed that among 1528 *Pseudomonas* isolates 6 of them were bla encoding gene . Among them were *bla* TEM_{12}, 2 of them were *bla* VEB_3 and other two of them were *bla* $bell_2$ (Castanheira et al., 2021).

bla IMP_1

Different groups of beta lactamase enzymes have been recorded over time that caused high mortality, morbidity and public implication for several decades .The rate of antibiotic resistant bacteria is increasing day by day. In the USA, around 2 million people are infected with resistant bacteria which are caused by resistant genes. Already more than 7270 enzymes of the beta

lactam group have been discovered. Among them, *bla* $_{IMP}$ gene is well known gene that encodes imipenem ase enzyme along with other sub group such as *Verona Integron Metallo beta lactamase (VIM) German Imipenemase (GIM)*. This *IMP* enzyme included into beta lactamase along with carbopenemase enzyme. Recently 88 variants of *IMP* were documented. It was mostly transferred between gram negative bacteria and discovered first in 1980 from *Pseudomonas aeruginosa* bacteria from the strain named GN17203.The gene was mainly found in the Asia region. For instance, the countries which reported the prevalence of this gene are mostly Asian and 69% of this reported gene are found in countries such as Malaysia , Singapore ,Thailand ,Turkey and Vietnam . Furthermore, 36 % of this gene was found in Japan and 25% in China . It was most frequently and consistently discovered in Japan and Singapore according to the reports which showed It was commonly found in *Pseudomonas aeruginosa* along with other gram negative bacteria named *Actinobacter baumanii*, *Klebsiella pneumonia* (Pongchaikul&Mongkolsuk, 2022). The main reason for showing bacterial resistance is that antibiotic resistant genes operate in bacteria through plasmid exchange at the gene level that build up resistance to antibiotic like *bla Tem* like other genes, it was preceded by the plasmid such transposonintegron that functioned by the horizontal gene transfer and showed resistance as long as the gene remained. It has been noticed that, after the death of the resistant strain, sometimes DNA which carries antibiotic resistant genes can stay in the environment via deoxynucleotide enzymes. Antibiotic resistant genes make bacteria resistant by three mechanisms Firstly, they alter the target site of the bacteria wall which makes antibiotics hard to bind and proceed. Secondly they can hydrolyze and modify the antibiotic which makes them inactive .Thirdly, it can change the penicillin binding protein which causes antibiotics to fail to bind (Jian et al., 2021). It is a class B metallo *beta lactamase* that are not normally inhibited by the inhibitor such as tazobactam ,clavulanic acid disc. The enzyme of *bla* $_{IMP}$ encoded a gene cassette by occupying with resistant gene along with integron and transposon . These are finally inserted in the plasmid. The integron played a crucial role as they control the movement of the resistant gene and plasmid are very necessary which transfer the genetic material to the different bacteria and ultimately these bacteria showed resistance (Palzkill, 2012).

Bla $_{NDM}$

Carbapenem antibiotics are one of the popularly used antibiotic to treat several infection related with the gram negative bacteria. Mostly they are used to treat bacteria; group of ESKAP (*E.coli, Staphylococcus aureus, Klebsiella pneumonia, Actinobacter baumanii*, *Pseudomonas aeruginosa ,Enterobacter spp*) among all over the world. They are one of the most used antibiotics worldwide which are also known as Beta lactam antibiotics. Here carbapenemase is an enzyme that breaks down beta lactam antibiotics to make them nonfunctional. They have the ability to hydrolyze several antibiotic groups such as penicillin, cephalosporin. These carbapenemase are serine based enzyme that depends on serine residue to their active site for hydrolyzing beta lactam antibiotic (Aurilio et al., 2022). Furthermore, *bla NDM* is most recently found gene and

their enzyme is *NDM*. It was reported first in 2009 in a hospital of New Delhi in India whereas isolate was collected from *Klebsiella pneumonia* from a patient of Sweden who was travelling in India. This resistant gene can hydrolyze all the *beta lactam* except aztreonam. From its founding moment till now, there are 13 variants emerged from site mutation surrounded by the gene that encodes beta lactamase. Particularly, *NDM 1* is the most common variant yet and other variants such as *NDM 2, NDM3* have similarity found with it. It was found by several studies that these genes have enough capable genetic material for transferring gene encoding *NDM 1*. These genes are found in various countries such as India ,Pakistan ,Australia ,Nederland, UK , Middle East ,Guatemala (Mohapatra, 2013). It has genes that can inactivate several groups of antibiotics such as erythromycin, ciprofloxacin chloramphenicol. *bla ndm1* has an open reading frame that encodes 269 amino acids and shows horizontal gene transfer. According to a study, they consist of three dimensionalstructure with 3 helix and 7 beta strands and their core contain two beta sheets. Among them, one is based on seven anti parallel strands and the other one consists of five antiparallel strand. Their seven connecting strand are situated above the Beta sandwich. Their hydrogen interaction among helices and beta sheets involves several hydrogen bond (Saini & Bansal, 2012). This *bla NDM* 1 belongs to the subclass of B1 enzyme and they have two zinc ion on their active site . In *NDM 1*, Interaction occurred between the substrate by zinc ion that located on the active site. The invasion occurred when this zinc ion turned in a water molecule that gives proton for generating a new hydroxide which attack the carbon atom of the carbonyl group of *beta lactam* ring for hydrolyzing. It already caused several disease over the world . Occurrence of *bla NDM* $_1$ was spotted in a patient of Canada who recently traveled India and infected with *Pseudomonas* aeruginosa and the strain was ST654. According to survey, It was revealed that *bla NDM* $_1$ was the third most common carbopenemase encoding gene which was accountable for the 19% of the positivity .In addition 18.5% of the patient admitted on Pakistan military hospital with *Enterobacteriaceae* contain *blaNDM* $_1$ positive. (Wu et al., 2019)

1.6:Edible Seeds and Their Characteristics

Seeds are a good source of nutrition and consumed by humans for many years. It is very popular among different parts of the world and utilized for purposes such as cooking, drinking, oil production, medicinal etc. For these study, Six types of different seeds such as flaxseed, sesame seed, sunflower seed, chickpea seed, fennel seed and pumpkin seeds which carry various benefits . Their characteristic and nutrias level of different seeds described below:

Flax Seed

Flaxseed is one of the most ancient seed products that was cultivated in ancient Egypt and China for medical ayurvedic purpose .It is very well known and vastly used for edible drinks and it is very effective for reducing diabetics, constipation, heart disease ,cholesterol etc. It is very nutritional and healthy which is why use of this seed is increasing for various approaches such as cooking, dieting, skin care etc (Brazier, 2023). It was first cultivated in USA for producing fiber

of clothing and paper. This seed is filled with rich source of nutrition component such as protein, lipid , carbohydrate, essential fatty acid such as alpha linolenic acid and antioxidants . Typically flax seed is found in two forms : brown and golden. Notably , both of this flax seed is composed of omega 3 fatty acid , 18% of monounsaturated fatty acid and 73% of polyunsaturated fatty acid. They carried high amount antioxidant property such as tocopherol and beta carotene. Also they are very rich in protein and carry 80% of globulin, and 20% of the glutelin. Furthermore, High diabetic mellitus is caused by the trouble in metabolism of carbohydrate, protein and it Is accountable for 1.3 million of deaths in 2008. On this regard, flax seed can significantly reduce the risk of diabetic because it has component like omega 3 fatty acid and lignin present in flaxseed can also reduce the chances of cancer. It also can reduce the risk of colon formation which is helpful to prevent tumor. Now it has a lot of benefits but recent data shows several bacteria has been found in flaxseed that ultimately cause infection illness. Study showed that total 121 people has been infected with various *Salmonella* linked infection (Hylton et al., 2019) 31. In 2014 flax and chia see were cause food borne illness in Canada and 63 cases of sickness. Along with these, 31 cases in USA. Also, in 2016, they were recalled due to the possible *salmonella* contamination).

Sesame Seed

Sesame is another type of edible seed and its scientific name is sesamum indicum which is one of the earliest human produced crops. It is used for various purposes such as cooking and dietary oil production etc. It has been used for more than 5000 years and mostly found in Pakistan , India, China, Sudan, Tanzania . These seeds are very well recognized for its significant quality of sweet fruity odor and huge oil quantity available in this seed. Like other seeds, it is also filled with various type of nutrient such as protein, lipid, carbohydrate,vitamin mineral etc. They are also composed of high quantity of protein such as globulin , glutenin , alcoholic protein in vitro protein which enhance the digestibility. Moreover, they have good quantity of lipid, vitamin E , carbohydrate which makes it a better supplements and essential item that can be helpful for protecting kidney and liver, maintaining antioxidant balance and protecting cardiovascular system (Wei et al., 2022). However several outbreaks have been reported which are related to sesame seed products in recent times. In Canada outbreaks occurred from a sesame product named tahini which was imported from Israel (*Salmonellosis Outbreak Linked to Imported Sesame-based Products*, 2021). This is mainly caused by bacteria called *Salmonella* from different periods of 2011 to 2021. In 2019 eight Canadian cases were reported related to these products followed by six cases in USA Between 2016 -2017 multiple cases have been reported related with sesame seed and tahini consumption and total 47 cases were reported among different European countries such as UK ,Greece , germany,luxembourg . (Coulombe & Tamber, 2022).

Pumpkin Seed

They are highly nutritious edible seeds filled with different types of nutrients such as protein , fat, fiber, minerals and vitamins. The unsaturated fat present in pumpkin seed makes it very feasible for treating conditions such as urinary tract infection. kidney stone high blood pressure. Along with these, it has a high antioxidant component such as flavonoid, phenolic acid which is very helpful for decreasing inflammation and fighting many diseases. They also contain essential components that are very helpful to reduce BPH which is a condition that causes enlargement of bladder and it can also be very helpful for problems such as overactive bladder (King, 2024). In 2016, Oregon state in the USA declared possible way of contamination for pumpkin seed by stating that it may be a possible source of contamination for *Listeria*.The contamination may occur through microorganism such as *E.coli* and *Salmonella* (Center for Food Safety and Applied Nutrition, 2020).

Sunflower seed

Like other edible seeds sunflower seeds are also very healthy and beneficial which are used in both drinking and cooking purposes. These seeds appeared from the centre of the sunflower and it can be found in both black shell seed and white striped version. It is very beneficial for increasing the immune system, reducing blood pressure and diabetics and it can decrease the chance of fatty liver disease in women . It also enhances the red blood cell formation along with widening the blood vessel. Along with this, seed has a mineral named antioxidant that eliminates the cellular damage in the human body. Moreover, It has an essential zinc component that can help to increase sense taste, wound healing and many more . Despite all its nutritional quality, It is the source of possible food borne illness. Several bacteria such as *E.coli, Salmonella*, *Listeria*, and *Pseudomonas aeruginosa* could spread from this material .Study showed that it can be a major source for the aflatoxin and mold production(McCulloch, 2024). Furthermore,Recently in 2018 an outbreak has occurred related to the sunflower seed consumption in the USA which is caused by *Listeria Monocytogenes* (Jeschke, 2024).

Chickpea Seed

It has different names that vary from culture to culture . Garbanzo bean , Bengali gram Egyptian pea are few of its names. It's mainly a middle eastern food and considered as healthy dry food. They are compact with several nutrition components such as vitamins , calories , protein fat etc .This food can be helpful for reducing hunger due to its protein and fiber that act as a slow digestion food. It has minerals such as magnesium that are essential to prevent heart disease. Some articles showed that it can be very accommodating for reducing cancer as it has legumes. Nevertheless, It can also be a great source of food-born infection by several harmful microorganisms. a report showed that they can be contaminated by *E.coli* and *Salmonella enterica* (Elliott, 2023).

Fennel Seed

Among edible seeds, it is very popular for its nice aroma and often used for medicinal herbal purposes for thousands of years. It compacts with necessary nutrients that makes it unique and useful for digestion .Fennel has an important component named galactagogue which is very helpful for the women for lactation and enhancing breast milk supply. It has an anti-inflammatory element that makes it more prominent in overcoming digestive disorder. It can also be essential to reduce inflammation. In addition, bacteria can spread these seeds. In Germany two cases of *Salmonella senftenberg* were reported in 2007 (Lubeck, 2024).

