Risk of antibacterial resistance in street-vended and expired food items collected from different places of Dhaka city, Bangladesh.

A Dissertation submitted to the Department of Pharmacy, East West University, as the Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy.

Submitted by

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Declaration by the Research Candidate

I, Nargis Islam, hereby is declare that this dissertation entitled "Risk of antibacterial resistance in street-vended and expired food items collected from different places of Dhaka city, Bangladesh" submitted to the Department of Pharmacy, East West University, in partial fulfillment for the requirement of the Degree of Master of Pharmacy, is an authentic research work done by me under the guidance of Professor Dr. Sufia Islam, Department of Pharmacy, East West University, Dhaka Bangladesh. The content of this dissertation in full or in parts, have not been submitted to any other Institution or University for the award of any Degree or any Diploma of Fellowship.

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Certificate by the Supervisor

This is to certify that the dissertation, entitled "Risk of antibacterial resistance in street-vended and expired food items collected from different places of Dhaka city, Bangladesh" is a bona fide research work done under our guidance and supervision by Nargis Islam (ID # 2015-3-79-005), in partial fulfillment for the requirement of the Degree of the Master of Pharmacy.

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Dedication

This research paper is dedicated to my

respected Thesis Supervisor

&

my beloved family

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Abstract

Street food contamination has become an important public health issue and a great concern to everybody. This is due to the lack of an adequate understanding of the basic food safety issues. The place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors are the major sources contributing to microbial contamination. Street foods are available in almost each and everywhere such as in a market, fair, park or other public places. The popular street foods are phucka, chotpothi, belpuri, samucha, daalPuri, lassi, pakura, halim and many more. Expired foods are those which are not safe to eat and the goal is to ensure quality for a period of time after buying it. Food borne illnesses of microbial origin are a major health problem associated with street foods. People, who patronize street food, have been reported to suffer from food borne diseases like diarrhea, cholera, typhoid fever, jaundice and food poisoning. Therefore, microbial contamination, food safety, street food condition and other factors are important to avoid street food contamination. Identified foodborne bacteria and antibiotic resistance isolates can create a public health problem. Therefore, the present study was designed to evaluate the antibacterial susceptibility against the microorganism present in the street and expired food items in different areas of Dhaka city, Bangladesh. Twenty five (25) samples were taken from different places in Dhaka city. The tested samples were singara, samucha, noodles, fuchka, mayonnaise, kabab,beguni, sandwich, chicken shashlik, chicken patties, vegetable, and different types of biscuits, ghugni and many more. All these are native foods available in the street and different places.We have used the seven antibacterial discs such as ciprofloxacin, erythromycin, gentamycin, kanamycin, penicillin G, tetracycline, and vancomycin. Our study shows that penicillin G and vancomycin are resistant antibiotics against the isolated bacteria. Erythromycin and tetracycline are intermediate antibiotic that means a higher dose of the antibiotics is needed to prevent the bacterial growth. Gentamycin, kanamycin and ciprofloxacin showed susceptibility against the microorganisms. The study confirms considerable contaminations in street/ expired foods and antibiotic resistance have been identified against the microorganisms.

Key words: Street foods, expired foods, antibacterial sensitivity.

Chapter 01

Introduction & Literature Review

1.1 Street Food

Street food is exactly means the food available in a public place, such as from a vendor on a street. Typically, street food is tasty, ready-to-eat food or drink sold on the street, in a market, fair, park or other public place. It is sold by a hawker or vendor from a portable stall, cart or food truck (Streetfoodinstitute.org, 2013).With variations within regions and cultures street food vending is found across the world. Sold by vendors and peddlers street food is the ready to eat food or drink sold on street and public spaces (Nasvinet.org, 2017).Vendors usually use portable booth, food cart or truck to sale the food items. The chief characteristics of street food is that street foods are reasonably priced and flavored and easily available. Street food started in Asia, is massive in America, and now Europe's getting a taste of the action too (Street Food UK, 2014).

Fig 1.1: Street-vended food

1.2 Features of street foods in Bangladesh

From ancient Greece to Pompeii, from China to Turkey, street food has a long and colorful history. Today, it has become an urban mainstay in large cities and small towns alike, and continues to evolve and tempt the passersby on streets around the world(Streetfoodinstitute.org, 2017) Street foods are very much popular in the developing countries today. Its expansion is linked with urbanization and the need of urban populations for both employment and food. As a developing country a lot of street foods are found in the street and open places in Bangladesh

(especially in Dhaka). Street foods are very much popular to the Bangladeshis living in the urban areas due to its cheap price and sharp taste (Akheruzzaman, 2014).

1.3 Common problems of street foods

There are 128 varieties of street foods found in Bangladesh. Among them phucka, chotpothi, belpuri, samucha, daalPuri, lassi, pakura, halim are most popular (Akheruzzaman, 2014). Despite the importance of the street food and the street food vending the fact that the street food vending involves many problems cannot be ignored. The present condition in which most of the street food vendors cook and sale are unsuitable.The place is not clean, well lit and far from source of contamination. Most of the street food vendors do not practice hygienic method of covering food and water. Food not covered is exposed to flies, birds, rodents etc which may cause food borne pathogens. Street food vendors also lack proper food handling and waste disposal training.A majority of food vendors are not aware of causes of food poisoning and food borne disease which can spread through their food if not handled properly. Many studies have indicated that Water is a critical raw material in many street-vended operations (Nasvinet.org, 2017).

1.4 Expired foods

Most consumers mistakenly believe that expiration dates on food indicate how safe the food is to consume, when these dates actually aren't related to the risk of food poisoning or foodborne illness. The actual term "Expiration Date" refers to the last date a food should be eaten or used (Sifferlin, 2013).

According to USDA Expired Food describe as:

‘Sell by’ this is the date by which manufacturers suggest that retailers remove the product from shelves. The goal is to ensure quality for a period of time after you buy it. That can be several days to several weeks, depending on the item. For instance, milk, assuming proper refrigeration, should last five to seven days past its sell-by date before turning sour.

‘Best by’ and **‘use by’** those terms tell you when to eat (or freeze) a product for the best quality. A jar of salsa may not taste as fresh and tangy as it’s supposed to, for example, and crackers may be soft instead of crisp after those dates (Consumerreports.org, 2015).

"Guaranteed fresh" date This usually refers to bakery items. They will still be edible after the date, but will not be at peak freshness.

"Pack" date This one is for canned or packaged goods, as a rule, but it's tricky. In fact, it may be in code. It can be month-day-year-MMDDYY. Or the manufacturer could revert to the Julian calendar. January would then be 001-0031 and December 334-365. It gets even weirder than that. (Lawrence, 2015).

