Microbiological analysis of hospital cafeteria fast foods in preference to the presence of antibiotic resistant bacteria and their association with pathogenicity

Ph.D. Dissertation

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF DHAKA IN THE FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF NUTRITION AND FOOD SCIENCE
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DHAKA-1000

SUBMITTED BY
REGISTRATION NO. - **77**
SESSION: **2018-2019**

Dedicated To My Beloved Parents

Certification

It is hereby certified that the student bearing Reg.No.77, Session, 2018-2019 has carried out the research work entitled **"Microbiological analysis of hospital cafeteria fast foods in preference to the presence of antibiotic resistant bacteria and their association with pathogenicity"** for the partial fulfillment of her Ph.D. Degree in Nutrition and Food Science from the University of Dhaka under our academic supervision in Microbiology laboratory, Institute of Nutrition and Food Science and Microbial Genetics and Bioinformatics Laboratory, Department of Microbiology, University of Dhaka.

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Abstract

Antibiotic resistance is an emerging national and global threat to the food chain. Rising popularity of fast foods along with their capacity of harboring different multiple antibiotic resistant pathogenic bacteria displays a leading issue related to public health across the globe. The research focused on the identification of various multidrug-resistant (MDR) bacterial species that are currently in circulation, along with the existence of virulence and antibiotic resistance determinants among the isolates of eight hospital cafeteria fast foods (sandwich and burger), as well as the evaluation of the level of knowledge and related practices involved with food handling amidst the food handling personnel of these hospital cafeterias in Dhaka, Bangladesh. The total viable bacterial counts from burger and sandwich samples ranges from 2.1×10^2 to 4.0×10^6 (CFU/g) and 7.0×10^2 to 1.0×10^6 (CFU/g) respectively. According to the total bacterial count, the majority of the sandwich and burger samples were in an unsatisfactory condition. A total of 137 bacterial species were isolated from the sandwich and burger samples, of which 87 (63.50%) were Gram-negative and 50 (36.50%) were Gram-positive. Among the Gram-negative isolates, 52.87% were retrieved from sandwiches, while 47.12% were from burgers. In case of Gram-positive isolates, 58% and 42% were retrieved from sandwiches and burgers respectively. The phenotypic characteristics of the isolates were screened through selective identification (morphological) in different selective media and Gram staining followed by different biochemical tests and gene specific PCR of ribosomal gene (16S rRNA) sequencing. DNA sequence homology and phylogeny analysis were employed to find out the close relatives. The Gram-negative isolates were distributed into 10 different genotypes using Randomly Amplified Polymorphic DNA (RAPD) typing namely *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Citrobacter* spp. and *Acinetobacter* spp. The Gram-negative isolates were widely dispersed among the various bacterial species, with *Klebsiella* spp. accounting for the highest percentage (42.53%) and *Salmonella* spp. and *Acinetobacter* spp. the lowest (1.15%). Based on various biochemical tests, the isolated Gram-positive organisms were divided into 6 groups encompassing *Bacillus* spp., *Planococcus* spp., *Micrococus* spp., *Streptococcus* spp., *Clostridium* spp. and *Staphylococcus* spp., with the highest percentage of isolates from *Bacillus* spp. (58%). and lowest in *Staphylococcus* spp. (2%). About 16.09% of the Gram-negative and 6%

of the Gram-positive isolates were strong biofilm formers, while 13.79% and 6% of the Gram-negative and Gram-positive strains showed β-hemolytic properties respectively. Using 17 antibiotics from 11 different antibiotic classes, the susceptibility pattern against antibiotics was examined. Approximately 80.99% of the Gram-negative and 54% of the Gram-positive isolates were resistant against multiple antibiotics. The study isolates had significantly higher percentages of resistance to particular antibiotic classes; with the maximum resistance against ampicillin and penicillin preceded by meropenem and the lowest resistance against imipenem. The virulence and antibiotic resistance genes among the Gram-negative isolates were detected by employing gene specific PCR. Among the harbored virulence genes, all *Pseudomonas* spp. were *opr*I positive and 12.5% were *opr*L positive. *Salmonella* isolate was *inv*A positive and 50% of the *E. coli* strains were *uid*A positive. In case of antibiotic resistance genes, bla_{SHV} showed the highest prevalence (20%) among the class A β-lactamase; amidst the carbapenemases resistance, bla_{OXA-48} showed the highest prevalence (12.22%) followed by bla_{OXA-1} (2.22%), bla_{KPC-1} (2.22%). Through the fluoroquinolone resistance, *gyr*B showed the highest prevalence (50%) followed by *qnr*S (41.67%). Most of the food handlers had poor knowledge (62.50%) and practice scores (71.67%). The training condition ($P<0.05$), educational status ($P<0.05$) and monthly income ($P<0.05$) significantly influenced the practices of the food handling personnel as revealed by univariate analysis. According to the outcomes of multivariate logistic regression, food handling personnel practice score was significantly influenced by the level of education ($P<0.05$) and training status ($P<0.05$, AOR=0.049, 95% CI =0.011, 0.225). Food handler's knowledge and practice score was significantly related ($P<0.05$) to each other. Prevalence of such virulence and resistant determinants along with poor food handling knowledge and practices in hospital cafeteria fast food indicates the risky situation regarding hospital acquired pathogen, increases the risks for already infected and vulnerable patients, impedes treatment and threatens recovery as well as increases the health costs of the country and may jeopardizes the attainment of sustainable development goals (SDGs) particularly the SDG 3.

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Abbreviations

Symbols	
%	Percentage
&	and
≤	Less than equal
≥	Greater than equal
°C	Degree Centigrade
µg	Microgram
µl	Microliter
g	Gram
M	Molar
ml	Milliliter
mm	Millimeter
mM	Millimolar
General Terms	
ARG_s	Antibiotic Resistance Genes
AMR	Antimicrobial Resistance
VFG_S	Virulence factors-associated genes
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
cfu	Colony Forming Unit
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleoside tri-phosphates
e. g.	Examplia gratia
ESBLs	Extended-Spectrum β-Lactamases
et al	And others
FAO	Food and Agriculture Organization
WHO	World Health Organization
kb	Kilo base
kbp	Kilo base pair
MDR	Multi Drug Resistant
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
RAPD	Randomly amplified polymorphic DNA
rpm	Rotation Per Minute
sec	Second
UV	Ultraviolet

Chapter 1:
Introduction and Review of literature

1.0 Introduction and Review of literature

1.1 General Introduction

In the modern, globalized society, Food-borne diseases (FBD) is a crucial public health issue. Due to the high rates of illness and mortality caused by different food-borne diseases, their effects on both health and economy are increasingly recognized (Abebe et al., 2020). Outbreaks and isolated incidence of food borne illness is regular in most of the countries in the globe (Kirk et al., 2015). Food-borne illnesses retard socioeconomic development by challenging healthcare systems, unstabilizing economies at national and international level, and harming trade and tourism. It is terribly challenging to evaluate the true prevalence of food-borne illnesses as there is little or no FBD surveillance system in maximum countries, specifically in developing countries. Even in countries with excellent surveillance system, incidents are often unpublicized to the authority affiliated with public health (Abunna et al., 2016). As reported by World Health Organization, roughly 600 million (or almost every tenth people) fall ill and mortality of 420,000 persons occur annually from eating contaminated food (WHO, 2023). In accordance with World Health Organization, about 30% population in developed countries go through food borne illness annually, whereas nearly 2 million deaths in developing countries caused by these foodborne illnesses (Andargie et al., 2008) .

An emerging concern related to public in Bangladesh is the food borne illnesses despite significant advancements in the public health sector. Because of the dense population, underdeveloped construction, contaminated water, poor sanitation and hygiene situations, Bangladesh has a significantly high incidence of food related illnesses and other food safety risks (Al Banna et al., 2021). The most important food related illnesses in Bangladesh includes diarrheal diseases along with enteric fever, hepatitis etc. (Tabashsum et al., 2013), (FAO, 2023). In Bangladesh, both urban and rural areas have experienced high rates of morbidity due to foodborne illnesses over the past few decades (Al Mamun et al., 2013) . Every year, Bangladesh has about 30 million persons who experience from one type of food related illness (Al Banna et al., 2021; FAO et al., 2012) . The information on the worthiness of the food related diseases are scanty in Bangladesh.