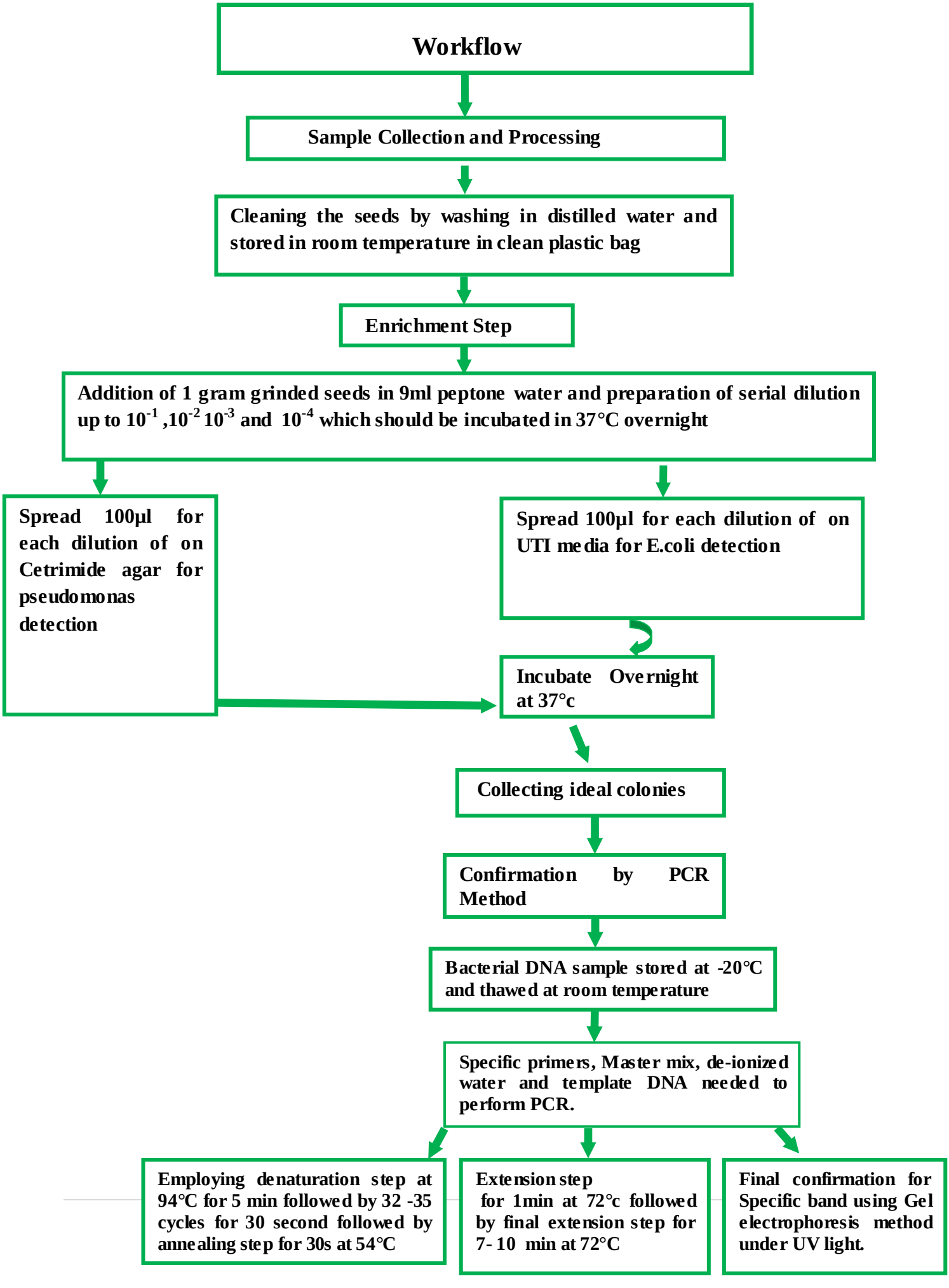
Chapter 2: Procedure

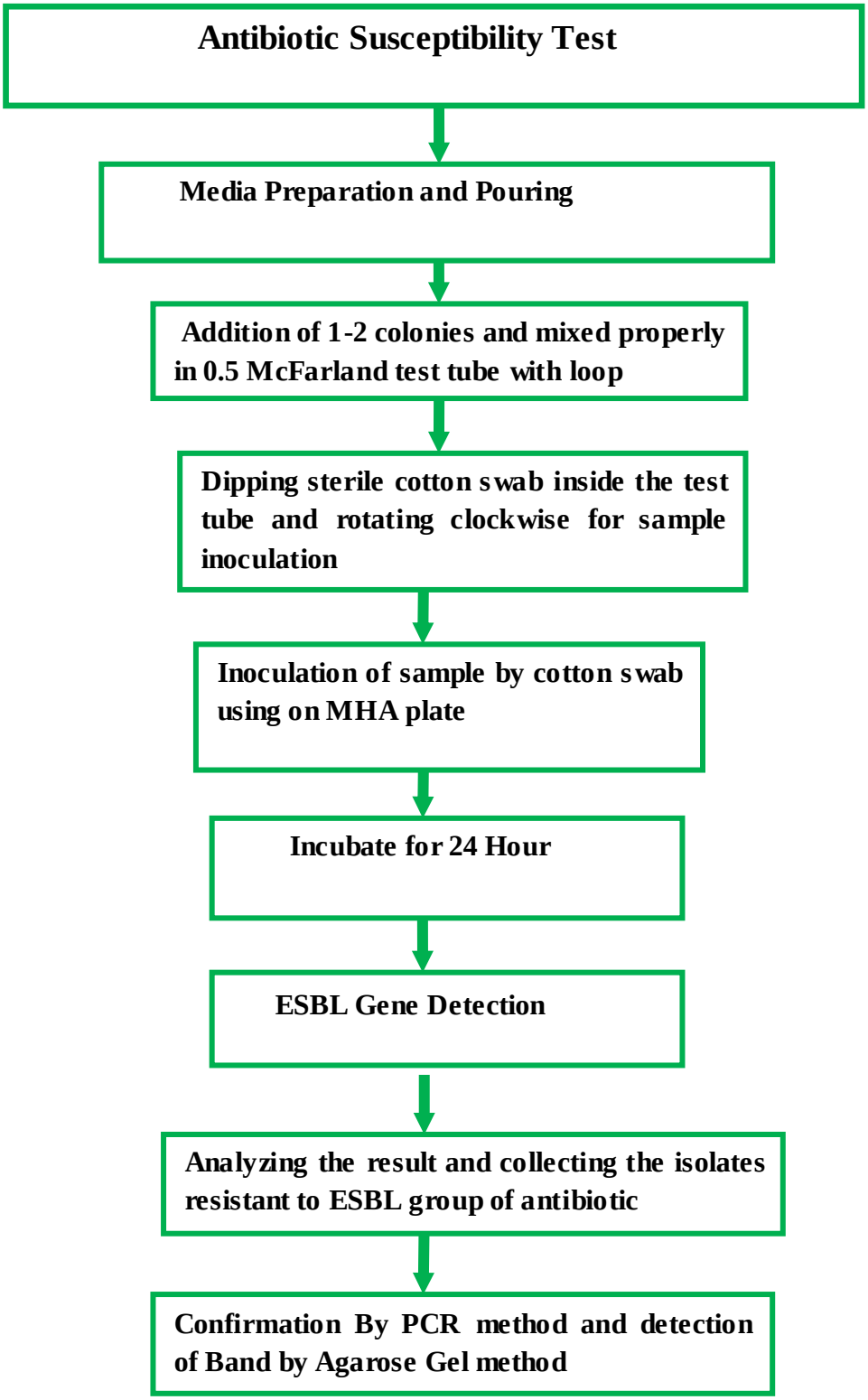
2.1: Major Equipment and Material

- Test Tube
- Dilution Bottles
- Petri dish
- Micro pipette
- Incubators
- Centrifugation machine
- Ethanol
- Mortar

Media

- Cetrimide Agar
- Nutrient Agar
- Mueller–Hinton Agar
- UTI Media


Workflow
Sample Collection and Processing
Cleaning the seeds by washing in distilled water and stored in room temperature in clean plastic bag
Enrichment Step
Addition of 1 gram grinded seeds in 9ml peptone water and preparation of serial dilution up to 10^{-1} ,10^{-2} 10^{-3} and 10^{-4} which should be incubated in 37°C overnight
Spread 100µl for each dilution of on Cetrimide agar for pseudomonas detection
Spread 100µl for each dilution of on UTI media for E.coli detection
Incubate Overnight at 37°c
Collecting ideal colonies
Confirmation by PCR Method
Bacterial DNA sample stored at -20°C and thawed at room temperature
Specific primers, Master mix, de-ionized water and template DNA needed to perform PCR.
Employing denaturation step at 94°C for 5 min followed by 32 -35 cycles for 30 second followed by annealing step for 30s at 54°C
Extension step for 1min at 72°c followed by final extension step for 7- 10 min at 72°C
Final confirmation for Specific band using Gel electrophoresis method under UV light.


Antibiotic Susceptibility Test
Media Preparation and Pouring
Addition of 1-2 colonies and mixed properly in 0.5 McFarland test tube with loop
Dipping sterile cotton swab inside the test tube and rotating clockwise for sample inoculation
Inoculation of sample by cotton swab using on MHA plate
Incubate for 24 Hour
ESBL Gene Detection
Analyzing the result and collecting the isolates resistant to ESBL group of antibiotic
Confirmation By PCR method and detection of Band by Agarose Gel method

2.2:Selection of location for Sample

Sample has been collected from five major big market of Dhaka city. These are Kawran Bazar(Tejgaon), Rampura Bazaar (Rampura), Kaptan Bazaar (gulistan), Mogbazaar (Malibagh) and A G B Colony Bazaar (Motijheel). They were selected because products from all over Bangladesh arrived in these places which could be a key factor to understand the presence of bacteria among edible seeds from a variety of places.

Sample collection and preparation

51 samples of 5 different categories were collected from various parts of Dhaka city..Later, seeds should be cleaned properly with distilled water and they were crushed properly using a mortar.Later they were sun dried and stored in a clean plastic bag and stored at room temperature. It should be made sure samples are carried in an undamaged container to avoid contamination.

Sample Processing

In this step, grinded seed powder was enriched properly in PBS for enhancing the growth of bacteria. Later ,it was done by the addition of grinded seed powder aseptically in a conical flask which market with individual sample code.

1)Afterwards 1 g grinded sample was added in 9 ml of NACL mixed accordingly. Afterwards ,ten fold serial dilution up to 10^{-3} should be prepared and The outcome should differ based on the presence of microorganisms in different media .

Identification of Bacteria

Several types of bacteria were targeted and random colonies collected from culture plates for identification.Several media used for the characterization of specific bacterial isolates .Cetrimide Agar, Nutrient Agar, Mueller–Hinton Agarused for detection of gram positive and gram-negative bacteria

Organism	Media
E.coli	UTI Media
Pseudomonas aeruginosa	Cetrimide Agar Media

Table 1: Name of Selected Organism

2.3:Disc Diffusion Method

To perform disc diffusion method, 100 µl of microorganism from inoculums preparation of bacterial stain was placed in MHA plate using a spreader Afterwards, Microbial growth were analyzed measuring zone of inhibition. Firstly media plates were checked for growth after 24 hours ,These samples were already cultured on both UTI and Cetrimide agar media. It is known that UTI agar is mainly used for the detection of *E.coli*. These organisms known for causing several infections and fatal disease. Furthermore, this process was initiated by collecting two or three colonies from cultured media using a inoculums loop in a saline solution which was mixed and vortexed properly in 2 ml of saline solution in test tube. For this test, mcfarland 0.5 suspension standard was used and by measuring proper bacterial suspension which is equal to 1×10^{8} and make sure turbidity correct. Later sterile cotton was used for dipping inside the test tube and rotated clockwise by the side of the tube with gentle pressure to remove excess fluid. After these, the sample was inoculated using a streaking method. The swab was streaked on the surface of the MHA plate from each corner of the plate via back and forth motion for three times to make sure it was inoculated properly.

Plate was rotated 60 degree to cover every part with the bacterial sample. After these , cultured MHA plates were incubated overnight in the incubator.

Media preparation

To prepare MHA media, 6 gram Mueller Hinton agar was added in 100 ml water and boiled for using a Bunsen burner followed by sterilization in autoclave machine .Antimicrobial activity was determined by observing microbial growth on culture plate by measuring the zone of inhibition on the surface .