In most cases, manufacturers decide on dates and terms based on their own product testing. According to a report from the NRDC and Harvard University, they use a number of methods, such as lab tests and taste testing, to set them. And consumers have no way of knowing the background. In many cases, dates are conservative, and if you go beyond them, you may not notice any difference in quality, especially if the date has recently passed (Consumerreports.org, 2015).Street foods often reflect traditional local cultures and exist in an endless variety. There is much diversity in the raw materials as well as in the preparation of street food beverages, snacks and meals.The street foods also play an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income groups and are appreciated for their unique flavors and convenience. Most street foods are considered both finger food and fast food and are more reasonably priced than restaurant meals. 2.5 billion people worldwide eat street food every day, according to a 2007 Food and Agriculture Organization study. Street foods also assure food security for low income urban population and livelihood for a significant proportion of the population in many developing countries. Street foods are described as wide range of ready-to-eat foods and beverages or prepared at home and consumed on the streets without further preparation. These food items are usually sold by vendors and hawkers in the streets or other similar public places. While street vended foods are appreciated for their unique flavors as well as their convenience, they are also important in contributing to the nutritional status of the population. In contrast to these potential benefits, it is also recognized that street food vendors are often poor, uneducated, and lack knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand

washing, sources of raw materials, and use of potable water. Consequently, street foods are perceived to be a major public health risk (Redzwan Habib, 2017).

1.5 Safety of Street Foods

Some studies on street foods chemical composition have shown that a several numbers of banned food additives were being used by the street vendors in different food preparations. Presence of banned coloring like Metanil Yellow, Orange II, Rhodamine B, Auromine Orange G were detected in street foods found in Dhaka. Non-food-grade additives such as textile coloring agents were used to make the appearance of the street foods better. Street food is harmful for our health because when the sellers make some alur chop, samucha, beguni, singara, they use burnt oil. They also use harmful chemical in food to delicious. Street foods are mainly prepared from flour, meat, fish, vegetables, egg etc. These ingredients often contain microorganisms. That's why most of the time the microbial condition of the street foods are not satisfactory. These foods are prepared in open places by the street vendors. The water they use is not filtered rather it contains bacteria and microorganisms like *E. coli* speciesof*Alcoligeus, Proteus, Bacillus, Salmonella.* Due to the handle of foods with unclean hands microbes get contaminated with these foods. Many street food sellers they don't cut their nail regularly but we know that in nail 150000 Lakh bacteria can be remain there. Most of the time vendering machine and utensils are not cleared properly so the undesirable microbes take place in the street foods.A study of BCSIR shows that freshly squeezed or freshly prepared fruit juices sold by local market vendors in Dhaka city contain a lot of microbes (Rane, 2011).

1.6 Food Borne Illness

Food borne illnesses of microbial origin are a major health problem associated with street foods. In addition, resistance of foodborne microorganisms in multi-drug made the food safety situation more vulnerable in public health. Approximately, 30 million people in Bangladesh are suffering from foodborne illnesses each year. Diarrheal diseases are the most common food poisoning cases in Bangladesh and in some cases, these can cause death. The diseases are caused by either toxin from the microbe or by the human body's reactions to the microbe. The traditional processing methods that are used in the preparation, inappropriate holding temperature, and poor

personal hygiene of food handlers are some of the main causes of contamination of street foods. Also the foods are not effectively protected from flies and dust. In Bangladesh, street foods are mostly prepared and processed manually and sold to the public at various lorry terminals, by the roadside or by itinerant vendors. A study of the socioeconomic conditions and determination of the hygienic and sanitary practices of street food vendors in Dhaka City Corporation was carried out by FAO 2010. The study result demonstrated that 25% street food vendors are illiterate and cannot write their names and have no formal education. As street food business requires low investment, most of the vendors (88%) were found to own the business. They reportedly work for 13–18 hours a day without having toilet facilities. Most of the vending shops (68%) were located on the footpath irrespective of areas surveyed and 30% vending carts were placed near the municipal drain and 18% near the sewerage. Microbiological study of different foods items, drinking water, and hand swab samples showed the prevalence of overwhelmingly high numbers of aerobic bacteria, coli form bacteria, and pathogens. People, who patronize street food, have been reported to suffer from food borne diseases like diarrhea, cholera, typhoid fever, jaundice and food poisoning (CDC, 2016).

Table 1.1 Causes of Food Borne Diseases

Group of Pathogen	**Example of Pathogen**	**Sign & Symptoms**
Bacteria	*Salmonella* spp. *Campylobacter jejuni* *Shigella*spp. STEC O157:H7 *Listeria monocytognes* *Vibrio* spp. *Yersinia* spp.	Abdominal pain; diarrhea; chills; fever; nausea; vomiting;chills; dehydration; jaundice;Anorexia.
Parasites	*Cryptosporidium* spp. *Cyclospora*spp. *Trichinella*spiralis *Giardia lamblia* *Toxoplasma caris* *Entamoebahistolytica*	Severe diarrhea; low grade fever and severe intestinal distress; abdominal pain; cramps.
Toxins **Enterotoxins**	*Staphylococcus aureus* *Clostridium perfringens* *Bacillus cereus*	Abdominal pain, diarrhea, vomiting, nausea,

Botulinum toxins	*Clostridium botulinum*	Vertigo; double vision; difficulty in swallowing, Speaking and breathing; weak muscles; respiratory paralysis, frequently fatal.
Fish toxins	Scombrotoxin Ciguatera toxin Paralytic shellfish toxin	Abdominal pain, dizziness, vertigo, numbness,tingling, and muscle pain, diarrhea, nausea, vomiting.
Group of Pathogen	**Example of Pathogen**	**Sign & Symptoms**
Mushrooms	Amatoxin Phallotoxin	Severe seizures of abdominal pain, persistent vomiting And watery diarrhea, extreme thirst, and lack of urine production.
Miscellaneous	Niacin Monosodium glutamate	Visual hallucinations and ataxia in a child.

(Jay, Loessner and Golden, 2005)

1.7 Microbial contamination in street food

Street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. Major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors. Various studies have identified the source of food safety issued involved in street foods to be microorganism belonging to the genus *Bacillus, Staphylococcus, Clostridium, Vibrio, Campulobacter, Listeria, Salmonella* (Rane, 2011).

1.8 Antibiotic sensitivity

An antibiotic sensitivity or susceptibility test is done to help choose the antibiotic that will be most effective against the specific types of bacteria or fungusinfecting an individual person(Cold et al., 2017).A sensitivity analysis is a test that determines the "sensitivity" of bacteria to an antibiotic. It also determines the ability of the drug to kill the bacteria. Sensitivity analysis starts with a bacterial sample. The grown bacteria is known as a culture and bacteria in the culture will grow and multiply. The bacteria will form colonies, or large groups of bacteria, that will each be exposed to different antibiotics.These colonies can be susceptible, resistant, or intermediate in response to the antibiotics:

- Susceptible means they can't grow if the drug is present. This means the antibiotic is effective against the bacteria.
- Resistant means the bacteria can grow even if the drug is present. This is a sign of an ineffective antibiotic.
- Intermediate means a higher dose of the antibiotic is needed to prevent growth.