Fast food is a category of mass-produced, efficiently conditioned and distributed food that is sold in some eateries, concession stands, and convenience stores. Beside these, having high levels of sodium, cholesterol, saturated fats, refined grains, and sugars, fast food items are frequently highly processed, precooked, or frozen (Britannica, 2023). It is a quick substitute for home-cooked meals. Fast food consumption has increased as a result of societal trends that are more mobile, have a large number of itinerant workers, and place less of a focus on family-oriented activities (Nashwa et al., 2017). Globalization, economic growth, and rising incomes have all contributed to an increase in fast food consumption in Bangladesh, similar to other Asian countries (N. Islam & Ullah, 2010). A number of earlier studies have shown that a variety of bacterial species were present in fast foods such as *Enterobacter* spp., *Salmonella* spp., *Citrobacter* spp., *E. coli* spp., *Klebsiella* spp., *Providencia* spp., *Proteus* spp., *Staphyloccous* spp., *Flavobacterium* spp., *Streptococcus* spp., *Pseudomonas* spp. etc. (Easa, 2011; Sabuj et al., 2018; Shaltout et al., 2016; University et al., 2015). The general public, hospital staff, visitors, outpatients, and society at large all receive food from hospital cafeterias concurrently (Lesser & Lucan, 2013). Foodborne disease outbreaks can increase hospitalization costs for the public health department by causing diseases and fatality among patients, companions of the patient, and service personnel in hospitals and the general population (Vemula et al., 2014). A hospital outbreak of a foodborne illness can cause service interruptions, life-threatening illnesses, and also fatalities for any infected person, especially the existing vulnerable patients (Ghosh & Alim, 2022). Microbiological contamination of food and drinking water is a crucial route of food related disease transmission across the world (Al Mamun et al., 2013). An important worldwide public health issue is the microbial contamination of food. Over the past few decades, the majority of nations have observed a significant rise in the prevalence of diseases brought on by microorganisms in food (WHO, 2022). In addition to these, inadequate storage facilities, inadequate food safety knowledge, poor hygiene, cross contamination, inadequate education and training of the food handlers, etc. make food more susceptible to contamination (Alemayehu et al., 2021; Ghosh & Alim, 2022; Grace, 2015). In recent years, it has been demonstrated that multidrug-resistant gram-negative strains, specifically those belonging to the Enterobacteriaceae family, are highly prevalent in food (Dougnon et al., 2021). A huge number of pathogenic Gram-positive bacterial strains from different bacterial species such as *Staphylococcus spp., Streptococcus spp., Enterococci spp., Clostridium spp., Bacillus spp., Corynebacterium*

spp., Listeria spp. are particularly notable today for posing problems with resistance, significantly burdening the public health system, and increasing healthcare costs (Kulkarni et al., 2019; Woodford & Livermore, 2009). Resistance to antibiotics is a notable health issue globally and if the present trend of incorrect and extreme use of antibiotics continues, it is reported that antibiotic resistance will result in 10 million fatality globally by the year 2050 (T et al., 2022). A serious risk to human health is posed by foodborne bacteria that are antimicrobial-resistant (AMR) and that cause bacterial infections. Furthermore, the threat level rises due to the possibility that treatment options may be jeopardized due to some of these bacteria's capacity to make biofilms (Q et al., 2022). Since the determinants of antibiotic resistance can spread to other bacteria with clinical relevance to humans, contaminated fast food bearing drug-resistant bacteria can impose a serious risk to mass people health.

A variety of tests, including phenotypic virulence assays like hemolysin production (Saha et al., 2020), biofilm formation (M. N. Hoque et al., 2020), and antimicrobial susceptibility tests (Abbey & Deak, 2019), as well as virulence and resistance gene specific PCR, can be used to identify the potentially pathogenic bacterial strains. Additionally, these microbes are anticipated to be very contagious, increasing the likelihood that they will significantly raise human morbidity and/or mortality (NIH, 2021). There is limited surveillance on the foodborne pathogen in Bangladesh, the prevention and control of pathogen transmission via food necessitates accurate knowledge of their virulence and resistance patterns. Therefore, this study aims to investigate the circulating diverse microorganisms, reveal their antibiotic resistance pattern, different potential virulence and antibiotic resistance genes of hospital cafeteria fast foods which will provide insights into the characteristics of microbial flora associated with hospital fast foods and thereby facilitate adopting effective measures to minimize foodborne pathogen transmission.

1.2 Review of Literature

1.2.1 Fast food-A rapid influx

To fit the new jet age, ideas, relationships, and lifestyles have changed, and eating habits are no exception (Hossain & Islam, 2020) . Modern society has shifted from eating conventional foods to fast foods as a result of advances in science, technology, and industry (Didarloo et al., 2022). In 1951, Merriam-Webster first used the term "fast

food."(Islam & Ullah, 2010). In accordance with Merriam-Webster, the terminology fast food is used to define food that can be prepared and served rapidly. Generally speaking, it applies to any food that is provided to customers for takeout and requires little preparation time. Therefore, quick availability was the main reason behind its creation. These includes specialty items like fried chicken, pizza, hamburgers, and sandwiches (Hossain & Islam, 2020).

1.2.1.1 Global fast food consumption

When the White Castle was opened in the US in 1916, the first fast food restaurants were born. American-founded fast food chains like McDonald's, KFC, and Pizza Hut are now multinational businesses with locations around the world (N. Islam & Ullah, 2010). Fast food outlets currently make up the biggest segments of the food industry having more than 200,000 restaurants and $120 billion sales annually in the U.S. only. Fast food is in high demand worldwide. This is evidenced by the fact that international companies like McDonald's and Yum! Brands generate 65 percent and 50 percent of their sales abroad, respectively (Hossain & Islam, 2020).

1.2.1.2 Entrance of fast food industry in Bangladesh

In Bangladesh, fast food culture first emerged in the early 1990s. Fast food culture has primarily targeted the younger segment of the market and the workforce in the corporate sector in Bangladesh. Bangladesh has experienced a rapid influx of fast food restaurants (N. Islam & Ullah, 2010). On Bailey Road in Dhaka, the first fast food restaurant opened for business in Bangladesh. Some fast food outlets in Bangladesh with names like Swiss, Helvetia, etc. were created through a franchising system. The first international fast food franchise entered Bangladesh in the beginning of 2000. By obtaining a franchise from Transcom Foods Limited (TFL), Pizza Hut and KFC entered the Bangladeshi market (Hossain & Islam, 2020). (Sabuj et al., 2018)

1.2.2 Potential microbiological hazards in fast foods

Food borne diseases are the result of ingestion of food safety hazards. Microbiological hazards play crucial role in food borne diseases. Several studies concluded that a large number of pathogenic organisms present in fast foods (Sabuj et al., 2018), (University et al., 2015), (Shaltout et al., 2016), (Easa, 2011).

A Nigerian research conducted on microbiological screening of fast foods studied four food samples from four different fast food restaurants. The bacterial, coliform and total fungal count were more than the recommended levels. Different bacterial species including *Streptococcus sp., Bacillus sp., Staphylococcus sp., Flavobacterium sp., Proteus sp.,Escherichia coli, Klebsiella sp., Salmonella sp.* and *Pseudomonas aeruginosa* and four fungal species namely *Penicillium sp., Rhizopus stolonifer, Mucor sp*. and *Aspergillus flavus* were identified (University et al., 2015).

A systematic review conducted on evaluating the risks of fast foods on public health and screening of microbiological quality of those foods in developing countries concluded that different potential pathogenic bacterial species were present in fast foods beyond the maximum allowable limit and are potentially harmful to human health (Mengistu et al., 2022).

Another study on Nigerian fast foods revealed that bacterial colony count ranged from $2.4x10^4$-$4.2x10^6$ which is well above the bacteriological quality standard. Different bacterial species such as *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Pseudomonas putida*, *Escherichia coli*, *Proteus vulgaris*, *Micrococcus luteus*, *Bacillus cereus*, *Pseudomonas chlororaphis* and *Bacillus subtilis* were characterized and identified from those fast foods (Oladipo & Fajemilo, 2013).

The study conducted in Alice, South Africa on 252 ready-to-eat food samples observed the presence of *Listeria* spp., *Enterobacter* spp. *Aeromonas hydrophila* , *Klebsiella oxytoca* , *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas luteola* but not any *Salmonella* spp. or *Escherichia Coli.* Maximum food samples failed to meet bacteriological quality standards (Nyenje et al., 2012).

A study analyzed the microbiological hazards of sandwiches examined 174 samples detected *staphylococcus aureus, E. coli,* and *Salmonella* spp. and no Listeria monocytogens (Bae & Park, 2011) .

Microbiological quality on sandwiches were investigated in a study in South korea revealed the presence of *Salmonella* spp., *Bacillus cereus, Escherichia coli, Staphylococcus aureus* etc. All samples were devoid of Vibrio parahaemolyticus (Hg et al., 2013).

In Bangladesh, a research conducted on Bangladesh Agricultural University campus, about 120 fast food samples were investigated. The study revealed the occurence of *Staphylococcus spp., Salmonella spp.,* and *E. coli* and their infections causing mean annual risks were about 100% in all cases (Sabuj et al., 2020).

Microbiological screening of available ready-to-eat foods in Dhaka, Bangladesh investigated 45 samples in a study. The research revealed the existence of antibiotic resistant bacterial strains in various foods (Banik et al., 2019).

A study on 72 food items that were vended in street in the campus of Bangladesh Agricultural University, Mymensingh, Bangladesh. A large number of bacteria of different spp. were revealed who were resistant to a large number of antibiotics ampicillin, amoxicillin, nalidixic acid whereas sensitive to ciprofloxacin, gentamycin, azithromycin and chloramphenicol (Sabuj et al., 2018) .