2.4:DNA Extraction By Boiling Method

This extraction method was initiated by taking a loop-full of bacteria with a sterilized inoculums' loop and put it in 400 µl of 1x or 10x TE buffer in an Eppendorf tube. Afterwards it was vortexed until they mixed properly. Later ,they were centrifuged properly in a centrifuge machine for 10 min within 13000 RPM at 25° C .After these the supernatant was discarded and again 400µl of TE buffer added in the tube and mixed properly with pellet for 15 seconds. Later they were kept in the heat block for 10 minutes at 100°C. The mixture was kept in the room temperature for 5-10 minutes and after these they were centrifuged again for 10 min within 13000 RPM at 25° C .Finally supernatant was transferred in another Eppendorf tube and kept in the -20°C.

2.5:Confirmation by PCR method

PCR applied mainly to detect the presence of specific bacteria DNA from the sample .For these

1) Bacteria samples was stored at -20°C and thawed at room temperature. Later they inoculated on media and incubated 37°C for 24 hours.

2) Later, DNA extraction was done using a boiling method.

3) To initiate PCR process, specific primer was used to detect target organism for each sample. This process require 11µ l of mater mix content which was prepared by the addition of 6µl of master mix, 3 µl of nuclease free water, 1 µl of forward primer and 1 µl of reverse primer for each sample isolates.

4)PCR methods included Denaturation, amplification and extension of infinite hold step processes which carried on different cycles.

5)PCR method required denaturation to separate double stranded single stranded DNA This step occur for 5 min at 94°C and later 32-35 cycle of denaturation for 30s,annealing step for 30s at 54°C and extension step for 1 min at 72°C and final extension step for 10 min at 72°C.

6)Later PCR products need to be electrophoresis using agarose gel. After staining the gel with ETBR, they should be visualized, Control may require measuring possible contamination. Finally specific bands will show at specific position of lanes due to using specific primers.

2.6:Gene Detection

In this section three types of gene were selected for detection which were bla TEM bla IMP and bla NDM. Among them they were detected by using detection method using specific primer and later the band size were checked.

2.7: Agarose Gel Electrophoresis

It is a popular method in molecular biology which can separate the DNA fragments based on their size. These DNA samples involve electric current for running the process and consist of components such as gel and molecule of interest. In this process various macromolecules placed through a gel named agarose Agarose can be extracted from seaweed which carries repeated agarobiose. Here ,DNA ought to travel through gel as all the molecules have the same amount of charge .In addition DNA was placed in a pocket referred as well. DNA could not pass freely and gel reacted like a sieve which disrupted its migration of the DNA. As a result DNA move slowly towards the positive pole Here, small molecules move faster and larger molecules move slowly which helps to separate the DNA molecule as they can migrate faster in this experiment. For these , DNA remained far away from the origin than the larger molecule by which DNA separated based on their size. Different type of factors may affect migration of DNA.

1) To initiate the gel run,at first 2% of agarose gel was prepared with the addition of 2 gram LE agarose, 2 ml of 50x Tae Buffer which was mixed in 96 ml of distilled water . Later it was boiled until the solution became mixed properly and became colorless.

2)4µl of ETBR added to the gel when it is a bit cool.

3) Comb was already placed in the gel tray later liquid gel poured in the gel tray while kept in room temperature made it cool .Furthermore comb was placed 1 inch from one end of the of tray and put it in a vertical position in a way that the teeth remain 1-2 mm above the surface of tray.

4)Finally, comb removed after gel has been solidified and assured no bubbles in gel.

Electrophoresis

1)Electrophoresis should be placed and embedded in the electrophoresis buffer and it should not be the same buffer used in agarose solution.

2) Afterwards , 5 µl of pcr product was loaded in the gel .

3) Later proceeds for gel run at 100v for almost 30 min.

4)Finally, DNA bands have been checked under UV light.

Here is some of the reference primer for target sequence shown below:

Gene Region	Primer Sequence	Base Size
Pass Pseudomonas aeruginosa	F:5-GGGGGATCTTCGGACCTCA-3 R 5-TCCTTAGAGTGCCCACCCG-3	100-956 BP
Eco (E.coli)	5'GACCTCGGTTTAGTTCACAGA3' 5'CACCACGCTGACGCTGACCA3	500 BP
bla IMP	5′- GAAGGCGTTTATGTTCATAC-3′ 5′- GTATGTTTCAAGAGTGATGC-3′	258 BP
bla TEM	5' AAAATTCTTGAAGACG-3' 5' TTACCAATGCTTAATCA-3'	1100 BP
bla NDM	5'-GGTTTGGCGATCTGGTTTTC-3' 5'-CGGAATGGCTCATCACGATC-3'	476 BP

. **Table 2** :Primer Sequence and their Bp size

PCR condition for the selected organism and gene described below

Primer	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycle
Eco (E.coli)	95°C , 3 min	94°C 45 Sec	58°C 45 sec	72°C 60sec	72°C 3 min	30 Cycles
Pass (Pseudo	95°C 2 min	94°C 20 sec	58°C 20 sec	72°C 40 sec	72°C 1 min	30
bla TEM	94°C 3 min	94°C 30 sec	50°C 30 sec	72°C 2 min	72°C 10 min	35 cycles
bla IMP	94°C 3 min	94°C 45 sec	61°C 45sec	72°C 45 sec	72°C 5 min	35 cycles
bla NDM	94°C 5 min	94°C 30 sec	58°C 30 sec	72°C 30 sec	72°C 7 min	35 cycles

Table 3: PCR condition for required organism and genes

3. Result

3.1:Result analysis for *E.coli* and *Pseudomonas*

For this study ,results were obtained from 51 different edible seeds which were collected from different areas from which 13.7 % (n=7) of samples were positive for *E.coli* and 43% (n=21) of isolates were positive for *Pseudomonas aeruginosa*. It shows samples are way more contaminated with *Pseudomonas* than *E.coli*. Among six categories of seeds, only flax seeds showed positive results for the *E.coli* and other type of seeds were negative. For *Pseudomonas*, sesame showed the most positive result by quantity and sunflower seed showed second most positive result by percentage. Serial dilution was done most of the time and overgrowth appeared in the culture media up to dilution number of 10^{-3} for all types of seeds. For these, serial dilution of flax seed was done up to 10^{-5} and serial dilution was done up to 10^{-7} for the other four categories of seeds to get a better result . For UTI media , only pink colonies were picked for the DNA extraction and oftentimes 10x TE Buffer were used during DNA extraction as results were not obtained via using 1X TE buffer. Two different media was used for the detection of *E.coli* and *Pseudomonas Aeruginosa*. Among them seven isolates were detected positive for UTI media that provides pink colony for *E.coli* and 21 samples were obtained positive for *Pseudomonas* aeruginosa detection were done in cetrimide agar media where they appeared as white naturally. Confirmatory processes were done by observing the fluorescence glow which is supposed to be obtained from the colony of the *Pseudomonas* isolates due to their unique characteristics. Here is the list of positive results obtain for both *E.coli* and *Pseudomonas* described in table :

3.1 Positive Results for E.coli

	Number of Isolates	Result
1	F1	+
2	F2	+
3	F3	+
4	F5	+
5	F6	+
6	F8	+
7	F9	+

Abbreviation

F= Flax Seed

S = Sesame Seed

SUF = Sunflower Seed

PK = Pumpkin Seed

FEN =Fennel Seed

Chic = Chickpea Seed

Table 4 : Positive isolate for *E.coli* based on serial

The pcr result for *E.coli* shown below :

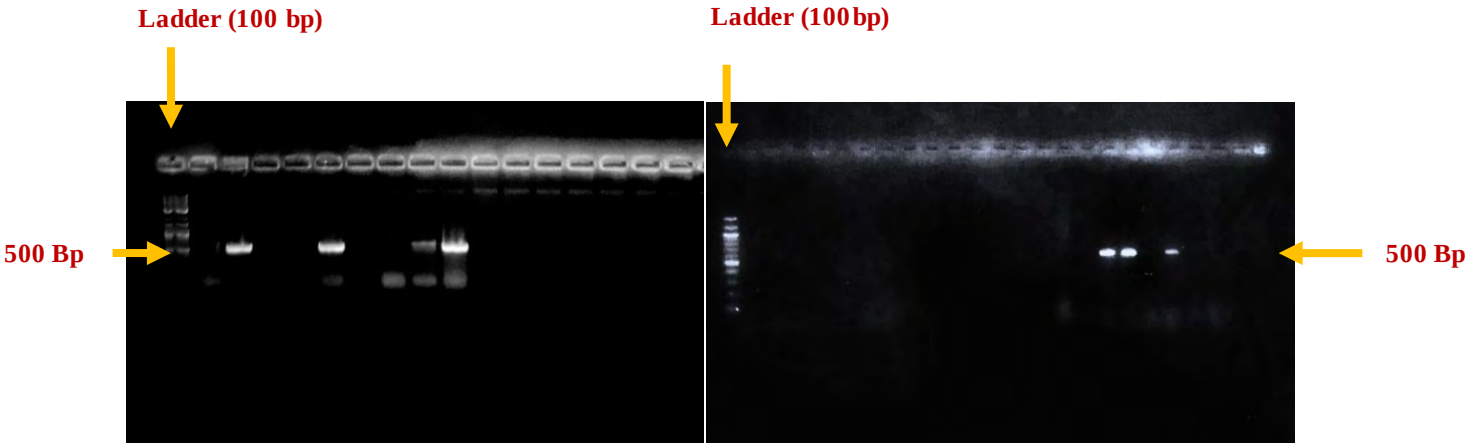


Figure 1:positive isolate from agarose gel run under UV light .Band occurred a 500bp for *E.coli*

Among five different places ,most contaminated seed was from old town an here is a chart to show the percentage of contamination among different areas.

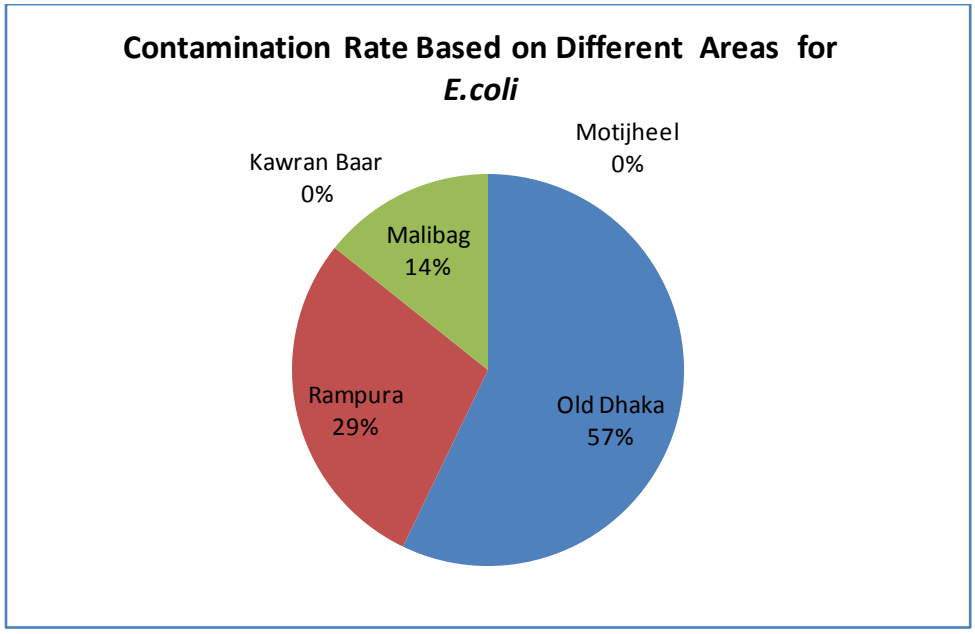


Figure 2 Contamination of *E.coli* from different places in Dhaka

In this pie chart, data represent that most contaminated seed was from old town (n=4) and second from Rampura (n=2) followed by third most polluted area was Malibagh (n=1)

For *Pseuomonas aeruginosa* in total 21 samples were positive. Among them, four of them were flax seed nine of them were sesame seed, five of them were sunflower seed three of them were pumpkin seed and one of them was fennel seed.