It is possible for bacteria and other pathogens to mutate. Antibiotics that work today may not work six months from now. Sensitivity tests are extremely important and useful tools, especially if the infection is caused by bacteria that has become resistant to some treatments (Case-Lo and Wu, 2016).

1.9 Zone of Inhibition

A Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth.With this method, approximately one million cells from a single strain are spread over an agar plate using a sterile swab, then incubated in the presence of the antimicrobial object (ex: an oxacillin disk, pictured below). If the bacterial or fungal strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on the agar plate, such as on the agar plate on the left-hand side of the photo below. If it is resistant to the antimicrobial agent, then no zone is evident, such as on the agar plate on the right-hand side of the photo below (Microchemlab.com, 2015).

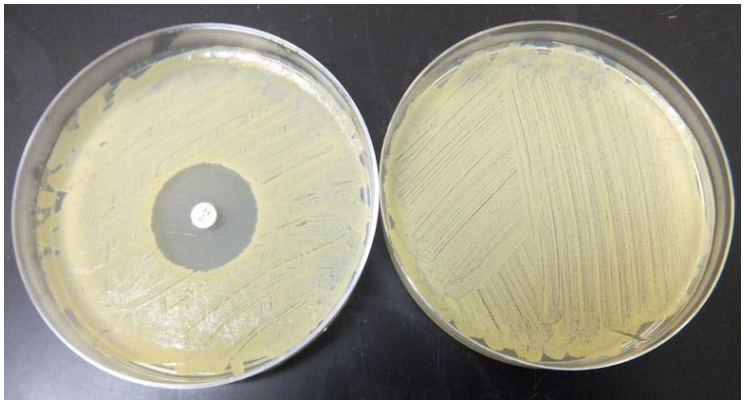

Figure1.2: Left Plate is of S. aureus with Oxacillin disk, Right Image is lawn growth of *S. aureus.*

1.10 Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria

Susceptibility determination is one of the most important procedures undertaken in clinical microbiology laboratories. Whilst the action of a product from one organism inhibiting the Several procedures utilizing the ability of an antimicrobial agent to diffuse through agar and inhibit the growth of the test bacterium were described in the early days of development and use of new antimicrobial agents. Most variations in the procedure were the application of the antimicrobial agents to the agar, such as cutting wells into the agar and cylinder plate. In 1994, Vincent and Vincent used filter paper discs impregnated with penicillin, and in 1945 Mohs described the radial streak method using 15 mm discs and the use of a sensitive control organism. The use of tablets instead of filter paper discs was first described by Hoyt &Levine in 1947.in early years of antimicrobial susceptibility testing there was no standardization between methods or even between laboratories. This problem was recognized by Gould & Bowie in 1952, WHO described a technique comparing the zone diameters produced by varying concentrations of antimicrobial agent incorporated on paper discs against control organisms. The test organism was then examined against a disc containing a single concentration, and the zone diameter compared with those produced by the control strains. Stokes describes a disc diffusion procedure

whereby the zone diameter of an antibiotic against a test organism and control organism could be compared on the same agar plate. Variations of the "Stokes test" are still the most commonly used method for routine susceptibility testing in clinical laboratories in Britain today. In 1966 Bauer et al. described standard procedures for performing disc susceptibility test and compared zone diameters with the minimum inhibitory concentrations. The continued need to standardize procedures not just between different laboratories but between different countries has led to various groups describing standard methods (Laura, 1990).

1.10.1 Agar disk-diffusion method

This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth (Tendencia, E. A. ,2004).

Agar disk-diffusion testing developed in 1940 is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing . Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, *Haemophilusinfluenzae*, *Haemophilusparainfluenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones .

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are

measured.the table shows the growth media, temperature, period of incubation and inoculum size required by CLSI standards (Balouiri, Sadiki and Ibnsouda, 2015).

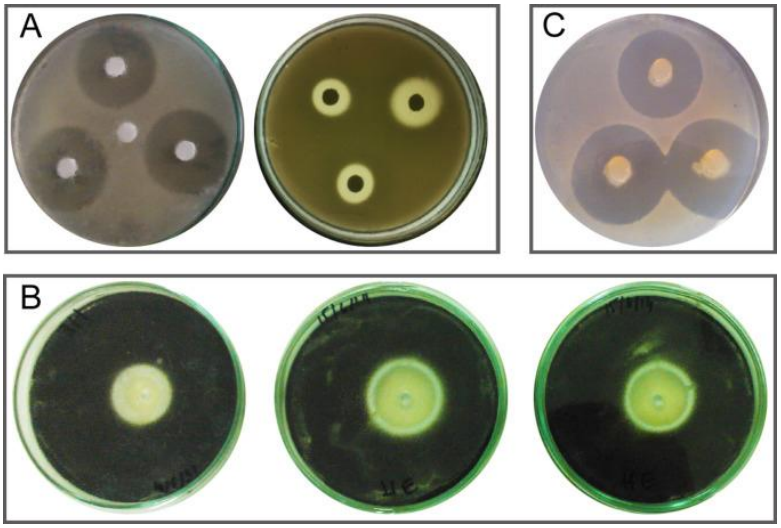

Fig 1.3 : Agar diffusion methods: (A) disk-diffusion method of microbial extract using *C. albicans* as test microorganism, (B) agar well diffusion method of essential oil using *Aspergillusniger* as test microorganism, and (C) agar plug diffusion method of *Bacillus* sp. against *C. albicans*.

1.10.2 Dilution Method

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited is recorded as the minimal inhibitory concentration or MIC. The MIC is thus the minumum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth. A procedure similar to broth dilution is agar dilution. Agar dilution method follows the principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited.

1.10.3 E-test

E-test is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein. This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.

1.10.4 Automated Antimicrobial Susceptibility Testing System

Several commercial systems have been developed that provide conveniently prepared and formatted microdilution panels as well as instrumentation and automated reading of plates. These methods are intended to reduce technical errors and lengthy preparation times. Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading and interpretation. Some examples of these include: Vitek System (bioMerieux, France), Walk-Away System (Dade International, Sacramento, Calif.), Sensititre ARIS (Trek Diagnostic Systems, East Grinstead, UK), Avantage Test System (Abbott Laboratories, Irving, Texas), Micronaut (Merlin, Bornheim-Hesel, Germany), Phoenix (BD Biosciences, Maryland) and many more.