1.2.3 Sources of contamination of fast foods

Contamination of fast food can emerge at any stage of consumption during production, processing, distribution, or preparation. Food product contamination is challenging to prevent because there are many possible points of contact between microorganisms and the product during production, processing, storage, distribution, and consumption (Zwirzitz et al., 2020) . Inadequate ingredient control, contaminated raw materials, poor sanitation procedures, and subpar equipment design have all been linked to bacterial cross-contamination and recontamination incidents (Fu et al., 2016). Inappropriate outer clothing, lack of cleanliness, inadequacy of holding temperature and ventilation systems along with cross-contamination posed the highest risk factors for sandwich contamination. Instead of unsanitary environments, the majority of the contributing factors were related to food handlers' disregard for sanitation regulations (Giwa et al., 2021). The lack of proper hygienic conditions and handling in slaughterhouses is the primary source of contamination throughout the meat based product production process. In meat based product, different organisms such as *Escherichia coli, Bacillus cereus, and Staphylococcus sp.* were found. The sources of these organisms were unhygienic conditions and improper handling, feeding and housing before slaughtering, contaminated water, spreadable contaminations from skin and feces, contents of digestion system etc. (Bintsis, 2018). When transporting, handling, processing, and displaying RTE foods, the microbial load on raw materials becomes an even more crucial factor. A large number of factors such as washing area, hands of food handlers, utensils used, preparation surface area, frequent and diverse utility of refrigerator, inappropriate storage conditions, the water used, display of food in an open area are all contributing factors for bacterial contamination of fast foods *(*Giwa et al., 2021*)* Lack of

food handling knowledge, poor educational status, lack of training , improper food handling practices are the contributing factors of contamination of fast foods (Ghosh & Alim, 2022).

1.2.4 *Microbial communities in hospital environment*

A diverse population of microorganisms are present in hospital environment. The bacterium *Staphylococcus aureus* is the most widespread. Other common pathogens like *Escherichia coli, Enterococci*, and *Candida* are common offenders and mucous membranes and skin are their potential habitat. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one drug-resistant strain that can be particularly harmful and challenging to treat. Based on the factors like location, number, biofilm formation, intrinsic resistance of organisms to different cleaning agents, and regional conditions, pathogenic microorganisms can survive in the hospital environment for hours or months (Chirca, 2019). Numerous hospital associated pathogens, including vancomycin-resistant *enterococci* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii, Clostridium difficile,* and *Pseudomonas aeruginosa*, have been shown to survive in the environment for long time. Because of their persistent presence in the environment, these microorganisms can transmit and spread in medical facilities (Suleyman et al., 2018). Hospital cafeterias are typically located close to the main hospital building or the treatment area. Food from hospital cafeterias is served concurrently to outpatients, visitors, staff members, the general public, and society (Ghosh & Alim, 2022; Lesser & Lucan, 2013). Furthermore, because hospitals use a lot of antibiotics, it is reasonable to assume that bacteria from the patients receiving treatment are a source of bacterial contamination in the cafeteria environment that are resistant to antibiotics. Again, potential pathogens can spread from the food, environment to the diseased person and can increase hospital associated infections (Chirca, 2019). There is no effective alternative therapy for infections generated by pathogens that are resistant to drugs, and infections caused by multiple drug resistant bacteria that can survive antibiotics of "last resort" can result in the failure of empirical treatment. The hospital setting is an effective reservoir and vector of bacteria that are resistant to antibiotics, despite stringent sanitization protocols. Hospital-associated infections can start outbreaks when pathogens spread to patients via healthcare workers and visitors. They can also linger on hospital plumbing and surfaces for months to years,

pick up new antibiotic resistance genes through horizontal gene transfer, and cause outbreaks of hospital-associated infections (Blake et al., 2021).

1.2.5 Antibiotic resistance- A public health issue

Now a days, the different antimicrobial agents are use indiscriminately. It hinders the achievement of the expected therapeutic outcomes and causes the emergence of antibiotic resistance. One of the greatest challenge to modern development, food security, and global health is antibiotic resistance. As the effectiveness of the antibiotics used to treat those declines, a growing number of infections, including salmonellosis, tuberculosis, gonorrhea, and pneumonia, are becoming more challenging to treat. Longer hospital stays, higher medical expenses, and higher mortality are all consequences of antibiotic resistance (Antibiotic Resistance, WHO, 2023). Resistance to antibiotics imposes a serious challenge to global mass people health, causing at least 1.27 million deaths worldwide and nearly 5 million deaths in 2019. Each year, more than 2.8 million infections in the US are resistant to antibiotics (CDC, 2022) .

1.2.5.1 Antibiotics

Antibiotics came into worldwide prominence with the introduction of penicillin in 1941. Antibiotics are a group of medicines that are used to treat infections caused by germs. Antibiotics either eliminate bacteria or prevent them from growing, allowing the body's natural defenses to get rid of them (MSD, 2023). The most effective class of medications ever created for enhancing human health is undoubtedly the antibiotics. In addition to this fundamental use, antibiotics have also been employed in the prevention, diagnosis, and treatment of infections in both plants and animals as well as in the promotion of animal family growth. All of these uses led to the widespread release of antibiotics into natural ecosystems.

1.2.5.2 Major classes of antibiotics

In general, antibiotics cannot be switched from one infection to another because they are specific to the type of bacteria they are treating. By either killing the bacteria (bactericidal) or preventing it from growing and reproducing (bacteriostatic), antibiotics are used to treat infections brought on by bacteria, such as those caused by *Staphaylococcus, Streptococcus*, or *E. coli* or others. None of the viral infections are

treated by antibiotics. Classification of antibiotics with their mode of action (MSD, 2023), (Orthobullets, 2023) is given in Table 1.1.

Table 1.1 Categorization of antibiotics with their mode of action

Antibiotic class	Antibiotic name	Mode of action
Aminoglycosides	Gentamycin, tobramycin, kanamycin, streptomycin	Hinder synthesis of protein
Cephalosporins	Ceftriaxone, cephalexin, cefuroxime, cefamandole	Inhibit cell wall synthesis
Chloramphenicols	Chloramphenicol	Obstruct protein synthesis
Fluoroquinolones	Ciprofloxacin, norfloxacin, levofloxacin	Interfere with DNA synthesis
Macrolides	Azithromycin, erythromycin, clarithromycin	Impede protein synthesis
Nitrofurans	Nitrofurantion	Deactivate essential cell components
Penecillins	Ampiciliin, penicillin G, amoxycillin	Inhibit cell wall synthesis
Tetracycliunes	Tetracycline	Inhibit protein synthesis
Sulfonamides	Sulphamethoxytrimethoprim	Inhibit folic acid synthesis
Polypeptides	Bacitracin, colistin, polymyxin B	Disrupt bacterial cell walls
Carbapenems	Imipenem,, meropenem,, doripenem	Inhibit cell wall synthesis

The antibiotic class known as beta-lactams, which includes the subclasses of carbapenems, cephalosporins, monobactams, and penicillins, is distinguished by a chemical structure called a beta-lactam ring.

1.2.5.3 Basic mechanism of action of antibiotics

There are numerous ways in which antibiotics work to produce their effects. Many of these substances—generally termed as "β-lactam antibiotics"—work by preventing the synthesis cell wall of bacteria. Inhibition of protein synthesis is another mode of action of antibiotics and protein synthesis in bacteria is inhibited by the aminoglycosides chloramphenicol, erythromycin, and clindamycin. Antibiotics like polymyxin B and polymyxin E (colistin) bind to phospholipids in the cell membrane of bacteria and retard it from acting as a selective barrier, allowing important macromolecules to leak out and causing the cell to die. This happens because the bacterial cell membrane contains

phospholipids (Britannica, 2023). The synthesis of folic acid (folate) is competitively inhibited by some antibiotics, such as the sulfonamides. The aminoglycosides, tetracyclines inhibit the conserved sequences of the 16S r-RNA of the 30S ribosomal subunit and chloramphenicol inhibit the 23S r-RNA of the 50S subunit to bind t-RNA to the A site of the ribosome. Quinolones are the inhibitors of DNA replication (Kapoor et al., 2017).