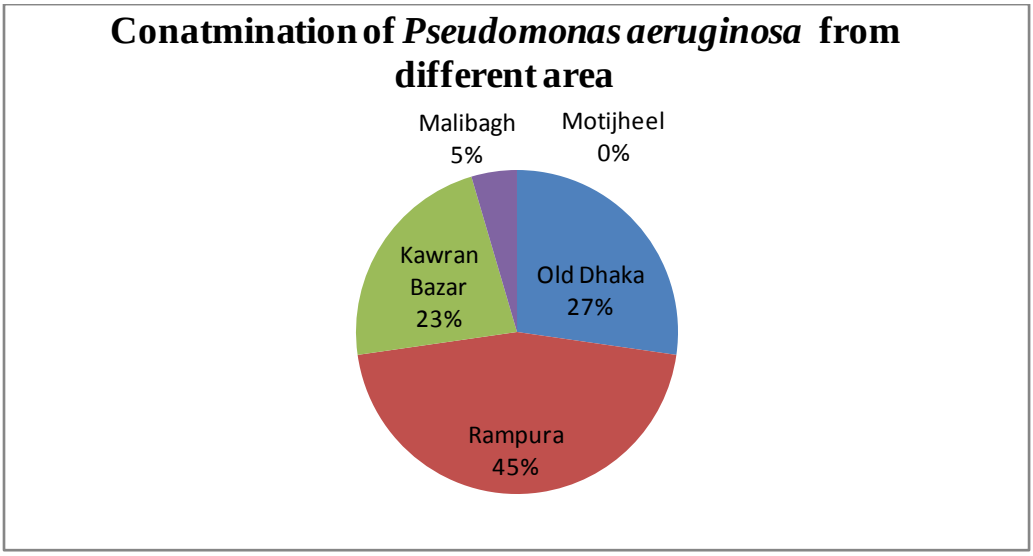


Figure 3 Frequency of contaminations for *P.aeruginosa* based on different area

The result from pie chart represents the total data of positive results from *Pseudomonas* that occurred from different areas. In this chart, 6 of the samples were from old Dhaka. Among them 3 of them were flax seed and three of them were sesame seeds. Most positive results for these organisms occurred from Rampura area 45% (n=10). Among them, 5 of them were sesame seed , 1 of them were flax seed, 4 of them were sunflower seed. Kawran bazar second least prevalence which is 23%. Among them three of the were pumpkin seeds and one of them was fennel seed. Motijheel has the least frequency of sample which is 5% ((n=1) a sunflower seed was positive from this area.

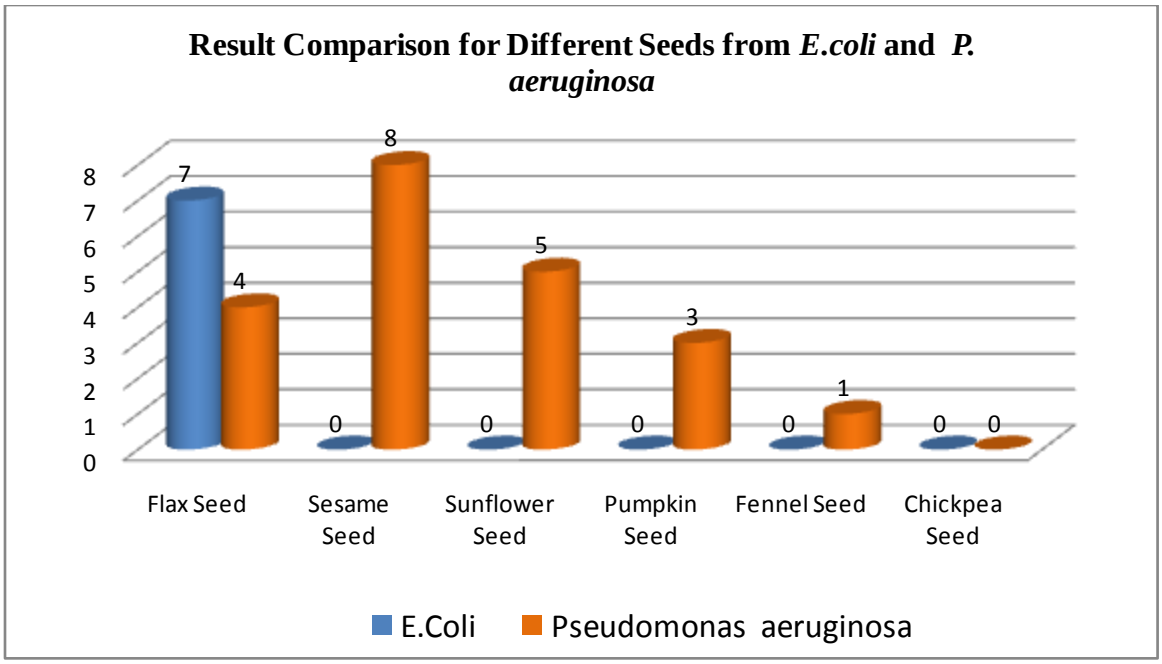


Figure 4 : visual representation of positive result for *E.coli* and Pseudomonas by quantity .

This Diagram showed the visual representation of results. From 51 samples in which flax seeds carried the most positive results (n=7) for *E.coli. For Pseudomonas aeruginosa*, sesame seed contained the most positive result and second most was sunflower seed followed by sunflower seed, pumpkin seed and fennel seed.

This table shows the detail about the isolate which has been consider as positive in detail

Seed Category	Positive Isolates(%)	Status
Flax Seed	4 (25 %)	+ (Green Glow)
Sesame Seed	8 (57%)	+ (Green Glow)
Sunflower seed	6 (85%)	+ (Green Glow)
Pumpkin Seed	3 (42%)	+ (Green Glow)
Fennel	1 (25%)	+ (Green Glow)

Table 5: Positive isolate for *Pseudomona aeruginosa*

From above it can be seen that sunflower seed has highest prevalence for *Pseudomonas aeruginosa* followed by sesame seed. It can be seen that *Pseudomonas* aeruginosa has higher prevalence than *E.coli.*

The result occurred from agarose gel run showed below :

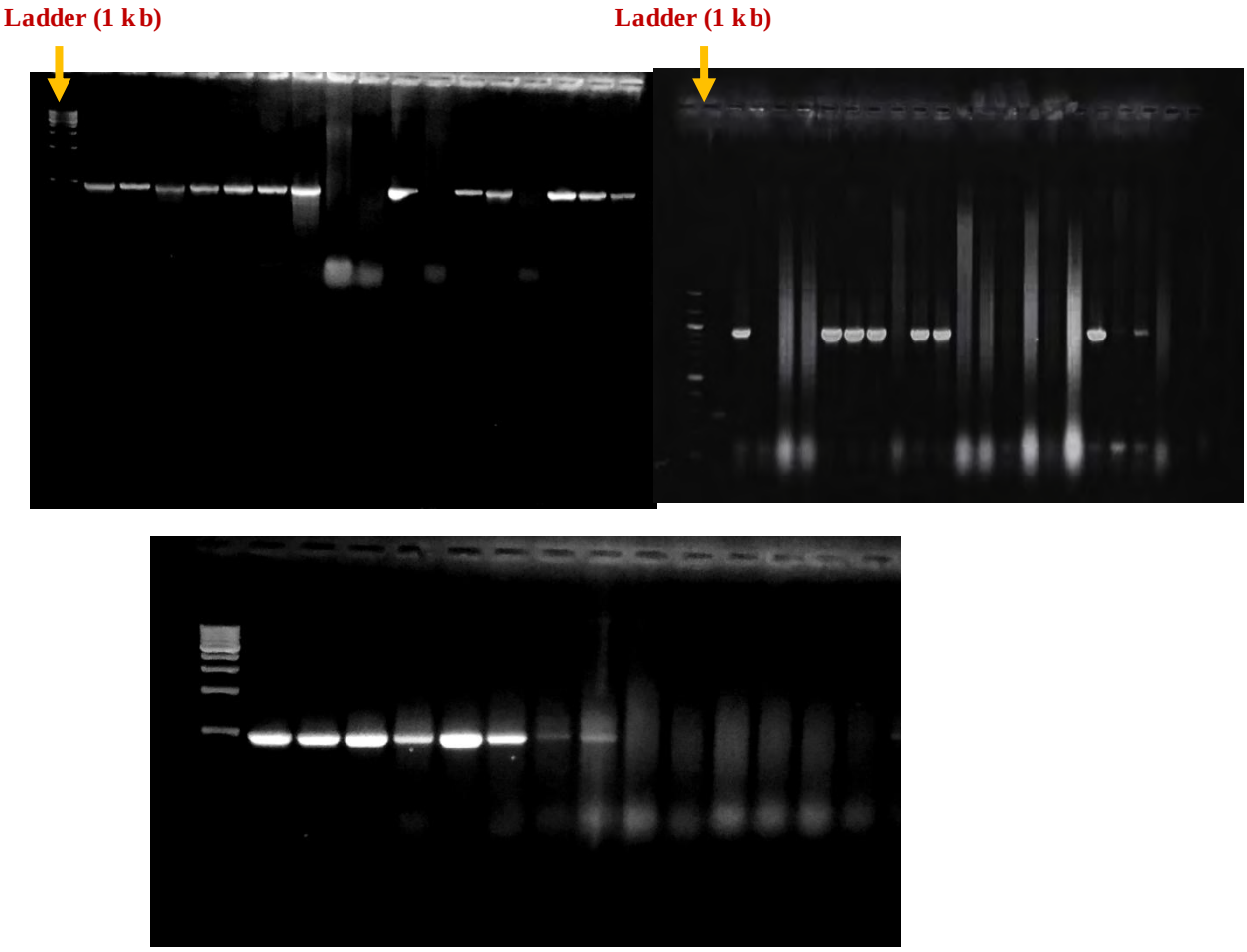


Figure 5 :21 of positive isolate from agarose gel run under. Band occurred a 100bp, 5 isolates were made copy from the positives. The colony which were obtained was confirmed by UV fluorescence.

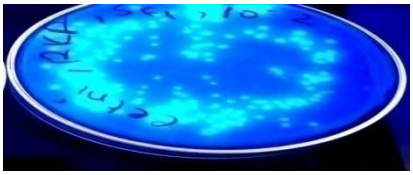


Figure 6 : *Pseudomonas* colony on cetrimide media

3.2 Antibiotic Susceptibility test

AST test analysis were done only for positive result from *E.coli* and *Pseudomonas aeruginosa*. They were revived from the T1N1 from the stock and later sub-cultured on nutrient agar media from which colonies were used for AST analysis using the Kirby Bauer Diffusion method. In total 12-14 antibiotic discs of different groups were used. For *E.coli* most sensitive antibiotic group were carbopenem and fluoroquinolone which exhibited 13.7% of sensitivity and most resistant antibiotic group were tetracycline, polymyxin and streptomycin of amino glycosides that showed resistance for all positive results which is 13.7 %. For *Pseudomonas aeruginosa* ,tazobactam from beta lactam group and ciprofloxacin from fluoroquinolone were most sensitive for all the organism of *Pseudomonas aeruginosa*. They showed susceptibility for *Pseudomonas* aeruginosa which is 41.17% and 39.20 % of susceptibility. Also, this samples showed resistance for maximum discs of antibiotic. Among them penicillin from amino glycosides, clavulanic acid from beta lactamase and *nitrofurantoin* from *nitrofurans* group showed resistance for all the positive isolates.