1.10.5 Mechanism-Specific Tests

Resistance may also be established through tests that directly detect the presence of a particular resistance mechanism. For example, beta lactamase detection can be accomplished using an assay such as the chromogenic cephalosporinase test and detection for chloramphenicol modifying enzyme chloramphenicol acetyltransferase (CAT) may utilize commercial colorimetric assays such as a CAT reagent kit (Remel, Lenexa, Kansas).

1.10.6 Genotypic Methods

Since resistance traits are genetically encoded, we can sometimes test for the specific genes that confer antibiotic resistance. However, although nucleic acid-based detections systems are generally rapid and sensitive, it is important to remember that the presence of a resistance gene does not necessarily equate to treatment failure, because resistance is also dependent on the mode and level of expression of these genes 11.

Some of the most common molecular techniques utilized for antimicrobial resistance detection are as follows:

1.10.7 Polymerase chain reaction (PCR)

This is one of the most commonly used molecular techniques for detecting certain DNA sequences of interest. This involves several cycles of denaturation of sample DNA, annealing of specific primers to the target sequence (if present), and the extension of this sequence as facilitated by a thermostable polymerase leading to replication of a duplicate DNA sequence, in an exponential manner, to a point which will be visibly detectable by gel electrophoresis with the aid of a DNA-intercalating chemical which fluoresces under UV light.

1.10.8 DNA hybridization:

This is based on the fact that the DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine; or uracil for RNA). Therefore, a labeled probe with a known specific sequence can pair up with opened or denatured DNA from the test sample, as long as their sequences complement each other. If this "hybridization" occurs, the probe labels this with a detectable radioactive isotope, antigenic substrate, enzyme or chemiluminescent compound. Whereas if no target sequence is present or the isolate does not have the specific gene of interest, no attachment of probes will occur, and therefore no signals will be detected.Modifications of PCR and DNA hybridization. With these basic principles, several modifications have been introduced which further improve the sensitivity and specificity of these standard procedures. Examples of such development were the use of 5'-fluorescence-labeled oligonucleotides, the development of molecular beacons, development of DNA arrays and DNA chips, among many others (Amrls.cvm.msu.edu.,2011).

1.11 Factors affecting Antibiotic Susceptibility Testing

Many conditions can affect the accuracy of the AST results, which is described in detail below.

1. pH

pH of the medium is an important factor which influences the accuracy of the antibiotic susceptibility testing. If the pH of the medium is too low than the desired pH, certain drugs such

as amino glycosides, quinolones and macrolides lose their potency, on the other hand, antibiotic classes such as tetracyclines appear to have excess activity a lower Ph and the vice versa happens in the case of the higher pH.

2. **Moisture**

The presence of moisture content on the medium can counter act with accuracy of the susceptibility testing. It is important to remove the excess moisture present in the agar surface, by keeping it in the laminar flow hood for few minutes.

3. **Effects of medium components**

If the media selected for the antibiotic susceptibility contains excessive amounts of thymine or thymidine compounds, they will reversibly inhibit the action of certain antimicrobial agents such as trimethoprim groups. This reversible inhibition yields smaller or less distinct or even no zones and will be misinterpreted as resistant antibiotics. MHA is low in thymine and thymidine content and it can be used successfully to study the susceptibility of antibiotics. Also the medium containing excessive cation reduces the zone size, while low cation content results in unacceptably large inhibition zones.

4. **Amount of organism**

The amount of the organism used for the susceptibility testing is standardized using a turbidity standard. This is obtained by a visual approximation using McFarland standard of 0.5 or else it can be determined by using a spectrophotometer with Optical density of 1 at 600 nm wavelength. In addition to this, the antibiotic concentration for the susceptibility testing is pre-determined and is commercially available (vlab.amrita.edu,.2013).

Literature review:

A study in Mexico city shows that a potentially pathogenic agents named Nontuberculous mycobacteria (NMT) has been found in a natural ecosystem and for this reason food is considered another source of NMT transmission for humans. This study was done to evaluate the microbiological quality and occurrence of NMT in fresh-squeezed orange juice samples purchased from different street vendors. Total 102 samples were analyzed and all showed positive result for aerobic mesophilic bacteria (AMB). A total of 55% total coliform, 25% for fecal coliforms and 13% for Escherichia coli were detected in the sample. The study suggested that fresh-squeezed orange juice might represent a vehicle for NMT transmission in humans and so proper handling and washing of orange is recommended to prevent the contamination during juice preparation (Cerna et al,2016).

In Bangladesh a study has been done on microbiological contamination of street vended foods, where samples were taken from different places in Dhaka city. The main objective of this study was to identify the presence of common pathogens and to describe the molecular characterization of *E.coli*. From two sampling locations in total 50 food samples were tested for the presence of microorganisms.To detect the pathogenic *E.coli* DNA was isolated for the molecular characterization by PCR. Twelve percent (12%) samples were confirmed to contain different species of *E.coli*and *Shigella*. The study suggested that all these enteric pathogens could be the potential cause for food-borne illness (Islam et al.,2015).

A study has reported on the food safety in Vietnam where food-borne disease were highlighted and attracted a lot of attention in Vietnam.This paper highlights the food safety in Vietnam. Lack of ethics leads to the production and trading of unsafe foods in order to make profits which might produce adverse health effects on customers. Contaminated food contributes to the burden of food-borne diseases and food poisonings in Vietnam which causes panic in population. In this paper they also discussed about good experiences in food safety management from other countries and drew lessons learnt for Vietnam to better deal with the current food safety situation (Hung et al,2017).

Another study in Meknes, Morocco aimed to estimate the proportion of sausages products contaminated with *Salmonella* and to identify serovarsto determine the antimicrobial resistant

patterns of isolates and to detect the invA and SpvC genes. Salmonella were isolated,4 serogroups and 12 serotypes were recovered. The "ACSSuT" penta-resistance patternwas observed in two of the *Salmonella Typhimurium* strains. Finally the study showed that all 34 *Salmonella* were positive for invasion gene invA and negative for the virulence gene spvC (Ed-Dra et al, 2017).

Food borne outbreaks become a worldwide concern which involved the fresh production of fruits and vegetables. Lytic bacteriophage cocktails and levulinic acid produce wash were investigated for their effectiveness against the foodborne pathogens *Escherichia coli* O157:H7,*Shigella*spp, and *Salmonella* on broccoli, cantaloupe, and strawberries. Three treatment were used all were compared against a 200-ppm free available chlorine wash. Potable water and water with an increased organic content of 2.5 g/liter dissolved solids and total organic carbon were used to prepare the solutions. Bacteriophage cocktailsproduce wash (BCPW)was the most effective treatment and the chlorine wash in water with higher organic content was the least effective treatment tested. The finding of this study indicates that the combination of antimicrobial bacteriophage cocktailswith a commercial produce wash is a very effective method for treating produce contaminated with *E.coli* O157:H7, *Shigella*spp, and *Salmonella* even in the presence of high loads of organic matter (Magnone et al, 2013).