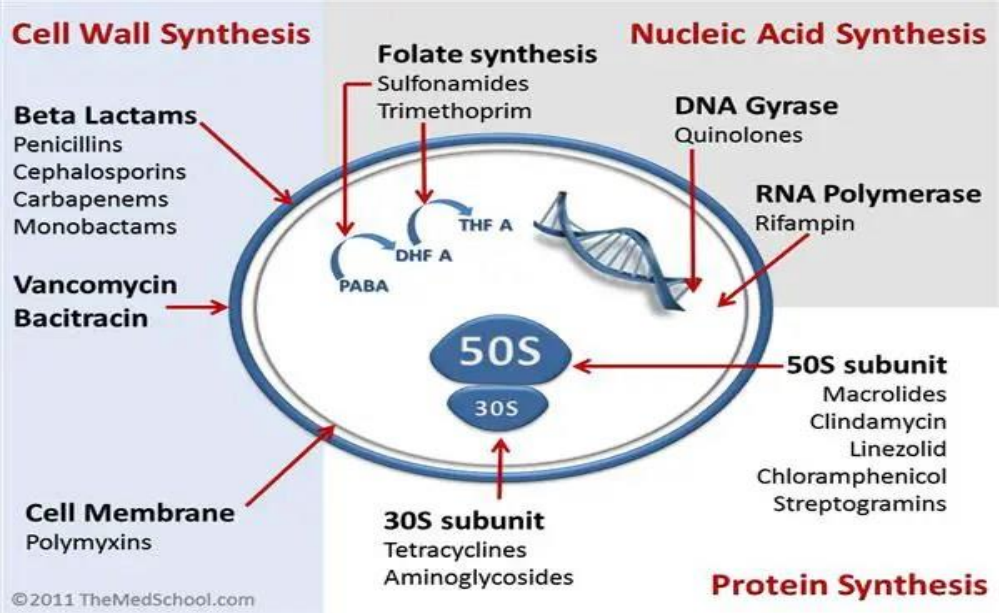


Figure 1.1 Mechanism of action of antibiotics (Kapoor et al., 2017)

1.2.5.4 β-lactamases

The enzymes responsible for hydrolysis of beta lactam is called β-lactamases. There are many prokaryotic genera that contain β-lactamases, the enzymes can cause resistance to β-lactam antibiotics (Silveira et al., 2018). Currently, more than 500 distinct beta lactamases have been identified in nature. These versatile enzymes are present in both Gram positive and Gram negative bacteria (Holten & Onusko, 2000). Beta lactamases are Gram-negative bacteria's primary defense mechanism. The most significant clinical and epidemiological effects are caused by extended-spectrum beta-lactamases (ESBL), *amp*C, and carbapenemases in hospital settings. The therapeutic options for infections acquired in hospitals and those developing in the community have been restricted due to the rising prevalence and global spread of these enzymes (Rada et al., 2019). As of now, the functional classification of Bush-Jacobi-Medeiros and the molecular structure

classification of Ambler have been used to classify β-lactamases (Sawa et al., 2020). By using motifs made up of the primary sequences that make up the protein molecules, the Ambler classification divides β-lactamases into four classes: A, B, C, and D **(Figure 1.2)**. The active center of -lactamases of classes A, C, and D is a serine, whereas β-lactamases of class B use metal zinc ions. Using the Bush-Jacobi-Medeiros method for functional classification, β-lactamases are divided into groups 1 through 3 based on how well they degrade β-lactam substrates and how well the inhibitor works **(Figure 1.2)**.

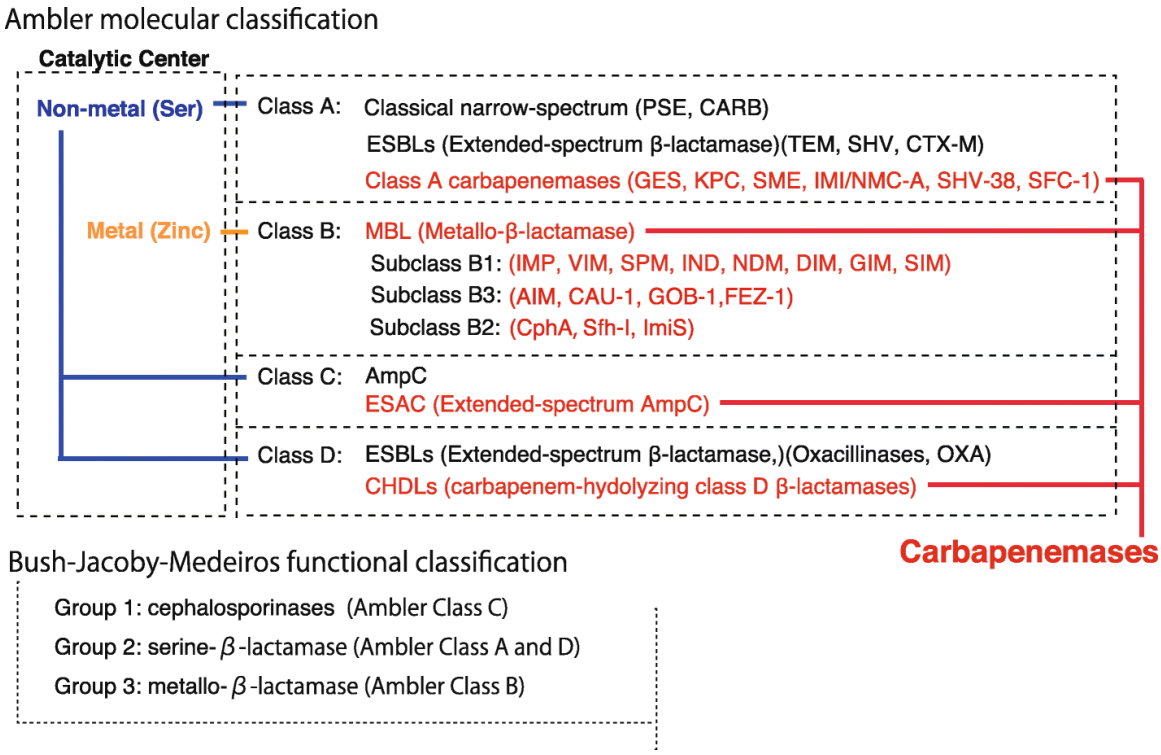


Figure 1.2 Classification of β-lactamases (Sawa et al., 2020)

1.2.5.5 Antibiotic resistance-Threat to public health: Global and Bangladesh perspective

1.2.5.5.1 Global perspective

Resistance in microorganisms can be transmitted to humans and the environment. Extensive use and misuse of antibiotics and other microbial stressors, such as pollution are responsible for this transmission. Human exposure to AMR in the environment can happen through contact with tainted waters, consumption of tainted food, inhalation of fungi, and other pathways that contain AMR-containing microorganisms. In accordance with the World Health Organization (WHO), AMR is an important health issue among the top ten challenges. Antimicrobial resistance poses a threat to the environment, the

safety and security of food and nutrition, economic growth, and social equity (UNEP, 2020). According to recent estimates, drug-resistant infections caused 1.27 million deaths worldwide in 2019. Up to 10 million deaths could occur every year by 2050 (Antimicrobial Resistance Collaborators, 2022) **(Figure 1.3)**. In the next ten years, if AMR is left unchecked, it could reduce GDP by US$3.4 trillion annually and send 24 million more people into extreme poverty (UNEP, 2020). AMR's growth and dissemination are significantly influenced by three economic sector value chains. Firstly, manufacturing of chemicals and pharmaceuticals. Secondly, agriculture and food including aquaculture, food crops, and the production of land animals. Thirdly, a variety of chemicals and disinfectants are used during the delivery of healthcare in hospitals, medical facilities, community healthcare facilities, and pharmacies (Environment, UNEP, 2020) (**Figure 1.4**) . Additionally, inadequate sanitation, wastewater, and related waste effluent in systems for handling human and animal waste, such as municipal wastewater, are significant sources of AMR. To achieve the Sustainable Development Goals (SDGs), addressing AMR is essential for which urgent multisectoral action is needed.

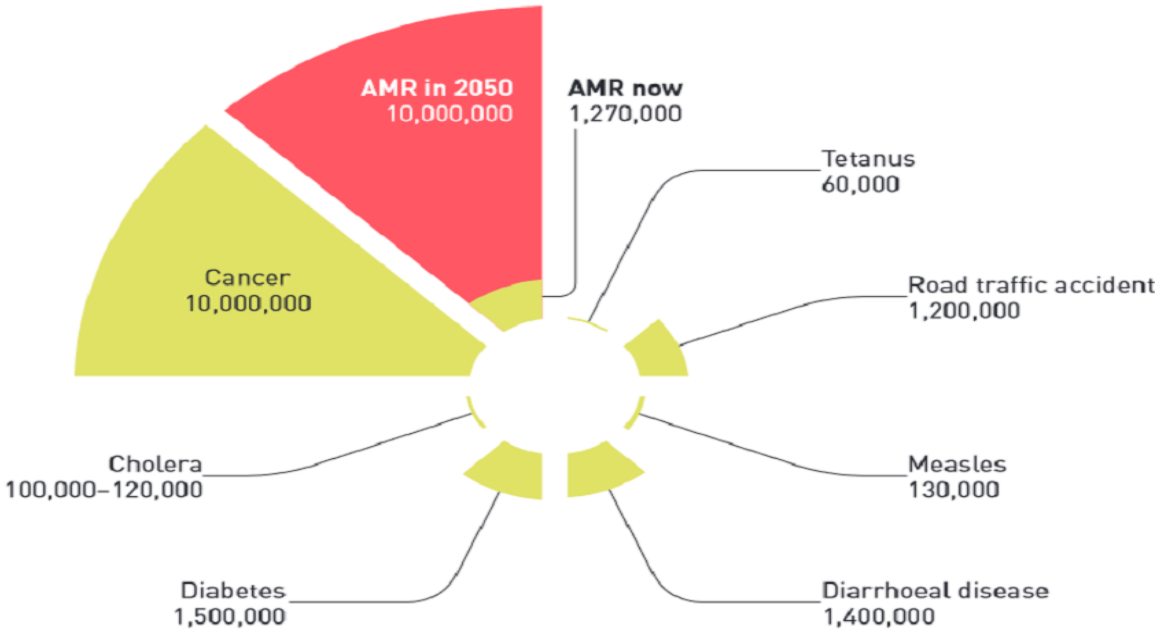


Figure 1.3 *AMR-related predicted mortality in comparison to current leading causes of death (O'Neill 2016; Murray et al. 2022)* (UNEP, 2020)