The result summary for positive *pseudomonas* isolates described below (Table 1)

Antibiotic Group	Antibiotic Susceptibility test			
	Antibiotic Name	Number of Sensitive Isolates (%)	Number of Intermediate Isolates (%)	Number of Resistant Isolates (%)
Aminoglycooside	Amikacin	**16** (31.3%)	**5** (9.8%)	**0** (0%)
	Gentamicin	**14** (27.50%)	**1** (2%)	**5** (9.8%)
Carbopenem	Meropenem	**18** (35.3%)	**0** (0%)	**3** (6%)
Fluoroquinolone	Ciprofloxacin	**20** (39.2%)	**1** (2%)	**0** (0%)
polymyxin	Collistin	**1** (2%%)	**3** (6%)	**17** (33.3%)
Penicillin	Amoxcillin	**0** (0%)	**0** (0%	**22**(43.17%)
Beta-lactamase	Tazobactam	**22**(43.17%)	**0** (0%)	**0** (0%)
	Clavulanic Acid	**0** (0%)	**0** (0%)	**22**(43.17%)
Glycylcycline	Tigecycline	**0** (0%)	**0** (0%)	**22**(43.17%)
Nitrofurans	Nitrofurantoin	**0** (0%)	**0** (0%)	**22**(43.17%)
Monobactam	Aztreonam	**11** (21.5%)	**5** (9.8%)	**5** (9.8%)
Cephalosporin	Cefepime	**15**(29.4%)	**3** (6%)	**3** (6%)
	Ceftazidime	**9** (17.65%)	**3** (6%)	**8** (15.7%)
Macrolide	Azithrromycine	**6** (11.76.%)	**0** (0%)	**15** (29.41%)

Table 6: AST result for *Pseudomonas aeruginosa*

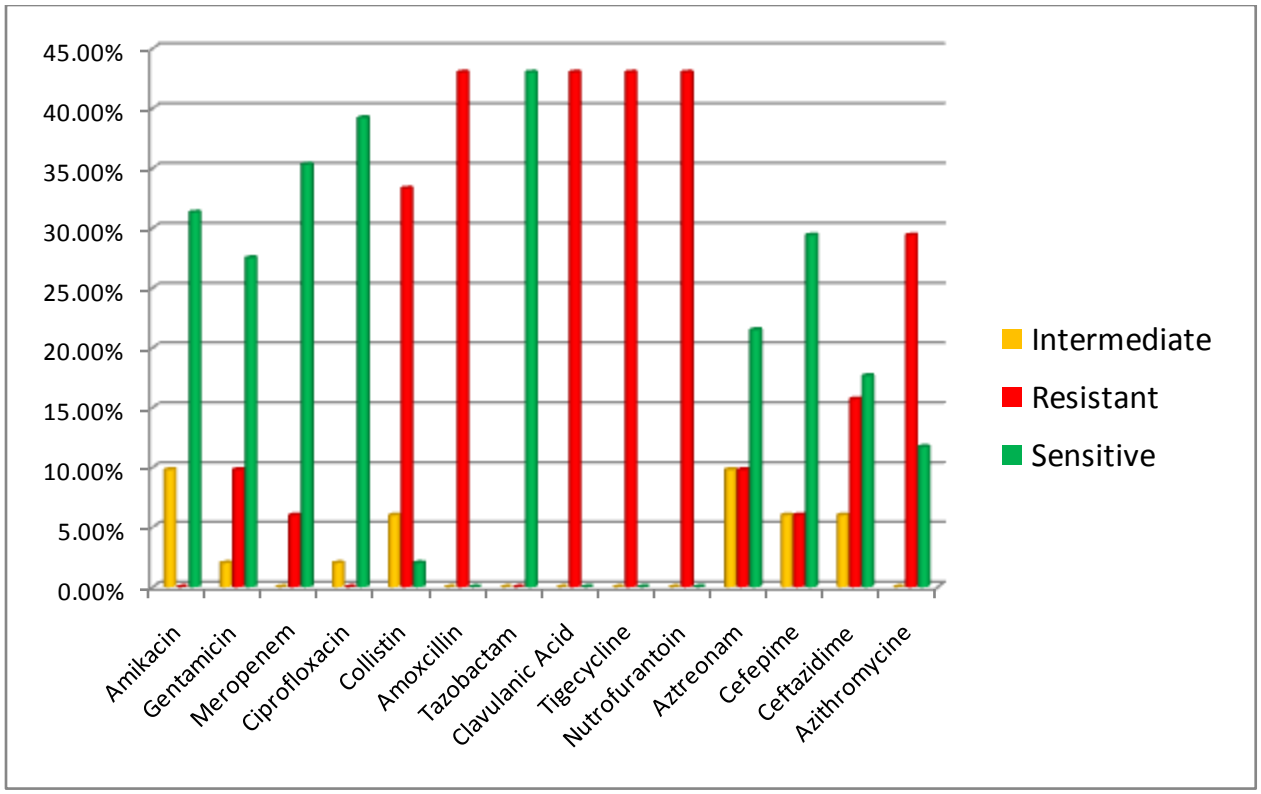


Figure 7: Comparative AST analysis for *Pseudomonas aeruginosa*

Here is the visual representation of AST result for *Pseudomonas aeruginosa* which prevailed most resistant disc was clavulanic acid from beta lactam amoxicillin from penicillin, tigecycline from glycylcycline, nitrofurantoin from nitrofurans which are fully resistant with the percentage of 43.17% and most sensitive group was tazobactam from beta lactam (43.17%) ciprofloxacin from fluoroquinolone (39.20%) and amikacin from amino glycosides group .

The result summary for positive *E.coli* AST analysis described below (Table 2)

Antibiotic Group	Antibiotic Name	Number of Sensitive Isolate (%)	Number of Intermediate Isolates (%)	Number of Resistant Isolates (%)
Aminoglycosides	Amikacin	**2** (4%)	**1** (2%)	**4** (7.85%)
	Gentamicin	**6** (11.76%)	**1** (2%)	**0** (0%)
	Kanamycin	**0** (0%)	**0** (0%)	**7** (13.7%)
	Streptomycin	**0** (0%)	**0** (0%)	**7** (13.7%)
Carbopenem	Imipenem	**0** (0%)	**7** (13.7%)	**0** (0%)
	Meropenem	7(13.7%)	**0** (0%)	**0**(0%)
Beta Lactam	Ampicilin	**0** (0%)	**0** (0%)	7 (13.7%)
Fluoroquinolone	Ciprofloxacin	**7** (13.7%)	**0** (0%)	**0**(0%)
Macrolides	Erythromycin	**0** (0%)	**0** (0%)	**7** (13.7%)
Phenicol	Chloramphenicol	**1** (2%)	**0** (0%)	**6** (11.76%)
Tetracycline	Tetracycline	**0** (0%)	**0** (0%)	**7** (13.7%)
Polymyxin	Collistin	**0** (0%)	**0** (0%)	**7** (13.7%

Table 7: AST result analysis for *E.coli*

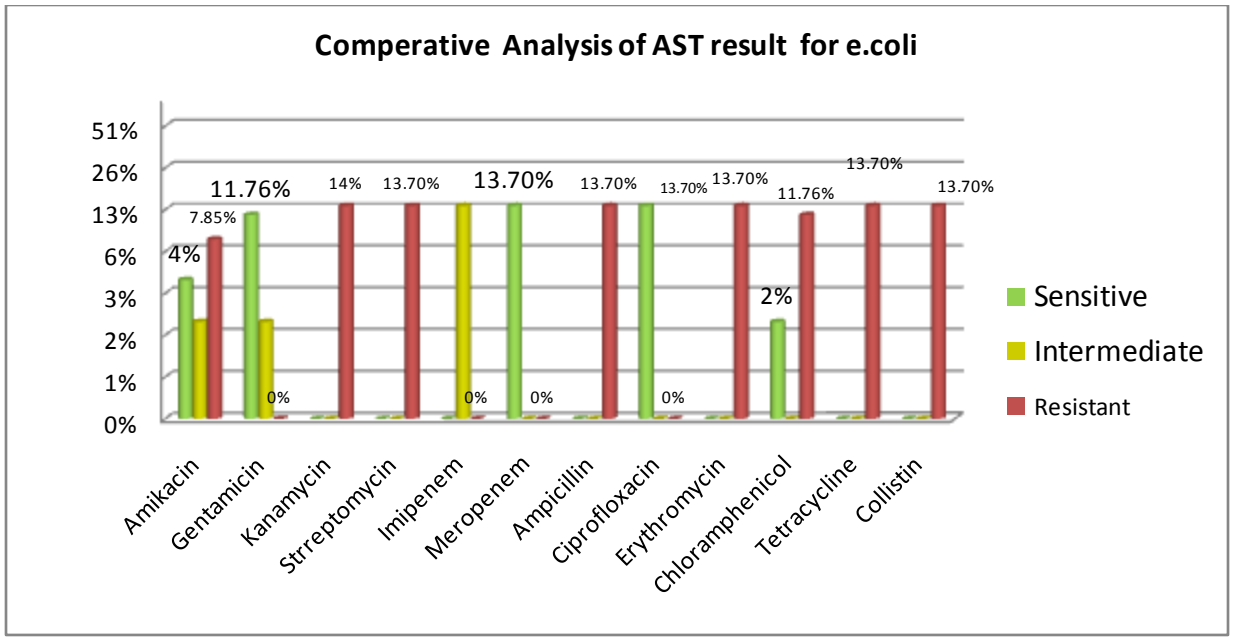


Figure 8: Visual representation of AST result for *E.coli*

Here It can be seen that ciprofloxacin and meropenem and gentamicin was most sensitive and tetracycline, erythromycin and ampicillin kanamycin and streptomycin were most resistant.

3.3 ESBL resistance gene analysis

Here gene detection was done for all the isolates that showed resistance to the extra spectrum beta lactamase group of antibiotics. For *Pseudomonas aeruginosa,*total 21 isolates showed resistance to the ampicillin and clavulanic acid which belongs to the ESBL group and for *E.coli* 7 isolates showed resistance to ampicillin and kanamycin which also belongs to the ESBL gene group. These resistance isolates were tested for detecting their resistance genes. In addition the resistance genes were analyzed such as *bla TEM* and *bla IMP* and *bla NDM* .For *E.coli* 7.8% (n=4) isolates were positive for *bla TEM* . Among them all of them were flax seed and *bla IMP*, and *bla NDM*, results were negative.

For *bla TEM* 4 of the samples were positive . The result for *bla TEM* shown below which occurred from the pcr detection using specific primer .

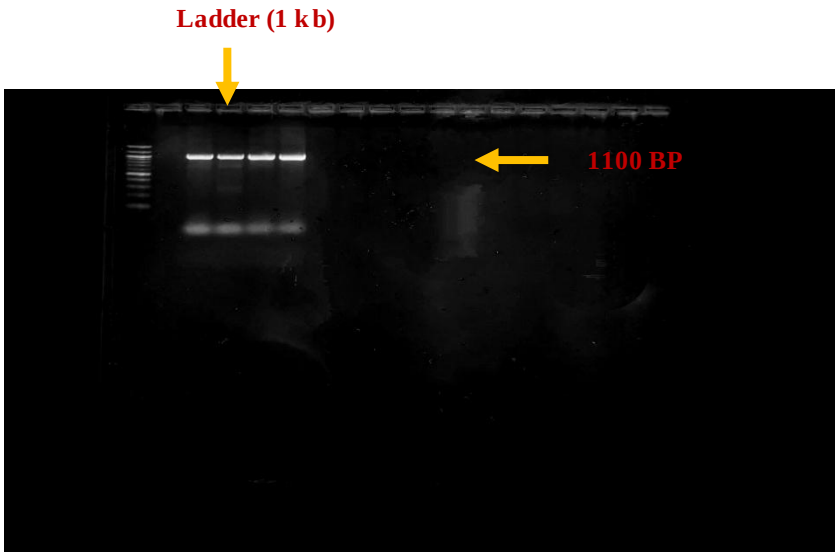


Figure 9 :*bla TEM* gene detection for *E.coli* by PCR method in 1100bp

For *Pseudomonas aeruginosa* bacteria one of the isolates were positivefor *bla IMP* gene and 3 of them showed positive result for *bla NDM*. The result for *bla* TEM_1 in *Pseudomonas aeruginosa* were negative.