An exploratory qualitative study was conducted to identify constraints to microbial food safety policy in Canada and the USA from the perspective of stakeholder groups along the farm to fork continuum. A discussion session were held about constraints to policy development and implementation where 37 stakeholders participated. Despite the plurality of stakeholdersand the range of content expertise, participants perceptions emerged into five common themes like-challenges related to measurement and objectives of microbial food safety policy goals, challenges arising from lack of knowledge, or problems with communication of knowledge coupled with current practices,beliefs and traditions; the complexity of the food system and the plurality of stakeholders; the economics of producing safe food and the limited resources to address the problem and inappropriate inputs to the decision-making process (Sargeant et al, 2007).

A study has been done to evaluate 17 plant essential oils and nine oil compounds for antibacterial activity against the foodborne pathogens *Escherichia coli* O157:H7 and *Salmonella* enteric in

apple juices. Carvacrol, oregano oil, geraniol, eugenol, cinnamon leaf oil and linalool were found as most active against *E.coli*. This study has evaluated that antibacterial agents could be divided into two classes: fast-acting and slow-acting. High-performance liquid chromatography analysis showed that the bactericidal results are related to the compositions of the oils. These studies provided the information about new ways to protect apple juice and other foods against human pathogens (Friedman et al, 2004).

A cross sectional study was conducted to assess the microbiological quality of local food items vended by the school-based street food vendors in Dhaka City. A total of 80 schools from 19 school-zones of Dhaka City and its outskirts were chosen for the study. A total of 110 food samples, one each from 110 school-based street food vendors, were collected for laboratory analysis. Face to face interviews were conducted with the food vendors using a pre-tested questionnaire. The food samples were analyzed for coliform counts in the Public Health Laboratory, Institute of Public Health, Dhaka, which is a national level central food testing laboratory in Bangladesh. Microbiological criteria recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) were considered to classify food samples as 'satisfactory' (total coliforms < 100 per g or ml) and 'unsatisfactory' (total coliforms ≥ 100 per g or ml).This study findings reflected poor microbiological quality for a considerable proportion of the school-based street vended foods indicating a health threat to the school children of Dhaka City (Mohammad et al,2013).

In Ghana, street vending foods are readily available sources of meals for many people but the biological safety of such food is always in doubt. The aim of this study is to ascertain bacterial isolate and determine total counts of bacterial species responsible for the contamination of the street vending food in Kumasi so as to determine the microbiological safety of such a food. This prospective study was conducted among street vending food at four bus terminals in Kumasi. From November, 2008 to February, 2009, 60 food samples comprising ice-kenkey (15), cocoa drink (15), fufu (5), ready-to-eat red pepper (normally eaten with kenkey) (5), salad (10) and macaroni (10) were purchased and analyzed. The food samples were purchased and transported to the laboratory in sterile plastic bags and analyzed for bacterial contamination. Most ready-to-eat foods in Kumasi were contaminated with enteric bacteria and other potential food poisoning

organisms with bacterial counts higher than the acceptable levels. Food vendors therefore need education on food hygiene (*P Feglo& K Sakyi, 2012).*

In Canada, a serious public health problem has been reported with the antimicrobial-resistant *Salmonella* species. The study is based on the antimicrobial susceptibility profiles of *Salmonella* isolates recovered from healthy broiler chickens at slaughter flocks in Alberta which were serotyped and tested for antimicrobials. Among 272 *Salmonella* isolates tested 64.0% were resistant to one or more antimicrobials, 10% were resistant to three or more antimicrobials and 1.8% were resistant to ciprofloxacin and five microbials. All isolates were susceptible to amikacin, amoxicillin, clavulanic acid, ceftiofur,cefoxitin,ceftriaxone,ciprofloxacin and nalidixic acid. Tetracyclinshowed the highest resistance (54.8%) followed by streptomycin(24.2%) and sulfisoxazole (8.4%). Strongest associations were observed between resistance to kanamycin and tetracycline and to amicillin and sulfisoxazole. This study gives a baseline information on antimicrobial susceptibility of *Salmonella* isolates of broiler chickens at slaughter in Alberta that can be a benchmark for further research (Mainali et al, 2014).

In Spain a geographical analysis of how commonly prescribed oral antibiotics are quantitatively and qualitatively responsible for the different local rates of erythromycin and penicillin resistance in *Streptococcus pneumoniae* is presented. From 1998 to 1999 a multicenter surveillance study yielded 1,684 consecutive *S. pneumoniae* isolates from community acquired respiratory infections. Data on antibiotic sales in the retail market for the same period were gathered, and the corresponding defined doses per 1,000 inhabitants per day were calculated. Macrolides and β-lactams were considered separately. Ample variation in both resistance rates and antibiotic consumption was seen. Multivariate analyses showed that integrated consumption of both macrolides and β-lactams accounted well for erythromycin (R^2 = 0.722; P = 0.002) and penicillin (R^2 = 0.706; P = 0.002) resistance. Macrolides were more important drivers for local differences in both erythromycin and penicillin resistance than β-lactams were. Consumption of once-a-day macrolides was key for local erythromycin resistance variations. Cephalosporins were slightly more important penicillin resistance drivers than aminopenicillins were (Cesar et al, 2001).

However, in Bangladesh only the microbial contamination of street foods has been reported and so far in our knowledge, no antibacterial susceptibility testing with the contamination has not

been carried out. Therefore, this present study was designed to evaluate the antibacterial susceptibility against the microorganism present in the street and expired food items in different areas of Dhaka city, Bangladesh.

Chapter 02

Research Objective

2.1 Research Objective:

The objective of this research work was therefore focused on the following point:

To identify sensitivity of different class of antibiotics against different enteric bacteria found from street and expired food.

Chapter 03

Materials & Method

3.1 Sample Collection

About 28 solid food samples were randomly chosen and collected from street vendors from different area. These samples were collected aseptically in different sealed poly bags to prevent their contact with any other source that can contaminate the samples.

3.2 Sample Category

Five different categories of food samples were collected. They were deep fried and fried items (Singara, aluchop, egg chop, pakora, nargiskabab, shikkabab, kathikabab), spicy items (Panifuchka, chhola), noodles, baked items (Cake, danish, biscuit, nimkey) and sweet items (Laddu, goja).

3.3 Sample Processing

Solid samples were crushed by mortar and pestle. Then 5 gm of sample were weighed for each broth.

3.4 Enrichment of the Organisms

3.4.1 Enrichment of *E. coli* and *Klebsiella spp*

5 gm solid sample were mixed well with 45 ml of Trypticase Soy Broth (TSB) + 0.3% yeast extract (YE) and then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37ºC for 18-24 h.