Figure 1.4 Different types of environmental AMR pollution sources (UNEP, 2020)

1.2.5.5.2 Bangladesh perspective

In Bangladesh as well as other developing nations, antimicrobial resistance (AMR) is now an emerging problem. In Bangladesh and other developing nations, there is little to no regulation on the use of a wide variety of antibiotics, despite the fact that there are very few verified data on the overall impact of antibiotic resistance on public health and the national economy. According to a systematic review research in 2020, antimicrobials are widely available without a prescription throughout the nation, and their irrational use is increasing across all industries, contaminating the environment and leading to the spread of resistance. Numerous supply and demand side factors play a significant role in the emergence and spread of AMR (R. Hoque et al., 2020). Among developing countries of Southeast Asia, Bangladesh was found to be a high degree of AMR, posing a regional and global threat (Bonna et al., 2022). In Chittagong in 2003, it was discovered that typhoid patients were not responsive to second-line therapy because first-line therapy had not even been attempted due to preexisting resistance (I. Ahmed et al., 2019). Self-medication, over-pescribing, inappropriate use of antibiotics, no regulatory controls, little or no training of rural healthcare providers are the main contributing factors of emerging AMR in Bangladesh (Bonna et al., 2022). A study on antibiotic resistance pattern of community-acquired urinary tract infections in Dhaka, Bangladesh revealed that approximately 65% of patients had uropathogens that were multi-drug resistant (MDR).

46% of Gram-positive bacteria and 71% of Gram-negative bacteria were MDRs, respectively. The study population had a high prevalence of community-acquired UTI caused by MDR organisms (M. A. Islam et al., 2022). Furthermore, because these outbreaks are caused by a variety of factors in countries like Bangladesh, extensive epidemiological studies are specifically needed.

1.2.6 Incidence of virulence and antibiotic resistance genes among bacterial population in Bangladesh

Due to the increased virulence properties of microorganisms and the severity of infection brought on by the prevalence of virulence and resistance genes, there is a high risk that treatment will fail (El-Baky et al., 2020). Pathogenic microorganisms with a high level of virulence have the capacity to spread widely and uncontrollably throughout human populations. Additionally, these microbes are anticipated to be very contagious, increasing the likelihood that they will significantly raise human morbidity and/or mortality (NIH, 2021). Different tests, including phenotypic virulence assays like biofilm formation (R. Hoque et al., 2020), hemolysin production (Saha et al., 2020), antimicrobial susceptibility tests (Abbey & Deak, 2019), as well as by different virulence and resistance gene specific PCR, can be used to screen the potentially pathogenic bacterial strains. Different study findings revealed that a large number of virulence and antibiotic resistance genes are present among different isolated bacteria in Bangladesh. A study on 100 *E. coli* strain from Bangladesh concluded that 98% of them were multi-drug resistant (MDR). Using 25 chosen isolates, PCR analysis revealed that the ESBL genes bla_{OXA} (48%) and $bla_{\text{CTX-M-15}}$ (32%), as well as the metallo--lactamase gene bla_{NDM} (80%), were the most prevalent. In 68% of the isolates, the *amp*C gene was found, and in 32%, *tet*C was positive. Notably, 34% of the isolates lacked carbapenem resistance. An extensively drug-resistant (XDR) isolate (L16) with a whole genome sequence (WGS) analysis showed the presence of the infamous sequence type 131 linked to multi-drug-resistant infections, numerous antibiotic resistance genes (ARGs), virulence genes, and mobile genetic elements that increase the risk of environmental transmission (Jain et al., 2021). A study on household water supply in Dhaka, Bangladesh showed the presence of $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CMY-2}}$. Quinolone resistance genes, *qnr*S and *qnr*B were detected. Among virulence genes, *lt* , *st* , *bfp* and *eae* were present (Talukdar et al., 2013). Another study conducted on *klebsiella pneumonia* in Chittagong, Bangladesh revealed the presence of bla_{NDM-1} ,bla_{SHV-11} gene in 64% and

38% of the strains respectively. Additionally , *uge* gene was present in 47% of samples., and 19% of samples carried bla_{NDM-1}, bla_{SHV-11}, *uge* genes together (Tanni et al., 2021).

1.3 Aims and objectives

In light of the limited options for treating patients who are seriously ill and on the verge of developing pan-drug resistance, antibiotics are being used more frequently in hospitals. However, developing nations like Bangladesh, where the overuse or misuse of antibiotics is a common occurrence, face a serious risk of clinical relevance. Public health risk is attributed to factors such as high selective pressure, a lack of hygienic knowledge, poor management in the medical industry, and the recent trend of medical tourism. Therefore, the urgent need to evaluate the level of drug resistance is to stop the impending danger.

So, the dissertation was designed to examine the incidence of antibiotic resistant bacteria in hospital cafeteria fast foods in Dhaka, Bangladesh and their association with pathogenicity.
Precisely the study will address the following objectives:

- Examination of bacterial load and isolation, identification & characterization of the bacterial strains associated with hospital cafeteria fast foods.
- Spectrum of drug resistance among all the Gram-positive and Gram-negative isolates in hospital cafeteria fast foods.
- Phenotypic characterization of both Gram-positive and Gram-negative isolates.
- Molecular characterization of the bacterial strains and phylogenetic diversity analysis of the isolates.
- Phenotypic virulence assays of both Gram-positive and Gram-negative isolates.
- Detection of different virulence and resistance genes among the Gram-negative isolates.
- Assessment of the food handling knowledge and behavior practiced in hospitals through questionnaire.

Chapter 2:
Material and Methods

2.0 Materials and Methods

Hospital cafeteria fast foods vehiculated with pathogenic microorganisms along with the existence of numerous virulence and resistance genes adorned with its increasing popularity possesses one of the most severe impendence to mass people health. Therefore, the current study is an endeavor to unveil the various MDR bacterial species that are currently in circulation as well as the occurence of virulence and resistance determinants amidst the isolates from fast foods of hospital cafeteria in Dhaka, Bangladesh.

The materials and methods described in this chapter refer to procedures, operations that are of relevance to the study as a whole. Compositions of all media are enlisted in **Appendix I.** Chemicals and reagents are listed in **Appendix II.** The list of apparatus are given in **Appendix III**. **Figure 2.1** illustrates the whole work design of the present investigation.

2.1 Location of the study

The research was carried on eight hospital cafeterias in Dhaka city, Bangladesh. The hospital cafeterias were located in A (23°42'40.1"N, 90°24'06.6"E), B (23°43'44.7"N, 90°24'11.7"E), C (23°46'25.8"N 90°22'45.5"E), D (23°46'26.8"N 90°22'23.4"E), E (23°44'24.6"N 90°23'48.6"E), F (23°44'18.5"N 90°23'49.4"E), G (23°44'22.8"N 90°24'34.0"E) and H (23°45'02.1"N 90°22'12.6"E) sites. The reasons behind the selection of these hospital cafeterias were the admission of socio-economically diverse populations and consumption of foods from these cafeterias by their attendant, relatives, outpatients as well as staffs of the hospitals.

2.2 Study samples

Total 80 samples, 5 samples of sandwich and 5 samples of burger from each of 8 different hospital cafeterias in Dhaka city, Bangladesh were included in the present study. Different raw ingredients, meat products are generally present in sandwiches and burgers in addition to different handling processes. The study selected sandwich and burgers as samples because of the increasing popularity and assumption of presence of large number of food-borne pathogens in these fast foods.