The pcr result for *bla IMP* and *bla NDM* shown below

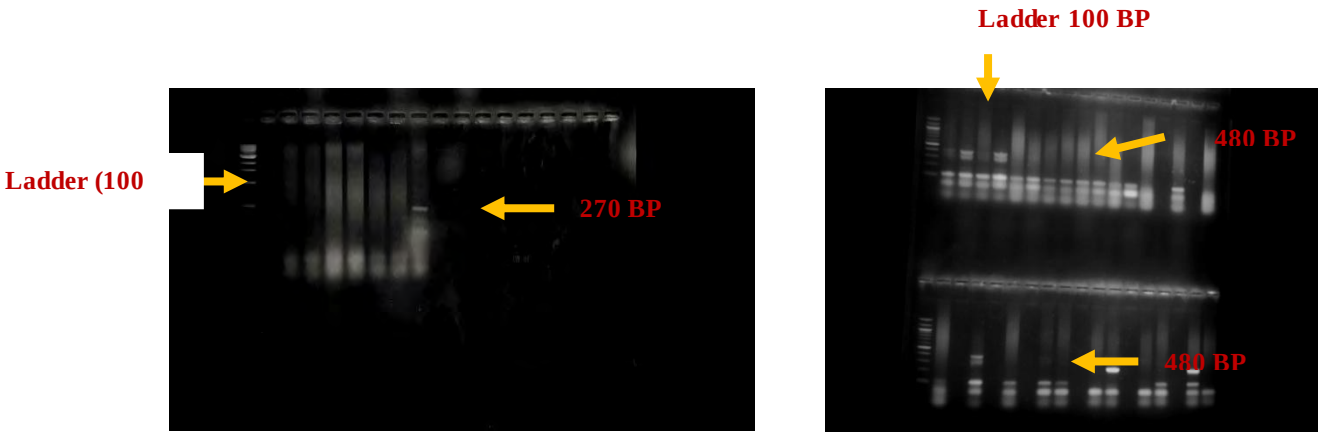


Figure 10: *bla IMP* gene detection for *E.coli* by **(** 11**)***blaNDM* detected for isolate in PCR method in 258bp 480 bp

Here is the table of result analysis for ESBL gen for *E.coli* arrived from flax seeds

Seed Catagory	Positive Isolates	*bla TEM*	*bla IMP*	*bla NDM*
Flax Seed	7	26%	0%	0%

Table 8: Occurrence of three type of ESBL producing gene from *E.coli*

This table represents the positive result for three different type of gene which arrived from different type of seeds category :

Seed Catagory	Positive Isolates	*bla TEM*	*bla IMP*	*bla NDM*
Flax Seed	4	0%	0%	0%
Sesame Seed	8	0%	6.66%	6.66%
Sunflower seed	6	0%	0%	6.66%
Pumpkin Seed	3	0%	0%	6.66%
Fennel	1	0%	0%	0%

Table 9:Positive results for three different gene of seeds category for *Pseudomonas aeruginosa*

4. Discussions

The number of infections caused by the food borne pathogens are increasing day by day .In recent years several outbreaks have occurred that are caused by various types of microorganisms and most of them are gram negative rod shaped bacilli. Here, the presence of bacteria was detected by the conventional PCR method in which a sample extracted from DNA extraction method was used. For the DNA extraction method, 1x TE buffer was used but 10x TE buffer is more preferred because it can protect DNA and restrain the degradation of DNA which is why it is better than 1x TE buffer. It is helpful as it can help the storage and protect it from the DNAse and RNAse enzyme and stopped the nuclease for protecting DNA for Both *E.coli* and *Pseudomonas aeruginosa* those are well known for spreading several infection. There were major outbreak occurred from consumption of sprout and cucumber consumption that resulted *E.coli* infection. Among them 25% infection occurred due to the consumption of the sprout in Germany (Buchholz et al., 2011). Another study by Karin and Katharina showed that 43% of the positive results contain strains of *Pseudomonas aeruginosa* among 1001 isolates (Schwaiger & Helmke, 2011). In Australia, local sprout samples were analyzed and this study revealed that among 14.8 % of the sample carried *E.coli* among 119 types of different samples such as alfalfa mung bean, lentil, sprouts. It also exhibited that this components were not fully fresh and decontaminated along with 6 of them are unsatisfactory to eat (Symes et al., 2015) Another analysis done by Rezalo and Motalebi revealed the percentage of *Pseuomonas aeruginosa* is relatively low among meat and they found 7.83% in meat product in Australia(Rezaloo et al., 2022). Furthermore, Result from a study found that mung beans and alfalfa were one of the main reservoirs of *E.coli*. In Norway an experiment by Robertson and Johanessen revealed that 12.9% of mung beans were contaminated with *E.coli* in norway (Lucy et al., 2002). *Pseudomonas aeruginosa* is also found in Norway and the prevalence rate was 73% among hospital patients (Heir et al., 2021). Contamination among different types of edible seeds has been recorded all over the world which displayed that these seeds were more prone to be contaminated with the fecal contamination. In a study by silva and nunes in southern Portugal, analyzed the quality of seeds by quantifying the average population of seeds aerobic and non

aerobic microorganism. It showed that of all other seeds, Flaxseed has the highest number of bacteria present in their culture and sesame seeds contain the second most persisting bacteria present in their enumeration followed by sunflower seed and pumpkin seed. It indicated that sesame seed and flax seed hold more bacteria than others, which was also stated in several other research articles (Silva et al., 2022). Similarly, Allamine and Djibrine in their study that exhibit the contamination level sesame seed in chad revealed that total samples were not satisfactory while *E.coli* contamination was found in the sesame seed sample (Allamine et al., 2024). The result in most of the cases showed the prevalence of *E.coli* is higher than any other bacteria which is in between 0%-20% between the samples .Several other bacteria also occurred but they showed less occurrence compared to *E.coli*. In Burkina Faso a study performed by Compaore and Bazie which expressed the condition of RTE (Ready to eat) food had lower moisture in sesame sold in the street. In this study different types of sesame were collected and their quality were analyzed. It has been noticed that the presence of fecal contamination through the culture method and ensured by the IMVIC test method (Compaoré et al., 2021).

In this study results from six categories were selected which are getting very popular for both drink and cooking purpose for their high nutrition quality. Contamination has been recorded in samples from each of the places indicating that sample was contaminated overall. From above the data in this experiment revealed that flax seed had higher prevalence of *E.coli* than *Pseudomonas.aeruginosa* and all the *E.coli* isolates were flaxseed positive. Here it might be stated that flax seed had higher chance of fecal contamination than *Pseudomonas aeruginosa*. Overall bacterial prevalence in this study showed that seeds were contaminated more with environmental pollutants than fecal matters. Among different types of seeds sunflower seed had a higher rate of *Pseudomonas* aeruginosa occurrence than other types of seeds stated that these seeds are less safe to eat than other seed products. Furthermore, among all seeds contamination was found on sesame in larger quantity isolates which stated that, the quality of sesame is not optimal and also poorer than other seeds components. Many of the reason might be accountable for this contamination .One reason could be that seed product might came in contact with the manure and other components which spread bacteria to the seeds such as unnecessary rotten roots and unplanned irrigation system. Another survey was conducted by the Food Safety Authority of Ireland which revealed the presence of different types of microorganism in edible seed products such as sesame seed, pumpkin seed, sunflower seed, poppy seed, hazel nuts, peanuts macadamia etc.This research found that around 0.8% of the sample was accountable for *E.coli* contamination and over 0.3% of those samples were considered as unsatisfactory. The microorganism was found mostly on pumpkin seed followed by nuts, sesame, and flaxseed. (Food Safety Authority of Ireland, 2012). In recent years, Shiga toxin producing *E.coli* has been major source of food contamination and it infected mainly children which is a matter of concern .Several study found that plant based raw food such as vegetables , nuts , edible seeds has been major reservoir for *E.coli* microorganism and they could be a part of major foodborne outbreaks.

In a study by Razzaq and Farzana, It has been found that vegetables are vastly contaminated with several microorganisms in Pakistan . It was noticed that among 145 samples 48% percent of the sample was polluted with *E.coli* and *Pseudomonas aeruginosa* was present in 60% of the sample. It also revealed that *E.coli* was present in almost all type of vegetable sample including, 50% of cabbage ,50 % of radish and carrots (25%) of the coriander. This study also established that in AST analysis *E.coli* was fully sensitive to gentamicin and ceftriaxone and it exhibited 48% sensitivity for kanamycin and 81% sensitivity to ampicillin and 93% sensitivity to erythromycin and most resistant to the carbenicillin. It also occurred from this study that most of the *P.*aeruginosa samples were fully resistant to ampicillin and nalidixic along with 98% resistant to kanamycin. In a relevant paper Tambekar and Mundhada expressed that *E.coli* was found in high numbers in plant materials. It showed *E.coli* was present in 38.3% of the sample (Razzaq et al., 2014).

A different study by Shah and Eppinger revealed that samples collected from rural areas also contain a high number of *E.coli* variants. It showed that in total among 260 samples, *E.coli* was present in overall 34% of the sample in Pakistan. Among them, 80% of the sample had shiga toxin producing *E.coli* and 83% of the sample had *ETEC E.coli*. They also analyzed the AST test in which they utilized several antibiotic groups such as beta lactam, aminoglycosides and others. It showed that 94% of the sample were resistant against tetracycline, 84% against the ampicillin , and 82% against CTX . The results indicated that they were most resistant against the beta lactam antibiotic group. The isolates were susceptible to GEN and CIP; it is very highly present in the vegetable sample (Shah et al., 2015).

The suitable temperature for the growth of *E.coli* is 37°C which is why it is found very often in the Asia region. In India, *E.coli* can be found on components such as sprout, seed, vegetable, fruit, water and many more. In a study by Malik and Gaind, It has been found that various fruits and vegetables are highly contaminated with *E.coli* bacteria. In this research, in total 150 samples of different fruits and vegetables were tested and formation of *E.coli* were found in 17.4% of the sample. Different types of sample were tested such as cabbage, ginger, cucumber, tomato in which *E.coli* was present mostly in ginger (100%) and cabbage (80%) followed by 75% of cucumber and tomato. Later, these samples were tested to analyze whether they had ESBL gene. For these, positive isolates from both of these organisms were chosen and 5.7 % of isolates were positive for genes. In addition, these bacteria can also be present in edible seeds. (Saksena et al., 2019).In India along with other bacteria *Pseudomonas* is also very common. In a study by Ruiz found that *Pseudomonas aeruginosa* is vastly present in the hospital isolates. It has been found that 53% samples were from *Pseudomonas* aeruginosa among different vegetable samples such as lettuce (90%) , Chard (70%) (Ruiz-Roldán et al., 2021).

Pseudomonas aeruginosa is known for its unique function by which it invades most individuals with low immune systems. It has been found that among 21 %of the patients with age range of

less than 1 year might get *Pseudomonas aeruginosa* related pneumonia that already has CF and 80% of the individuals with CF disease had chances to become infected with *Pseudomonas pneumonia*. Among several years the following organism was detected more frequently in plant based food. In a study it was revealed that among 11% of *Pseudomonas aeruginosa* was found in rice, salad, turkey and chicken food. Along with other samples, the salad showed 28% of contamination of *Pseudomonas aeruginosa* which indicated it could be present in a good number among vegetables (Ruiz-Roldán et al., 2021). According to a study by WHO, 1573 deaths occurred in 2007 by *Pseudomonas aeruginosa* and In 2015 around 4564 new deaths have been recorded due to this organism which exhibited the rate of infection by this organism has been increasing over years. It was found that these bacteria could contaminate potable water in china. A study by Allen and Geildrech showed the prevalence of this organism in 3 % of drinking water, 18% of bottled water and 9 % of tap water. In dairy products, meat, and processed food the presence of *Pseudomonas aeruginosa* was also detected. Another study showed that *Pseudomonas aeruginosa* can form biofilm in the milk tank by attaching to the wall at cool temperature (Chen et a1 , 2011). In a study it has been found that among 107 raw milk samples, 18% of the samples were contaminated with *Pseudomonas aeruginosa* (Garedew et al., 2012). In recent years the presence of *Pseudomonas aeruginosa* was detected in a high number among several food products. An experiment conducted in Spain showed that the number of *Pseudomonas aeruginosa* has been found in higher numbers and It prevailed that 53% of the vegetables were contaminated with *Pseudomonas aeruginosa*. Among them 10 sample were green bean and others were potato, cucumber and onion (Ruiz-Roldan et a1 ., 2021).

In a Microbial analysis by Deshmukh and Sonawane, the presence of *E.coli* in fennel seed was found. This research found that among 10.6 % were contaminated with *E.coli* while among 68% of the sample were contaminated with different pathogen. It indicated that they are contaminated by the fecal flora (Deshmukh et al,. 2020). Besides Asia *E.coli* found largely on African subcontinent on various components .Research was done in several countries of African region such as Jamaica, Costa Rica , Nigeria , Chad . In Costa Rica microbial inspection was conducted among various ready to eat food that includes ,Salad, vegetables. It has been found that 56% of the sample were contaminated with fecal coliform and they were not acceptable for human which portray the level of contamination in those sample an around 10 % *Pseudomonas aeruginosa* was detected in banana (Microbiological Evaluation of Ready-to-eat Foods Manufactured by Small Costa Rican Industries, 2010) (Saville et al., 2017).