3.4.2 Enrichment of *Salmonella spp* and *Shigella spp*

5 gm solid sample were mixed well with 45 ml of BPW (Buffered Peptone Water) broth and incubated at 37 ºC for 18-24 h.

3.4.3 Enrichment of *Vibrio spp*

5 gm solid sample were mixed well with 45 ml of APW (Alkaline Peptone Water) broth, then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37ºC for 18-24 h.

3.5 Selective Growth of the Organisms

3.5.1 Selective Growth *E.coli* and *Klebsiella* spp

Cotton buds were dipped into the enrichment broths and swabbed onto MacConkey and TBX(Tryptone Bile X-glucuronide) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism.

3.5.2 Selective Growth of *Salmonella spp* and *Shigella* spp

Cotton buds were dipped into the enrichment broths and swabbed onto BGA (Brilliant Green Agar) and XLD (Xylose lysine deoxycholate) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism.

3.5.3 Selective Growth of *Vibrio spp*

Cotton buds were dipped into the enrichment broths and swabbed onto TCBS (Thiosulfate citrate-bile salts sucrose) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism

3.6 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs. /sq. inch for 20 minutes. Screw cap test tubes, conical flasks, prepared media etc. were also sterilized.

3.7 Preparation of Petri dishes

The different types of prepared Agar solution were poured into each of the three Petri dishes in a way so that each Petri dish gets 12-15 ml agar medium. Agar medium was dispensed into each Petri dish to get 3-4 mm depth of agar media in each Petri dish. After pouring the agar medium, all Petri dishes were kept in room temperature so that agar medium can become properly solidified. Then sodium chloride were inoculated in the Petri dishes with the help of cotton buds and loops.

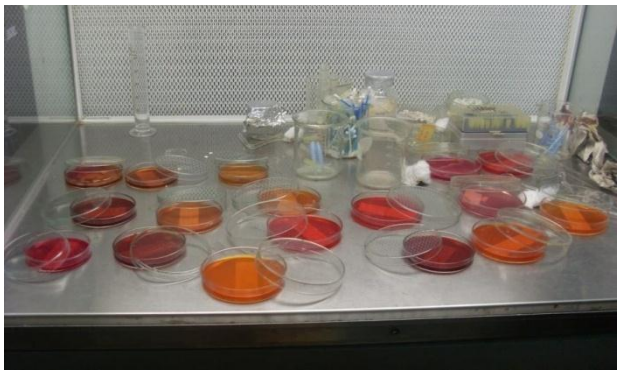

Figure 3.1: Petri dishes preparation

3.8 Incubation

Then all the prepared agar plates with respective samples were placed inside a bacteriological incubator at 36°C temperatures for 24 hours for obtaining growth of specific organism in specified plates.

Figure 3.2: Incubator

Standard Colony Morphology of Suspected Organism in Different Media After overnight incubation of the specific media, organisms were selected based onthe followingcriteria:

Table 3.1: Standard Colony Morphology of Suspected Organism

After overnight incubation of the specific media, organisms were selected based on the following criteria:

Organism	**Media**	**Appearance**
E. coli	MacConkey	Lactose Fermenting pink colonies Non-Lactose fermenting colorless colonies
	TBX	Blue colonies
Salmonella	BGA	Typical red colonies
	XLD	Red or clear colonies with black center
Vibrio	TCBS	Large yellow colonies
Shigella	XLD	Typical red colonies

3.9 Apparatus &Reagent used for Isolation and Identification of Specific Organism:

- Laminar air flow cabinet (ESCO,Singapore)
- Petridishes
- Autoclave (HIRAYAMA,Japan)
- Hot air oven (FN-500,Niive)
- Agar
    - MacConkeyagar
    - XLDagar
    - TBXagar
    - BGAagar
    - TCBSagar
- Inoculatingloop
- Spiritburner
- Handgloves
- Mortar andpestle
- Incubator
- Measuring Cylinder(100ml)
- Distilledwater
- Analyticalbalance
- Media preparationbottle
- Antibiotic disc
    - Gentamycin
    - Kannamycin
    - Vancamycin
    - Erythromycin
    - Tetracycline
    - Ciprofloxacin
    - Penicillin G

3.10 Cell counting

3.10.1 Theory:

In quantitative microbiology, we are concerned with determining the concentration of colony forming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of sample may be impossible. It is desirable to have "countable" plates – containing between 30 and 300 colonies. If fewer than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together.

3.10.2 Counting bacterial colonies

1. After an appropriate incubation period the plates were examined for colonial growth.
2. Colonies will form on the top of the agar as well as in the agar. Those on top of the agar will be larger but all colonies must be counted.
3. Plates were selected that appear to have between 30 - 300 colonies in and on the agar asthis gives the best statistical representation of the number of bacteria in the undiluted sample.Using a light box or colony counter (if one is available) and marker pen (put a dot above each colony as you count it), the number of colonies were counted in each of the dilutions having between 30 - 300 colonies.

3.10.3 Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria

Agar disk-diffusion method

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.the table shows the growth media, temperature, period of incubation and inoculum size required by CLSI standards (Balouiri, Sadiki and Ibnsouda, 2015).

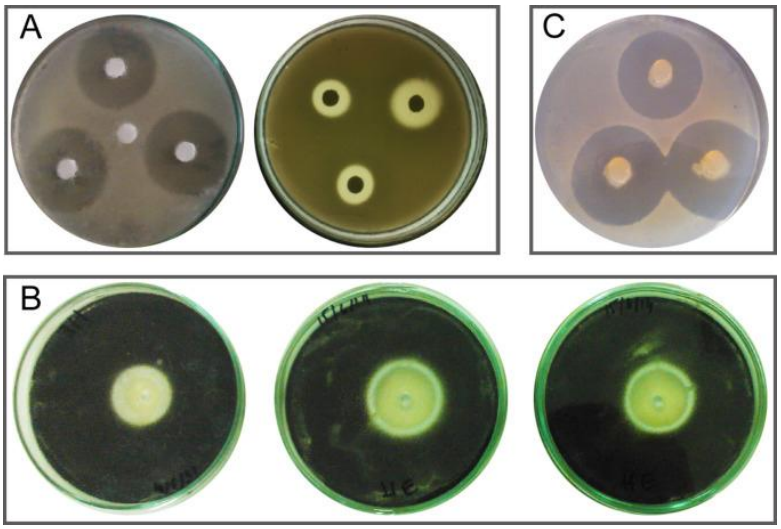

Fig 3.3 : Agar diffusion methods: (A) disk-diffusion method of microbial extract using *C. albicans* as test microorganism, (B) agar well diffusion method of essential oil using *Aspergillusniger* as test microorganism, and (C) agar plug diffusion method of *Bacillus* sp. against *C. albicans*.