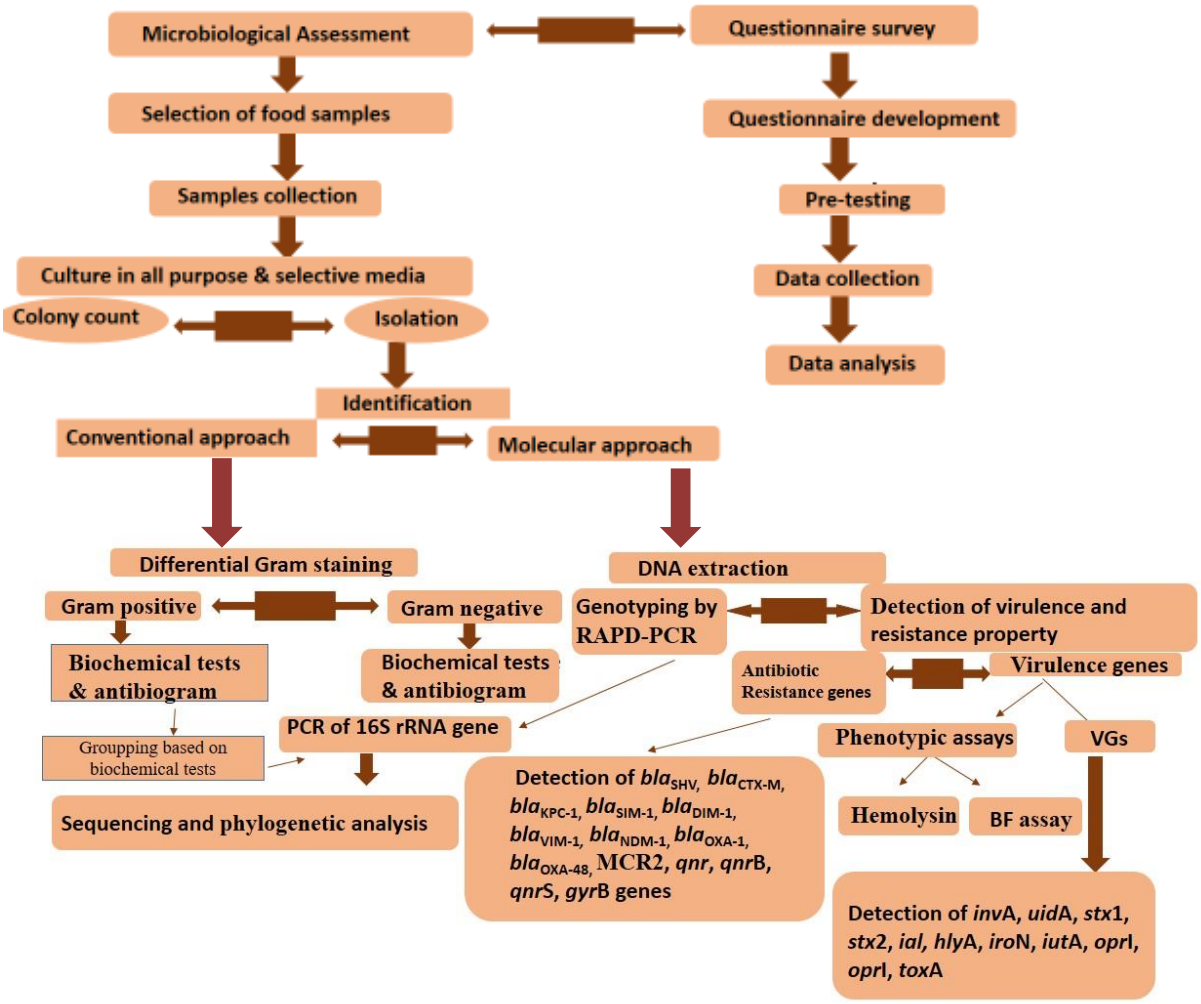


Figure 2.1 Complete design of research work

2.3 Sample collection

The study samples were collected from the eight hospital cafeterias between August 2019 and February 2021. Aseptic plastic bags were used for the collection of the samples. Then it was wrapped and kept in a refrigerated icebox (at 4^0C). The samples were brought to the research laboratory and processed within 2 hours of procurement. Institute of Nutrition and Food Science and Department of Microbiology, University of Dhaka provided the support for the microbiological assessment of the samples.

2.4 Processing and preparation of samples

After taking out from the sterilized plastics bag, sandwich and burger samples were kept in an aseptic petri-dish and about 10 grams of each sample was weighed. The next step was the blending of the measured 10 grams of each sample and mixing with already

prepared 90 mL peptone water (Clarence et al., 2009) in a conical flask. Then the flask was cotton plagged followed by shaking for the proper mixing of the sample. After that pipetting of 1 ml of the homogenized sample solution was done followed by transferring it into aseptic cotton plugged test tubes containing 9 mL of 0.1% peptone water. Then the sample solution shaked again for proper mixing. Thus, the preparation initial dilution was done. Other serial dilutions were contrived up to the 5^{th} dilution from this homogenization.

2.5 Isolation of bacterial species

50 µL of the samples of each dilution was plated on Plate Count Agar (PCA), MacConkey (MC) agar, *Salmonella-Shigella* (SS) agar, Eosine-Methylene Blue (EMB) agar, Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar, Potato Dextrose Agar (PDA), Nutrient Agar (NA), Mannitol Salt Agar (MSA), Phenylethyl Alcohol Agar (PEA), Citrate Agar, Blood Agar (Himedia, India; Oxoid, UK) using spread plate technique and Cooked Meat broth followed by incubation at 37 °C for 24 hours. Counting was carried out of normal plates of 25-250 colonies. The counts for each plate were expressed as colony forming unit of the suspension (cfu/ml). Pure cultures of the isolates were obtained through sub-culturing discrete colonies into differential and selective media. Pure isolates were then stored at 4 °C (F. Jahan et al., 2016).

2.6 Preservation of the isolates

Every isolate was streaked aseptically on NA plate and was incubated overnight at 37^0C. Following incubation, culture broth suspended thoroughly with 20% glycerol into aseptic eppendorf tube and kept into -20^0C. Two sets of eppendorf tubes were kept ready for every isolate and maintained at -80^0C.

2.7 Conventional approaches of identification of isolates

The conventional approach included morphological and cultural characteristics, gram staining, and biochemical tests. Isolates were subjected to different biochemical tests in accordance with the procedure for culture identification discussed in "Bergey's Manual of Determinative Bacteriology (2012)"(Levine, 1975)

2.7.1 Cultural and morphological properties

Each isolate was subcultured on NA plates and the resulting colonies were selected for gram staining. The morphological characteristics (shape, size, arrangement) were recorded. Moreover, the colonial morphology (appearance, form, elevation, margin and consistency) on MHA plate was also recorded.

2.7.2 Identification of isolates using biochemical tests

In accordance with the approach discussed in "Bergey's Manual of Determinative Bacteriology (2012)", a series of biochemical assessments were done for culture identification of the strains. For the presumptive identification of the strains, performed biochemical tests included Kligler Iron Agar (KIA), Motility Indole Urease (MIU), Nitrate reduction, Citrate use, Oxidase, Catalase, Coagulase, Sugar fermentation, Methyl Red-voges-Proskauer etc.

2.8 Phenotypic virulence assays

Biofilm formation capacity and hemolytic properties of the isolates were examined among different phenotypic virulence assays.

2.8.1 Capability of biofilm formation of the isolates

Standard methods were used to assess the biofilm formation potential of the isolates utilizing 96-well microtiter plate assays. The study used TSB as the control and 30% acetic acid in water as the blank to measure the absorbance of solubilized crystal violet (CV) at 600 nm on a plate reader. In the 96-well tissue culture plates, the assay was run twice. To evaluate the BF capacity of isolates on a 4-grade scale, the cut-off optical density (ODc), which was established as three standard deviations above the mean optical density of the negative control, was used. The scale classifies organisms into four categories, non-biofilm formers, NBF ($OD \leq ODc$); weak biofilm formers, WBF ($ODc < OD \leq 2 \times ODc$); moderate biofilm formers, MBF ($2 \times ODc < OD \leq 4 \times ODc$), and strong biofilm formers, SBF ($OD > 4 \times ODc$) (M. N. Hoque et al., 2020).

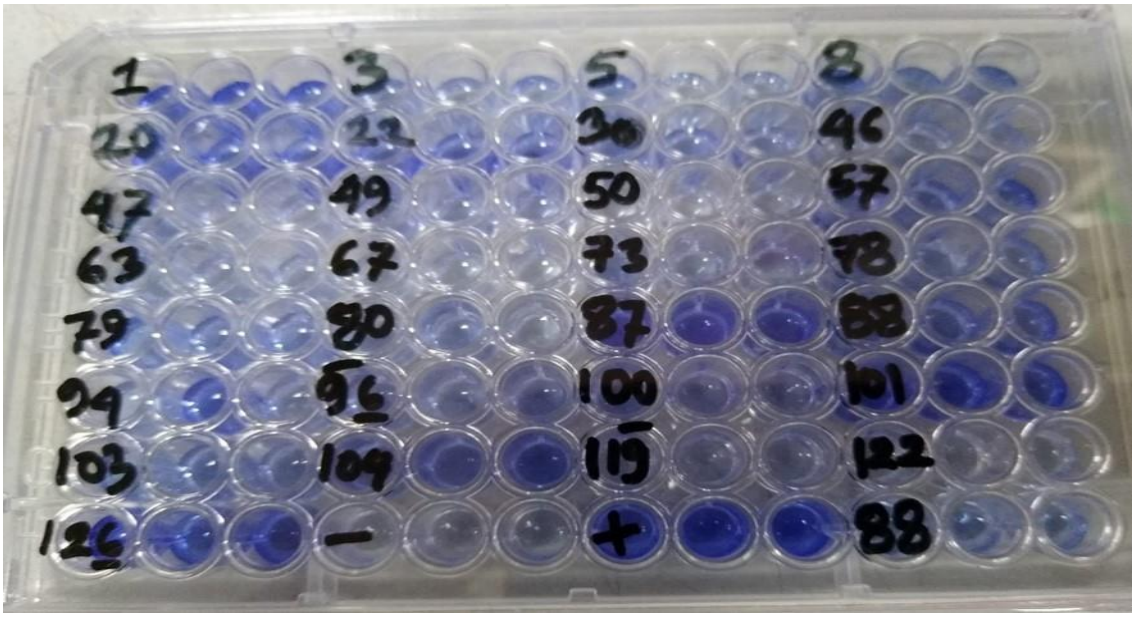


Figure 2.2 Biofilm formation in 96 well microtiter plate

2.8.2 Hemolytic properties of the isolates

The hemolytic properties of the isolates was investigated by streaking blood agar base plates with 5% sheep blood addition. After 24 hours at 37 degrees Celsius, the plates were screened for signs of a hemolysis pattern: β-hemolysis (clear zones around colonies), α-hemolysis (a green zone around colonies), or γ-hemolysis (no halo around colonies) (Saha et al., 2020).