Similarly, another study in Nigeria with several ready to eat foods such as rice, salad detected the presence of *E.coli* in the sample. It showed 68.45 % of the sample was contaminated with the *E.coli* pathogen and 11.31 % of the sample was contaminated with the *Pseudomonas aeruginosa*. The *E.coli* positive isolates were further analyzed with AST where they were susceptible for ciprofloxacin, gentamicin, streptomycin and for *Pseudomonas aeruginosa* they organism were resistant to the entire antibiotics disc (Cc et al., 2022). Vendor vegetables are one of the main

resources of contamination in Nigeria. In this country, another research was done to assess the level of contamination of the vendor's vegetable. It showed that among 2.4 % of the sample has *E.coli* indicated the fecal flora contamination which caused from the unhygienic maintenance of the sellers or during the production. This study also found plant based food can also be contaminated with various other gram negative bacteria. They stated that samples from vendors vegetable was polluted with microorganism such as 2.4 % of *Enterobacter* ,12.2% of *Klebsiella* and 7.3% of *Pseudomonas spp* organism . This bacteria showed 100% resistance to the antibiotic disc of amoxicillin and clavulanic acid along with 95% resistance to ampicillin in the AST test and they were more susceptible for the antibiotic disc named ciprofloxacin (Adedeji et al., 2021). The difference between the prevalence of both *E.coli* and *pseudomonas* shown below :

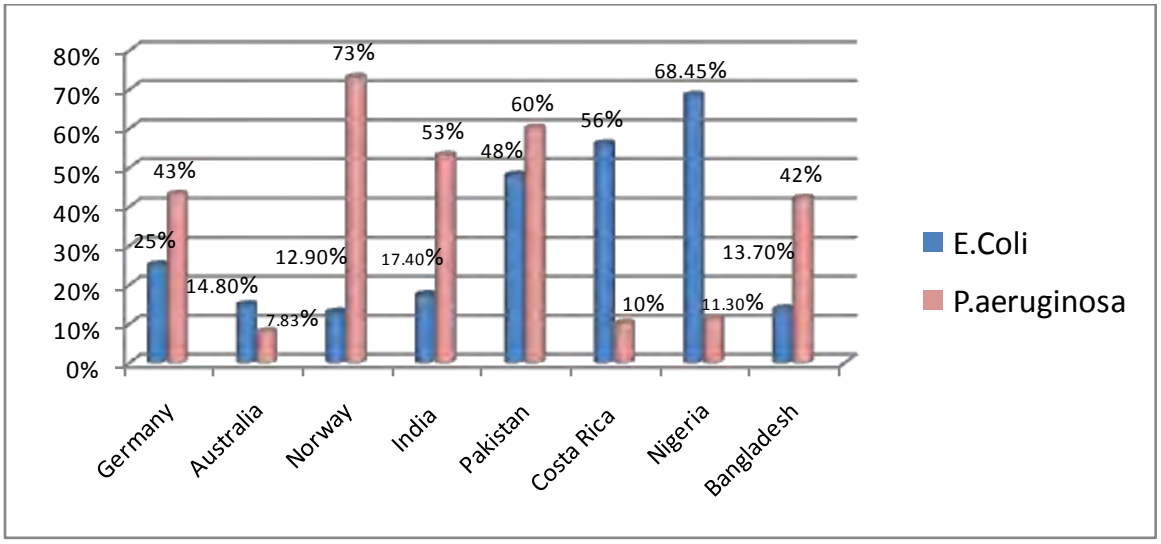


Figure 11: Prevalence rate of *E.coli* and *Pseudomonas* among different countries

From the above diagram It was noticed that the prevalence of *Pseudomonas aeruginosa* is mostly in Norway. It indicated that African countries such as Nigeria and Costa Rica have more contaminated samples in *E.coli* than *Pseudomonas aeruginosa*.The lowest prevalence of *Pseudomonas aeruginosa* observed in Australia which was 7.8 % showed that the samples were more contaminated with Fecal matter than environmental pollutants. The samples from India and Pakistan were more prone to be contaminated with *Pseudomonas.aeruginosa* according to data. Among African regions Nigeria and Costa Rica had the most prevalence in *E.coli*. In Bangladesh The prevalence of *E.coli* in seed samples was lesser than almost every country except Norway which unveiled that they are likely to be contaminated with *Pseudomonas aeruginosa*. Overall it could be said that prevalence are lesser than most other countries for *E.coli* and *Pseudomonas aeruginosa* in Bangladesh. So seeds quality are relatively good than most other countries.

In this particular study, AST analysis was done for *Pseudomonas aeruginosa* and *E.coli* .Almost all the samples were tested against the antibiotic group of Amino glycosides, Carbopenem, Beta Lactam, Fluoroquinolone , Macrolides, Phenicol, Tetracycline and Polymyxin. They are resistant against all the amino glycosides groups except gentamicin. This demonstrated that gentamicin can be used with urinary tract, gastrointestinal infection which is caused by *E.coli*. They are also resistant to the aminoglycosides antibiotics which stated that this flaxseed bacterium has the capability to produce enzymes that can inhibit the other aminoglycosides antibiotic. They are resistant to antibiotics like tetracycline, erythromycin and amikacin by which it could be stated that flax seed samples have the capability to produce enzymes for deactivating these two groups of antibiotics similar to the rest of other research. It also showed that they are susceptible to ciprofloxacin which indicates that this antibiotic is potent for inhibiting all the positive samples of *E.coli*. This ciprofloxacin and meropenem could also be very efficient for treating the infection related to *E.coli* and they can play a vital role to treat the resistant tendency of this bacteria.For Imipenem all the seeds were intermediate. Furthermore, among all the positive isolates of *E.coli* isolate number F8 and F9 showed the most susceptibility to four antibiotic group such as amikacin, gentamicin ciprofloxacin and gentamicin which indicated that there are more options to treat them and they have lower chance of infection. For *Pseudomonas. aeruginosa*, all positive 21 sample showed resistant towards tazobactam that was antibiotic disc from group lactam .

In this paper flax seed was the only seed category contaminated with *E.coli* which indicated that they were possibly more contaminated with fecal contamination. They can also be in contact with the manure for cattle, sewage, or in maintained distributed water during cultivation. Here, only 13.7 % of the samples were contaminated with *E.coli* and 42% of the samples were contaminated with *Pseudomonas* aeruginosa. For *Pseudomonas aeruginosa*, most contaminated sample based on the quantity was found on the sesame category and sunflower seed was most contaminated by percentage followed by flax seed which stated that this sample is more contaminated with environment contamination rather than fecal contamination. It also indicated that the sample might be contaminated with sewage run off , agricultural or industrial run off which formed a biofilm water system. It could be polluted due to storing in cold for a very long time(Bautista, 2014).

Different strains of *Pseudomonas aeruginosa* were captured over the years which showed high resistance for different antibiotic discs. A study by Jamaica showed that very high prevalence of *Pseudomonas aeruginosa* in vegetables along with high resistance of antibiotics. It was found that these samples are very resistant to aztreonam (36.4%)along with cefepime, ceftazidime and tobramycin (Allydice-Francis & Brown, 2012). Another study exhibited the presence of *Pseudomonas aeuginosa* in high numbers among raw vegetables and they were analyzed for antibiotic susceptibility tests . It has been found that isolates from *Pseudomonas aeruginosa* were

42% resistant to aztreonam 1.8 % of meropenem followed by 0.6% resistance to piperacillin and ceftazidime and for the rest of the sample showed susceptibility .

In this experiment, isolates which showed positive results also proceeded for AST analysis. As most commonly know six type of seeds were chosen for this study and among them, pumpkin seed showed the most susceptibility 54% followed by sunflower seed (51%) sesame seed (45%) and flax seed (28%). From these, it can be seen that flax seeds were the most resistant type of seeds that showed the *Pseudomonas* aeruginosa found in this seeds were more effective and they have the ability to cause pneumonia ,skin infection , UTI and several skin infection .This seed are also contaminated with *E.coli* indicated that they have came in contact with fecal contamination which make this seed more harmful than rest of the other seed. Also, sunflower seeds have been contaminated with a high percentage indicating that they have been polluted environmentally and no fecal contamination has occurred in this seed. This high amount of contamination could be really harmful for human health and food that has been made with this seed might cause severe sickness .They are more sensitive and have the ability to inhibit the antibiotic and deactivate their beta lactam antibiotic ring. From this data it can be stated that *Pseudomonas.aeruginosa* was mostly resistant to amoxicillin, ampicillin, clavulanic acid and kanamycin and susceptibility showed by ciprofloxacin. Similarly, the AST analysis from the sample showed that they were most susceptible to the meropenem from Carbopenem group which was 85.7% of the result followed by ciprofloxacin from Fluoroquinolone group (95%) .The isolates of *Pseudomonas aeruginosa* was most susceptible to tazobactam group (100%) .High susceptibility for this sample indicated that they this group of antibiotic would be very essential to treat the patient with this *Pseudomonas.aeruginosa* infection.They also showed that there are less chance of treatment failure options are available. Among other antibiotics such as, Aztreonam and ceftazidime are also shown susceptibility like other studies but the percentage of susceptibility are mild (52% and 43%). Some of the antibiotics showed full resistance in the sample , among them clavulanic acid , tazobactam and amoxicillin were fully resistance 100%) followed by 71% for azithromycin to the isolates of *Pseudomonas aeruginosa*. It indicated that these antibiotics would not be applicable for treating the *Pseudomonas* infection. Other discs such as ceftazidime cefepime and aztreonam showed resistance of 38 % ,24% and 14% of resistance .

Occurrence of these genes is recorded more frequently and beta lactam could make an impact on public health .This study was comprised of the result of the ESBL gene which are *bla TEM*, *bla IMP*, and *bla NDM*, gene and these are normally Carbapenem resistant. The isolates which showed resistant to the Carbopenem antibiotic group and beta lactam resistant antibiotic group which were picked for detection. It has been seen that among 7.8% (n=4) of the isolates from *E.coli* showed positive for the *bla TEM*, which is relatively lower than the occurrence of this

gene in most of the other countries. Here is the diagram which showed the difference of prevalence for three ESBL producing gene from *E.coli* and *Pseudomonas.aeruginosa*

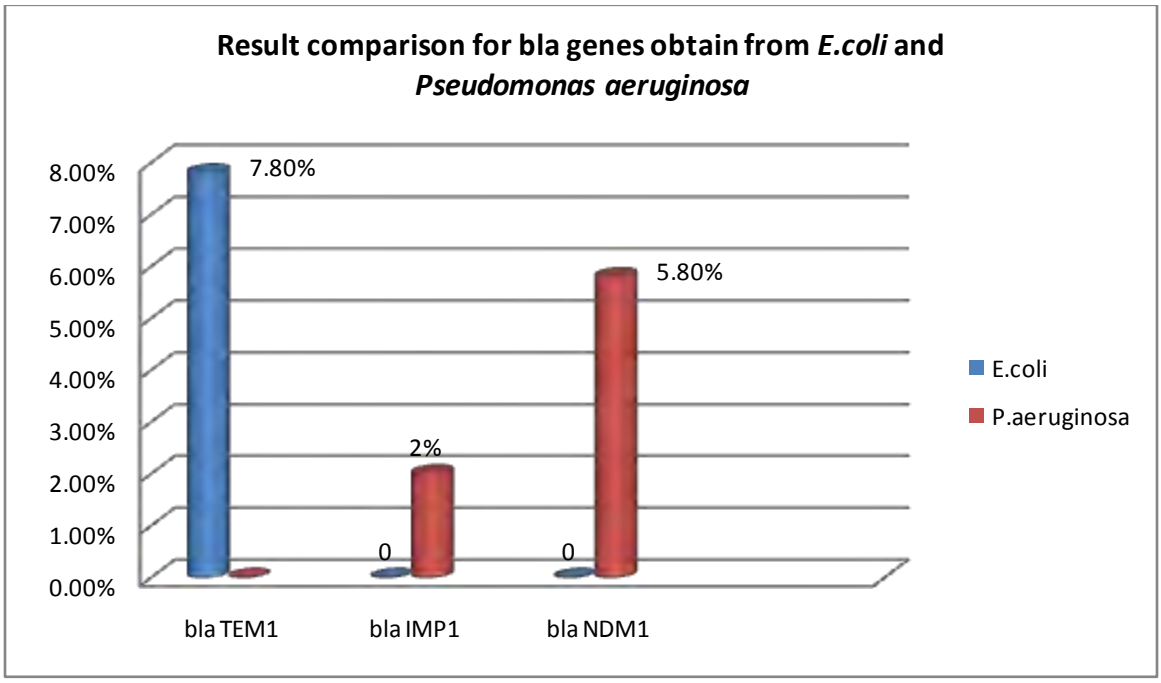


Figure 12: Comparative analysis of bla gene appearance for *E.coli* and *Pseudomonas aeruginosa*

This diagram showed that four of the *E.coli* isolates were positive for *bla TEM* gene and rest of them do not show the prevalence which indicated that they were not capable of producing ESBL enzyme and negative result for *bla IMP*, and *bla NDM*, showed that negative isolates were not capable of producing enzyme or there might be a hidden gene in the result. Furthermore, For *Pseudomonas* 2% of the sample (n=1) showed positive from 21 isolates for *bla IMP*, and 5.8% prevalence of *bla NDM*, . Overall it showed that both Imp and *NDM* gene were present and they can produce carbopenemase and New Delhi Metallo Beta lactamase 1 enzyme. .