Chapter 04

Results

Table 4.1: Bacterial colony morphology isolated from different street/ expired foods

Samples	**Agar Plates**				
	MacConkey	TBX	BGA	XLD	TCBS
Sweet Biscuit	Pink	No Growth	No Growth	No Growth	Yellow
	White dot				
	Mucoid Pink				
Nontabiscuit	Pink	No Growth	No Growth	No Growth	No Growth
Charabiscuit	Pink	No Growth	No Growth	No Growth	No Growth
Fruit cake	Pink	No Growth	No Growth	No Growth	No Growth
Vegetable curry	Pink	No Growth	No Growth	No Growth	Yellow
Patties	Pink	No Growth	No Growth	No Growth	Yellow
Chicken patties	No growth	Blue	No Growth	No Growth	No Growth
Somucha	Pink	No Growth	No Growth	No Growth	Yellow

Table 4.1 shows thateight samples (sweet biscuit, nonta biscuit, chara biscuit, fruit cake, vegetable curry, chicken patties, somucha, patties) are tested on different agar media (macconkey, TCBS,XLD, BGA,TBX) to observe the microbial growth of different types of microorganism. Among them seven samples except chicken patties shows microbial growth in macconkey agar media which is identified by its pink color. Among them sweet biscuit shows pink, white dot and mucoid pink colony. On the

other hand sweet biscuit, fruit cake, vegetable curry, somucha and singara shows their growth on TCBS agar media which is identified by its yellow color colony . This table also shows that there is no growth on XLD and BGA agar and TBX shows its blue colony on chicken patties. The reason for observing no growth in sample may include the following: a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

Table 4.2: Bacterial colony morphology isolated from different street/expired foods

Samples	**Agar Plates**				
	Macconkey	**TBX**	**BGA**	**XLD**	**TCBS**
Egg curry	Pink	No Growth	No Growth	No Growth	Yellow
Chicken	Pink	No Growth	No Growth	No Growth	Yellow
Aluvorta	Pink	No Growth	No Growth	No Growth	Yellow
Noodles	Pink	No Growth	No Growth	No Growth	Yellow
Kabab	Pink	No Growth	No Growth	No Growth	Yellow

Table 4.3: Bacterial colony morphology isolated from different street/expired foods

Samples	Agar plate				
	Macconkey	**TBX**	**BGA**	**XLD**	**TCBS**
Beguni	Pink	No Growth	No Growth	No Growth	No Growth
Sandwich	Pink	No Growth	No Growth	No Growth	No Growth
Singara	Pink	No Growth	No Growth	No Growth	Yellow
Mayonnaise	No growth	No Growth	No Growth	No Growth	Yellow
Ghugni	No growth	No growth	No growth	No growth	Yellow
Chicken shushlick	No growth	No growth	No growth	No growth	Yellow
Vegetable roll	No growth	Blue	No growth	No growth	Yellow
Phuchka	No growth	No growth	No growth	No growth	Yellow

Table 4.2 and 4.3 shows that thirteenfood samples (egg curry, chicken, aluvorta, noodles, kabab, beguni, sandwich, singara, mayonnaise, ghugni, chicken shushlick, vegetable roll, phuchka) were used to treat on different agar media to see the growth of microorganism. Among them they all shows pink colony on macconkey agar except mayonnaise and ghugniwhereas TBX, BGA, XLD shows no growth of microorganism on the other hand TCBS agar shows its yellow color colony in all the samples beguni and sandwich. The reason for observing no growth in sample may include the following:

a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

Table 4.4: Standard Chart of Microbial Sensitivity

Antibiotic (Antimicrobial Agent)	**DISC CODE**	**Resistant (< or = mm)**	**Intermediate (mm)**	**Susceptible (= or > mm)**
Ciprofloxacin	CIP-5	15	16-20	21
Erthromycin	E	13	14-22	23
Gentamycin	GM	12	13-14	15
Kanamycin	K-30	13	14-17	18
Penicillin G	P	22	23-25	26
Tetracycline	Te-30	14	15-18	19
Vancomycin	Va-30	9	10-11	12

(Reynolds, 2016)

Table 4.4 is the standard chart of the microbial sensitivity of different antibiotics which is classified into different ranges such as, resistant, intermediate, and susceptible. This terms are used to measure the antibacterial efficacy of the antibiotics against different microorganism.

Table 4.5: Zone of inhibition of the test samples against different microbes

Agar plate	Sample	Antibiotic Disc (mm)			
		Erythromycin	Tetracycline	Ciprofloxacin	Penicillin G
Macconkey	Somucha	10	20	35	0
	Singara	>10	>12	23	0
TCBS	Singara	15	20	30	0
	Phuchka	20	20	30	0
	Myonnaise	27	15	33	0

Table 4.5 shows thatfoursamples were collected from different places of Dhaka city. After the diffusion method antibacterial disc were placed on the samples to observe the zone of inhibition so that we can come to know about the efficacy of the antibiotics. From the table we can see that two agar plate were used to test the samples (somucha, singara, phuchka, mayonnaise) and four antibiotic disc were used (Erythromycin ,Tetracycline, Ciprofloxacin, Penicillin G). According to the standard table of zone of inhibition we can see that Erythromycin is resistant in macconkey agar in somucha and singara whereas it shows intermediate zone in TCBS (singara&fuchka) and susceptible zone (myonnaise) as well. On the other hand, Tetramycinshows susceptible zone in 3 of 4 samples, Ciprofloxacin shows susceptible zone in 4 of 4 samples and Penicillin G shows resistant zone in all samples.

Table 4.6: Zone of inhibition of the test samples against different microbes

Agar plate	Sample	Antibiotic Disc (mm)			
		Erythromycin	Tetracycline	Ciprofloxacin	Penicillin G
Macconkey	Egg curry	15	10	30	0
	Chicken	15	>15	35	0
	Aluvorta	10	17	21	
TCBS	Egg curry	20	15	30	0
	Chicken	15	20	37	0
	Aluvorta	17	15	25	0

Table 4.6 shows that three samples were collected to test the pathogens and after that to observe the inhibition zone to find out the efficacy of the antibiotics. From the table we can see that two agar plate were used to test the samples (chicken, aluvorta, egg curry) and four antibiotic disc were used (Erythromycin, Tetracycline, Ciprofloxacin, Penicillin G).Erythromycin shows intermediate zone in 5 of 6 samples whereas tetracycline shows intermediate zone in 4 of 6 samples , ciprofloxacin shows susceptibility in 6 of 6 samples and penicillin G have showed resistance in all samples.