2.9 Antibiotic susceptibility tests

The antibiotic susceptibility pattern of the selected isolates were tested with 11 distinct groups of antibiotics (17 antibiotics in total) including penicillins (ampicillin, 25µg; penicillin, 10 µg); aminoglycosides (gentamycin, 10 µg; kanamycin, 30 µg; streptomycin, 10 µg); cephalosporins (ceftriaxone,30 µg; cephalexin, 30 µg); chloramphenicol (chloramphenicol, 30 µg); macrolides (azithromycin, 30 µg); polypeptides (colistin,10 µg); quinolones (ciprofloxacin, 5 µg; levofloxacin, 5 µg); sulfonamides (sulphamethoxytrimethoprim, 25 µg); tetracycline (tetracycline, 30 µg); nitrofuran (nitrofurantoin, 300 µg) , carbapenem (imipenem, 10 µg ; meropenem, 10 µg) (Himedia, India; Titan media, India) **(Table 2.1)** using the standardized agar-disc-diffusion method known as the Kirbey Bauer method (Bauer et al., 1966). Although disc diffusion method has some limitations for colistin, as the colistin powder was not available, disc diffusion method had been used according to Dey et al., 2018 (Dey et al.,

2018). Commercially available antibiotic discs and Mueller-Hinton agar (Himedia, India) were employed for the antibiotic susceptibility test.

The following steps were taken to perform the antibiotic susceptibility test

2.9.1 Preparation of working culture

By following the standard guideline described by the Clinical and Laboratory Standards Institute (CLSI), turbidity stamdard of 0.5 Mcfarland wasprepared. For each isolate the inoculums was prepared as described below:

a. Each of the 87 isolates was revived from stock culture and again subcultured on MHA plate. Well isolated 2-3 colonies were selected from the fresh agar plate and transferred into a test tube containing 5 mL sterile Mueller Hinton Broth (MHB) (Himedia, India) **(Appendix-I).**

b. The inoculated broth was incubated for 2-4 hours at shaking incubator usually at 37°C till the turbidity achievement of 0.5 McFarland standard or equivalent turbidity of 0.1 OD at 600 nm, measured by a spectrophotometer (Visible Spectrophotometer 1200RS, China).

c. Sterile MHB was used to adjust the turbidity of actively growing broth culture to obtain turbidity optically comparable to the point of the 0.5 McFarland standards.

2.9.2 Inoculation procedure of the selected plates

In accordance with the following steps, inoculation of the plates with the working culture was done:

a. An aseptic cotton swab was soaked into the suspension ideally within 15 minutes of the turbidity adjustments of the inoculum suspension followed by rotating multiple times. Then the cotton swab was pressed firmly on the inside wall of the respective culture tube above the fluid level.

b. Then inoculation was done by streaking the swab over the entire dried surface of a MHA plate. The process was carried out twice, with the plate being rotated by roughly 60 degrees each time. Finally, a swab was taken from the rim of the agar. The procedure was done by maintaining standard biosafety issues into a Class II Biosafety Cabinet (Yakos65, China).

c. The lid was open for 3-5 minutes, but not exceeding 15 minutes for extra surface moisture absorption in advance of using the drug-impregnated disks

2.9.3 Impregnation of antimicrobial susceptibility disks on inoculated agar plates

Aseptic antibiotic susceptibility disks were dispensed onto the surface of every inoculated MHA plate. The individual discs were pressed down so that they can completely attach with the MHA surface. The disks were placed on the MHA surface in such a way so that the disks can maintain a distance of 24 mm from center to center. Within 15 minutes of the impregnation of the disks, the plates were placed inverted in the incubator (Memmert, Germany), which was set at 37°C.

2.9.4 Observation of plate readings and interpretation of findings

The zone of inhibition in each plate which is demonstrated by a uniform circle with a confluent lawn of growth was observed after 16-18 hours of incubation. Measurements were made of the disc's diameter as well as the diameter of the zones of total inhibition as determined by unaided eye inspection. Measurements for the zones were made to the nearest whole millimeter. In accordance with the Clinical and Laboratory Standards Institute break points, the results were finally classified as susceptible, intermediate, and resistant(Abbey & Deak, 2019), (Clinical & Laboratory Standards Institute, 2022). Multidrug-resistance (MDR) was described as acquired resistance to minimum one agent in three or more antimicrobial categories (Ap et al., 2012).

Table 2.1: Antibiotics with their groups, concentrations of the discs and zone interpretation according to CLSI guideline

Antibiotics groups	**Antibiotics**	**Concentration of the discs (µg)**	**Zone interpretation (diameter in mm)***		
			S	**M**	**R**
Carbapenems (Carboxypenems)	Imipenem (IMP)	10	≥23	20-22	≤19
	Meropenem(MER)	10	≥23	20-22	≤19
Aminoglycosides	Gentamycin (GEN)	10	≥15	13-14	≤12
	Kanamycin (K)	30	≥18	14-17	≤13
	Streptomycin (S)	10	≥15	12-14	≤11
Penicillins	Ampicillin (AMP)	25	≥17	14-16	≤13
	Penicillin (P)	10	≥17	14-16	≤13
Cephalosporins	Ceftriaxone (CTR)	30	≥23	20-22	≤19
	Cephalexin (CL)	30	≥18	15-17	≤14
Chloramphenicol	Chloramphenicol (C)	30	≥18	13-17	≤12
Macrolides	Azithromycin(AZM)	30	≥13		≤12
Polypeptides	Colistin (CT)	10	≥11		≤10
Quinolones	Ciprofloxacin (CIP)	5	≥26	22-25	≤21
	Levofloxacin (LEV)	5	≥21	17-20	≤16
Sulfonamides	Sulphamethoxytrimethoprim	25	≥16	11-15	≤10
Tetracycline	Tetracycline (TE)	30	≥15	12-14	≤11
Nitrofurans	Nitrofurantoin (F)	300	≥17	15-16	≤14

*S, M and R indicate sensitive, moderate and resistance zone respectively.

2.10 Molecular approach for identification of the isolates

Chromosomal DNA of the chosen isolates was extracted. **(Section 2.10.1)** followed by Random Amplification of Polymorphic DNA (RAPD) **(Section 2.10.3.1)** to categorize the isolates into distinct genotypes. In order to amplify the isolates' 16S rRNA gene, Polymerase Chain Reaction (PCR) **(Section 2.10.4)** was then carried out. For the purpose of performing phylogenetic analysis, the 16S rRNA gene amplicons of representative isolates of each genotype were sequenced **(Section 2.10.6).**

2.10.1 Extraction and purification of chromosomal DNA

Boiled DNA method was used to extract the chromosomal DNA of the pure isolates (Saha et al., 2020). Discrete colonies from NA plate was selected for subculture in a test tube containing 5 ml NB followed by overnight growth at 37ºC with aeration using shaking water bath set at 120 rpm. 1.0 ml of the culture was centrifuged at 13,000 rpm for 10 min by employing a centrifuge (Eppendorff, Germany). The cell pellets were suspended with 200µl PCR water after withdrawn of the supernatant followed by centrifugation at 120 rpm for 10 min. Then by using a heat block, they were boiled at 100^0C for 10 minutes accompanied by chilling in ice for 10 minutes and then centrifuged once more for 10 minutes at 10,000 rpm. 100-150 µl of the supernatant was collected into a fresh eppendorf tube

2.10.2 Quantification of DNA concentration

The concentration of extracted DNA was quantified as ng/ µl by using Nanodrops (Thermo Scientific, USA). The ratio between the readings at 260 nm and 280 nm (A260\280) provides an estimate of the purity of the DNA. Pure DNA preparations have A260\280value of 1.8.

2.10.3 Molecular fingerprinting of the Gram-negative isolates

Random Amplification of Polymorphic DNA (RAPD) was used to group the isolates into different genotypes.