Although prevalence rate is lower than the most of the other countries for both *bla IMP* , and *bla NDM*, gene .Among them *bla IMP* gene is one of them which is found already in 12 countries in Asia named china , India , Malaysia, Turkey ,Thailand , Iran Japan , Korea., Nepal, Vietnam, This gene is found most frequently in China and Japan (36%) and (25%). They were obtained from *Pseudomonas aeruginosa* and most of the sample was assembled from the hospital isolate. In a study by Fallah and Noori ,the strains of different bacteria were identified from a sample in which it has been noticed that 3..86% of the sample were *bla IMP*, and none of the isolates was containing *bla NDM* gene. The finding was accurate since this isolates were resistant to the beta

lactam group of antibiotic disc named tetracycline, ampicillin and tazobactam (Fallah et al., 2014).

Another study showed that prevalence of *bla IMP* occurred in 23% in Japan and 7% in France and 17 % in china analyzed from 171 isolates of hospital in which it has been noticed that the isolates which are producing ESBL gene are resistant to CIP. Those isolates were from *E.coli* and *Klebsiella* and they showed positive result of *bla TEM* (86%) *bla CTX* of 78% of the gene. Research conducted in Indonesia showed that *bla CTX* and *bla TEM* were present (40%)t in the sample from the broiler chicken.Here the samples were from the *E.coli* isolate and they were resistant to the beta lactam group of antibiotics. Moreover, in India several report showed that NDM_1 variant 66% of *E.coli* isolates was positive for *bla* NDM_1 variant and 75% of isolates were from *Klebsiella pneumonia* (Nagaraj et al., 2012).A study by Ahmed and Khalid showed that 5-18% of prevalence of *bla* NDM_1 variant in India and Pakistan hospital (Ahmad et al., 2018). In a hospital in India showed the presence of *bla* NDM_1, Among 34 isolates , 13 of them were positive for the antibiotic resistant *bla TEM* which is 38% and their band size was 1080bp (Faridah et al., 2023).

bla TEM is often known as the most founding gene and several study has shown that it occurred very often in patients worldwide. In a study by sah and dahai, It has been shown that *bla TEM* prevailed in high number among the isolates. This could be found on both hospital isolate and vegetable samples. Another study by usui it has been noticed that 7.7 of vegetable sample contain *bla TEM* gene Among 1050 urine samples, 165 of *E.coli* isolates were obtained as positive for *E.coli* (Usui et al ,2019). These samples were from people above 60 years old. The isolates which were resistant for ampicillin from beta lactam group were tested by pcr. It has been seen that overall 54.8 % samples were positive for the *bla TEM* gene (Sah et al., 2024). Another type of gene is *bla* NDM_1 gene that was found in Delhi and it encodes the *NDM* enzyme.
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In china the prevalence of *bla NDM1* has been noticed in many regions. A study by Wang and Li found that *bla NDM* gene obtained upon 0.16% of isolates which was isolated from the *Klebsiella pneumonia* bacteria (Wang et al., 2013). Furthermore, research conducted by nahid and khan demon stated the presence of *bla NDM* gene in 23.6% of isolates collected from 356 samples (Nahid & Khan, 2013). This *bla NDM* gene has prevailed and shown in many cases in china. Another research was operated by Liu and Chavda which focused on the sample collected from the *E.coli* isolates. In another study showed the prevalence of 29% in Japan In this study, Carbopenem resistant *E.coli* were used from which 98% of the isolate indicated the presence of the *bla NDM* of different variants. Most prevailed variant was *bla* NDM_5 which showed 58.5 % of prevalence and second most was *bla* NDM_1 which showed 26% of the presence from total. Other variants were also found such as *bla* NDM_7 and *bla* NDM_4 and *bla* NDM_9. In 2017, the presence of *blaNDM* was also detected in the sample collected from the German hospital .This sample

showed high prevalence (68%) of *bla NDM*$_1$ gene (Weber et al., 2019). Their prevalence described below in the diagram :

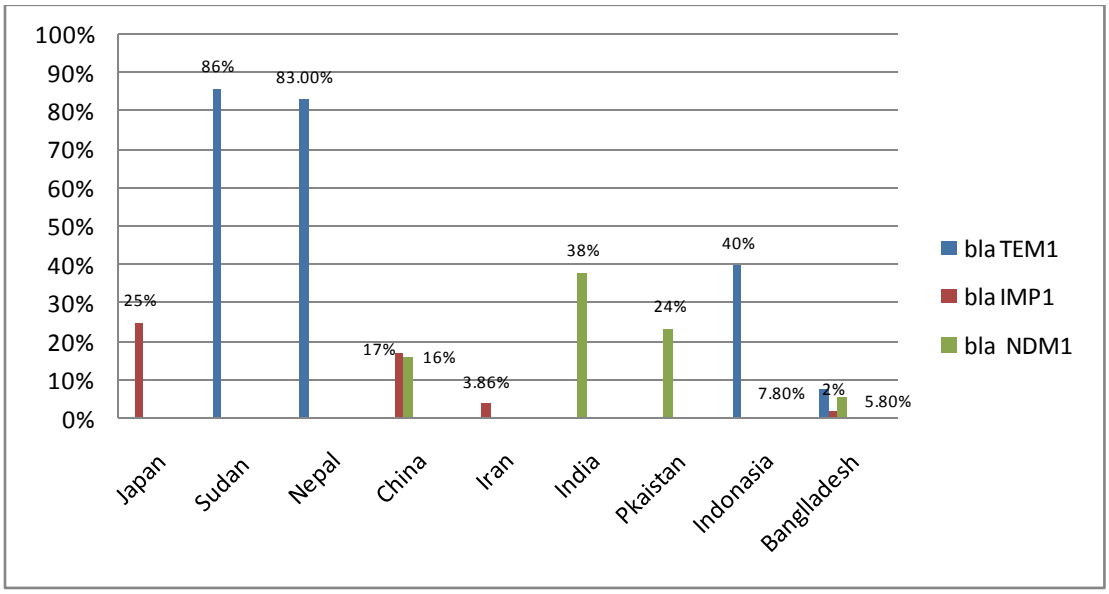

Figure 13: Prevalence of ESBL gene among different countries

Here, the most prevailed result showed on Sudan for *bla TEM* gene which showed 86% occurrence for these gene followed by Nepal which was 83% of the prevalence *bla IMP* gene was found mostly in Japan and Bangladesh has the lowest prevalence for all three type of genes .

5 . Conclusion

The usage of edible seeds is very common for its nutritionpurposes but prevalence of different microorganisms may cause potential health hazards for consumers. In this study, the most prevalent organism was *Pseudomonas aeruginosa* which was caused by several environmental pathways. Contamination has been detected by various confirmatory tests in this paper which showed the prevalence of bacteria in edible seeds in Bangladesh. Several issues are very common which need to be dealt with to reduce the rate of contamination. Among them wastewater treatment is a major issue for contamination. In developing countries it is a common practice of reusing the wastewater in fields to the urban areas. This waters often carried several dangerous particle such as industrial and institutional liquids along with groundwater, surface water that contain toxins , organic and inorganic particles .Using this water may eventually cause occurrence of several microorganism such as *Shigella ,Salmonella, Entamoeba* etc.This water could also mixed with different stream of water such as river ,pond which makes it more

contaminated. Furthermore, water from irrigation could be a major vehicle for contamination and source of several pathogens. They often can get mixed with the water of rainfall that came in contact with the rooftop that carried microorganisms such as *Listeria, E.coli, Salmonella* etc. The improvement of the irrigation system is essential to maintain better crop quality and eliminate essential pathogens. Here ,drip irrigation is a unique technology which provides the necessary nutrients direct to the plant. A strip of vegetation can be essential which will eventually remove the unnecessary pollutants from the irrigation system. Along with these, rotational grazing might be beneficial to remove the cattle from the field that might stop the inoculation of manure around irrigation system. The soil contamination can play a major role in the prevalence of the microorganism for infeectng seeds. To keep the irrigation system better, the manure pile should be kept far so that they cannot leach out to the irrigation system. A better diversion system would be very fruitful to separate the rain water and upper slopes from the irrigation system (Modderman, 2020). Utilizing an excessive amount of manure to the soil may cause soil contamination. Before using it to the soil, it is necessary to ensure that manure is dry enough and composed well so that they cannot be the reason for soil contamination.

During the dying process of seeds, contamination may occur as they are kept on the floor and often do not use any precaution for keeping seed away from dust. So it should be ensured that they have been soaked under the proper condition. Furthermore, the process of harvesting required a lot of equipment for conducting the processing and they could be the origin of contamination. For these, they should be cleaned properly and maintained after every few weeks. A lot of time farmers do not take proper way to decontaminate their equipment for many years which is why they could be a major reason for spreading various types of harmful pathogens.

When they are harvesting, the environmental condition should be checked as often polluted air and water might be the reason for the contamination. For these, the quality of water supply should be checked over time and their source should be as pure as possible. In developing countries harvesting is often done in a field which is close to the cattle farm. That is why there is always a chance that seeds are contaminated by the wastage or nonfood particles produced by cattle farms. It is necessary to take care of the wastage of other farms near the field and it should be made sure that they are handled with proper manpower.

Furthermore, most of the retailer market from which samples collected did not maintain the standard of proper handling as they were kept open most of the time. Along with these, they were stored in the dark place which might be another reason for spreading contamination from both of these microorganisms. Along with these, the proper temperature and condition for seeds storing are not maintained as it often requires keeping edible seeds in cold temperature. For these they should be kept in proper temperature when they are stored for a long time. The selling place should be open while maintaining proper cleanliness and dryness. A lot of microorganisms such as fungi and mold can grow in dark places. For these, the lighting of the room should be

maintained properly. From early ages many outbreaks have occurred due to the mice and different types of parasites. In storage the edible seeds were not often kept in a sealed package which is why they might come in contact with rats and many other types of wild animals by which different types of microorganism such as *Campylobacter spp ,Salmonella , E.coli* might be developed. So it should be monitored that the seeds are stored in a proper place and sealed bags are used.

Contamination might be spread during the harvesting time from workers .Many times farmers worked with various types of wound and lesions which became the reason for the occurrence of pathogens such as fungi and molds. *Pseudomonas aeruginosa* often causes different types of skin infection to the human being. It should confirm that farmers ,workers and sellers are taking proper precaution during working such as washing their hands, covering their wounds and sellers and retailers should be wearing gloves when they are maintaining the seedling and sprouts so that the chance of contamination would be minimized and they remain in good condition. In this study the main focus was to ensure the level of contamination which has occurred for many years in seeds. Different types of detection methods were used and it has been ensured that seeds are contaminated by *Pseudomonas aeruginosa*, stating the quality of seeds has been damaged by the environmental issue.

In AST analysis, a lot of samples showed resistance towards Carbapenem and beta lactam antibiotics but appeared to have negative results for gene detection which demonstrated that the outer membrane protein of the organism might be deleted. For these reasons they could not function properly.Another reason could be decreased cell permeability for the organism. For *E.coli* some of the gene was not detected but showed resistance which might be due to the lower affinity of penicillin binding protein for the organism which is why they could not bind and were not susceptible (W et al., 2018).

Moreover, The obtained results showed that the contamination level of seeds are very high and in many cases flax seeds are high at risk of consumption. The awareness about handling and maintaining its quality during transportation, processing is necessary so that consumer can get the proper benefit from this material when they are consuming it without getting sick. Government of this country should take proper steps to spread the awareness about maintaining these edible seeds and more organizations should be involved to maintain the proper quality of edible seeds. In rural areas several NGOs operates and conduct several awareness programmes. The collaboration between the local governments and NGO could be really beneficial for the people to know about necessary precautions and learn necessary measurements to take them as soon as possible.

6.Reference

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