Table 4.7: Zone of inhibition of the test samples against different microbes

Agar plate	**Sample**	**Antibiotic disc (mm)**			
Macckonkey		Erythromycin	Ciprofloxacin	Tetracycline	Penicillin G
	Noodles	10	21	10	0
	Kabab	12	20	15	0
	Beguni	10	17	15	0
	Sandwich	5	22	10	
TCBS	Kabab	20	25	10	0
	Noodles	15	22	20	0
	Chicken shashlik	10	25	12	0
XLD	Chicken patties	10	20	10	0

Table 4.7 shows that total 6 samples were collected and treated on macconkey, TCBS and XLD agar plate. Samples were collected to test the pathogens and after that to observe the inhibition zone to find out the efficacy of the antibiotics. From the table we can see noodles, kabab, beguni and sandwich were treated with macconkey agar and kabab, noodles, chicken shaslik were treated with TCBS agar and chicken patties was treated with XLD agar. Then four antibiotic disc were used (Erythromycin, Tetracycline, Ciprofloxacin, Penicillin G).we can observe that Erythromycin is resistance in 6 of 8 samples (except sandwich , chicken shaslik) ,Ciprofloxacin shows susceptibility in 4 of 8 samples and 2 of 8 samples shows intermediate zone of inhibition.

Whereas ,Tertracyclin shows resistance in 5 of 8 samples and Penicillin G has resistance in all samples.

Table 4.8:Zone of inhibition of the test samples against different microbes

Agar plate	**Sample**	**Antibiotic Disc (mm)**			
Macconkey		Gentamycin	kanamycin	Vancomycin	Penicillin G
	Fruit cake	23	10	0	0
	Vegetable	25	15	0	0
	Mishit biscuit	10	17	0	0
	Nonta biscuit	10	15	0	0
	Chara biscuit	20	17	0	0
	Patties	20	15	0	0
TCBS	Vegetable	23	20	0	0
	Patties	27	10	0	0
	Ghugni	25	15	0	0

In table 4.8 shows that total seven samples were used (fruit cake, vegetable, mishit biscuit, nonta biscuit, chara biscuit, patties, ghugni) . Fruit cake, vegetable, mishit biscuit, nonta biscuit, chara biscuit were tested in macconkey agar and vegetable, patties, ghugni are tested in TCBS agar. Gentamycin, kanamycin, vancomycin and penicillin G are used as antibacterial disc to observe the inhibition zone. Vancomycin and penicillin G shows resistance to all samples. On the other hand, gentamycin shows susceptibility in 5 of 7 samples, kanamycin shows intermediate zone in 7 samples.

Table 4.9: Zone of inhibition of the test samples against different microbes

Agar plate	Sample	Antibiotic Disc (mm)			
		Gentamycin	kanamycin	Vancomycin	Penicillin G
Macconkey	Sandwich	17	22	0	0
	Singara	15	30	0	0
TCBS	Patties	24	21	0	0
	Vegetable roll	20	15	0	0
	Somucha	25	15	0	0
TBX	Vegetable roll	20	20	0	0

Table 4.9 shows that total five samples were used (vegetable roll, somucha, singara, sandwich patties). Patties, vegetable roll, somucha are tested in TCBS agar and sandwich, singara were tested with macconkey agar and vegetable roll is also tested in TBX agar. Among five samples vancomycin and penicillin G shows resistance in all samples. Gentamycin has susceptibility in all samples and kanamycin shows susceptibility in 4 of 5 samples.

Chapter 05

Discussions & Conclusions

5.1 Discussions and conclusion

This study was conducted in different places of Dhaka city, Bangladesh showed antibacterial resistance against different pathogens. Street foods are very much popular in the developing countries due to its cheap price and sharp taste. A millions of people consume street foods like-fruit juices, meals, snacks etc everyday. Food borne illnesses of microbial origin are a major health problem associated with street foods. Approximately, 30 million people in Bangladesh are suffering from food borne illnesses each year (CDC, 2016). The increased global flow of antimicrobials brought with it the threat of antimicrobial resistance. Many governmental and agency reports have been published regarding the resistance of antibiotics. Various studies show that antimicrobials are frequently misused and overused in many developing countries, thus resistance to antimicrobials, has led to an increase in morbidity, mortality and cost of health care (Sharma,2005). To maintain the useful life of antimicrobial drugs in developing countries there is need to improve access to diagnostic laboratories, improved surveillance of the emergence of resistance, better regulation of the use of antibiotics, and better education of the public, doctors, and veterinarians in the appropriate use of the drugs.

The objective of this research work was therefore focused on the sensitivity of different class of antibiotics against different enteric bacteria found from street and expired food. The first vancomycin-resistant clinical isolates of enterococcus species were reported in Europe in 1988. Similar strains were later detected in hospitals on the East Coast of the United States. Since then, vancomycin-resistant enterococci have spread with unexpected rapidity and are now encountered in hospitals in most countries (Patrice, 2006). Another study shows that *Staphylococcus aureus*from patient cultures and hospital environmental samples for resistance to Penicillin-G. Only 4--7% of the 232 *Staphylococcus aureus* strains were sensitive to Penicillin-G. Patient strains (57) of *Staphylococcus epidermidis* were sensitive to penicillin-G in 29%. (kimberlin,1978).

According to our findings penicillin G and vancomycinshows resistant in all our twenty five samples i.e. these are not effective anymore and bacteria can grow even if the drug is present.This is a sign of ineffective antibiotics in our present study.

Study conducted by Judith shows that Erythromycin resistance was present in 22% of GBS

(group B streptococcus) isolates (Judith, 2004). In our study we have found Erythromycin and tetracyclinasintermediate antibiotic that means a higher dose of the antibiotic is needed to prevent the growth of microbes.

Fernandez reported that the combination effect of penicillin-gentamicin was more effective *in vitro* than the combination of ciprofloxacin-gentamicin against the low or high inoculum of enterococci (Fernandez et al, 1987). In our study we have found gentamycin, kanamycin and ciprofloxacin showed susceptibility against the microorganisms.

In our present study 25 food samples were tested with seven antibiotics to evaluate the antibacterial sensitivity. The standard chart of the zone of inhibition to categories the bacteria as resistant, intermediate and susceptible was used in this study. We have found penicillin G and vancomycin as resistant antibiotics as they did not show any zone of inhibition. We have also found erythromycin and tetracyclin as intermediate antibiotics. An excellent result was found against the microbes when treated with gentamycin, ciprofloxacin and kanamycin. There were some limitations in this study. Only seven antibiotics were used to see the antimicrobial sensitivity due to lack of methods and facilities. New method of identification of organisms should be implemented in our study.

The global crisis of antibiotic resistance has reached a point where, if action is not taken, human medicine will enter a post antibiotic world and simple injuries could once again be life threatening. New antibiotics are needed urgently, but better use of existing agents is just as important. More appropriate use of antibiotics in medicine is vital, but the extensive use of antibiotics outside medical settings is often overlooked. Doctors should be more careful to prescribe antibiotics and government should take actions for selling antibiotics without prescription.

Chapter 06

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