2.10.3.1 Random Amplification of Polymorphic DNA

Variations among the genotypes of Gram-negative isolates were screened through random amplification of polymorphic DNA (RAPD) employing primer 1283 (5′-

GCGATCCCCA-3′) (Weisburg et al., 1991). A reaction mixture of 10 µl was used for PCR reaction containing 5.5 µl of ready green master mix (Promega, USA), 1µl of template DNA, 1 µl of primer and 2.5 µl nuclease free water (Promega, USA) in each tube. The homogenate was subjected to cycling conditions including an initial denaturation for 5 min at 94 °C, followed by denaturation at 94 °C for 1.0 min, then primer annealing for 1 min at 39 °C , after that extension at 72 °C for 2.0 min, and finally delay at 72 °C for 5 min. 1.5% agarose gel made with ethidium bromide (5 µg/mL) was used for electrophoresis of PCR amplicons for separating the bands and finally visualization was done by using Image ChemiDoc™ Imaging System (Bio-Rad, Hercules, CA, USA) (Saha et al., 2020). In this RAPD PCR, the study used two different DNA markers of 1 Kb and 100 bp (Promega, USA) as control**.**

2.10.4 Polymerase Chain Reaction (PCR) of 16S ribosomal RNA (rRNA) gene

Polymerase Chain Reaction (PCR) using universal primers **(Table 2.2**) was employed to amplify the 16S rRNA gene of each extracted Chromosomal DNA of the selected isolates from each RAPD genotypes (Lane, 1991; Weisburg et al., 1991). The following steps were followed:

2.10.4.1 Reaction mixture preparation

Definite volume of primers, template DNA plus other components were mixed thoroughly for the preparation of reaction mixture in an eppendrof in the order provided in the **Table 2.3**. In case of preparation of reaction mixture for a large number of reactions, a master mix was prepared without template DNA and aliquoted into PCR tubes. Finally the addition of specific template DNA in the appropriately labeled PCR tube was done. A negative control having all components of mixture except template DNA was included in all PCR. A positive control (having known template DNA) was included in relevant cases. Placement of the PCR tubes was done in a thermal cycler (Veriti 96-Well Thermal Cycler, Applied Biosciences, USA) followed by setting of the amplification parameters correctly.

2.10.4.2 Amplification parameters of PCR

The amplification parameters for PCR of 16S rRNA gene that was set in the thermal cycler provided in **Table 2.4.** PCR tubes were refrigerated at - 20˚C after accomplishment of PCR until future analysis.

Table 2.2: Primers for 16S rRNA gene PCR

Primers	Sequence (5'→3')	Amplicon Size	Reference
27F	5'AGAGTTTGATCCTGGCTCAG-3'	~1400bp	(Lane, 1991)
1492R	5'-GGTTACCTTGTTACGACTT-3'		

Table 2.3: Components of PCR reaction mixture

Component	Component Volume	Final Concentration
$MgCl_2$, 25 mM Solution	2.0–8.0 µl	1.0–4.0 mM
5X Colorless GoTaq® Flexi Buffer Or, 5X Green GoTaq® Flexi Buffer	10 µl	1.0X
PCR Nucleotide Mix, 10 mM each	1µl	200 µM each
upstream primer (Table 2.6)	5–50 pmol	0.1–1.0 µM
downstream primer (Table 2.6)	5–50 pmol	0.1–1.0 µM
GoTaq® DNA Polymerase, 5 u/µl	0.25 µl	1.25 u/50 µl
template DNA	Variable	<0.5 µg/50 µl
Nuclease-Free Water to a final volume of	50 µl	

Table 2.4: Amplification parameters of 16S rRNA gene

Segment	**Condition**	**Cycle number**
Initial Denaturation	95°C for 5 minutes	1
Denaturation	95°C for 1minute 30seconds	**35**
Annealing	56°C for 1minute	
Extension	72°C for 1minute 30seconds	
Final extension	72°C for 7minutes	**1**

2.10.5 Amplicons analysis by agarose gel electrophoresis

The PCR products was resolved in 1% agarose gel. The successful amplification of the desired genes was then visualized using 1% agarose gel. For the preparation of 1% agarose, 1g of agarose (Sigma, USA) was added in 100 ml of 1X Tris-acetate EDTA (TAE) buffer **(Appendix II)** followed by heating of the mixture for 2.5-3 minutes in a microwave oven so that the agarose dissolve completely. Ethidium bromide (EtBr) was added to the boiling mixture at a final concentration of 0.5 g/ml after allowing it to cool

to about 45°C. The gel was applied to a gel casing that had been well-formed (comb-set) and was then left to set on a flat surface. The comb was taken off following the gel's solidification, and buffer (1x TAE) was added to the tank to fully encapsulate the gel. Mixing 5µl of PCR product with 1 µl of 6X loading buffer **(Appendix II)** was used to prepare the samples. The electrophoresis procedure was carried out for 50 minutes at 100 volts and 200 mA after the samples had been loaded into the wells. Alpha Imager HP Gel documentation system (Cell Bioscience, USA) was used to view the gel. With the help of a computer connected to the device, the photographs collected followed by analysis of the bands.

2.10.6 Ribosomal (16S rRNA) gene sequencing and phylogenetic exploration

The successful 16S rRNA gene amplicons of every representative RAPD category were sequenced by Macrogen Inc. Seoul, South korea.

2.10.6.1 Sequence alignment and identification

SeqMan Genome Assembler (DNAstar, USA) was used to combine the partial sequences of 16S rRNA gene of the selected isolates (Swindell & Plasterer, 1997). The identification of the isolates was made by 16S rRNA sequence comparison with GenBank database (Benson et al., 2015) of the National Center for Biotechnology Information (NCBI) using basic local alignment search tool (BLAST).

2.10.6.2 Construction of phylogenetic tree

Phylogenetic tree of the 16S rRNA gene sequences of the representative resistant isolates belonging to different RAPD groups was constructed using the different servers and bioinformatics software.

Briefly, MEGA11 (Tamura et al., 2021) was used to retrieve reference sequences from NCBI: http://www.ncbi.nlm.nih.gov database. ClustalW was used for alignment and trimming of the sequences. Phylogenetic tree was constructed with the aligned sequences using the Neighbor joining algorithm (Saitou & Nei, 1987) and Kimura-2 parameter model (Kimura, 1980) selecting 1000 bootstrap replication. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branch.

2.11 Identification of virulence genes

The virulence genes were identified by gene specific PCR among the Gram-negative bacteria. 11 different virulence genes were studied for different groups of bacterial strains .To detect the *Salmonella* strains; *inv*A (Invasion protein A) gene; an invasion gene had been used. The *Salmonella inv*A gene has been demonstrated to be an appropriate PCR target since it comprises sequences that are specific to this genus (Malmarugan et al., 2011; Saha et al., 2020). The selected genes for *E. coli* strains were *uid*A (β-D-glucuronidase), *stx*1 (shiga toxins 1), *stx*2 (shiga toxins 2), *ial* (invasion-associated locus) (Janik et al., 2020; López-Saucedo et al., 2003.; Saha et al., 2020). The virulence genes used for *Klebsiella* strains were *hly*A (alpha hemolysin), *iro*N (catecholate siderophore receptor) (Davies et al., 2016; Saha et al., 2020). *iut*A (aerobactin gene)(Clarence et al., 2009) and *uid*A (β-d-glucuronidase) genes were used for *Enterobacter* spp. (This study) . The selected genes for *Pseudomonas* strains were *opr*I (Outer membrane lipoprotein I), *opr*L (peptidoglycan-associated lipoprotein *opr*L) (Gholami et al., 2016a; Matthijs et al., 2013) that are associated with nosocomial infection; *tox*A (exotoxin A gene); (Abd El Tawab et al., 2016; A.Khattab et al., 2015). Elaborated description of primer sequences of selected virulence genes with their anticipated amplicon size are given in **Table 2.5.**

Table 2.5: Sequences of the primers employed for detection of virulence genes and annealing temperature of the primers with their expected amplicon size.

Name of primer	Primer Sequence (5′-3′)	Annealing T_a (^{0}C)	Targeted gene	Size (bp)	References
S139	5′-GTGAAATTATCGCCACGTTCGGGCAA-3′	55 (1min)	*inv*A	284	K. Sultana et al., 2021
S141	5′-TCATCG CAC CGT CAAAGGAACC-3′				
UidA-F	5′-AAAACGGCAAGAAAAAGCAG-3′	48 (1min)	*uid*A	147	Saha et al., 2020
UidA-R	5′-ACGCGTGGTTACAGTCTTGCG-3′				
Stx1-F	5′-CTGGATTTAATGTCGCATAGTG-3′	46 (1min)	*stx*1	150	
LT-R	5′-CGGTCTCTATATTCCCTGTT-3′				
Ial-F	5′-GGTATGATGATGATGAGTCCA-3′	58 (1min)	*ial*	650	
Ial-R	5′-GGAGGCCAACAATTATTTCC-3′				
Stx2-F	5´-GGC ACT GTC TGA AAC TGC TCC-3´	61 (1min)	*stx*2	255	López-Saucedo et al., 2003
Stx2-R	5´-TCG CCA GTT ATC TGA CAT TCT G-3´				
HlyA-F	5´-GCATCATCAAGCGTACGTTCC-3´	56 (1min)	*hly*A	534	Saha et al., 2020
HlyA-R	5´-AATGAGCCAAGCTGGTTAAGC-3´				
IroN-F	5´- AAGTCAAAGCAGGGGTTGCCCG-3´	77 (1min)	*iro*N	667	Davies et al.,2016
IroN-R	5´- GATCGCCGACATTAAGACGCAG-3´				
IutA-F	5´-GGCTGGACATCATGGGAACTGG-3´	67 (1min)	*iut*A	300	(T. Ahmed et al., 2020)
IutA-R	5´-GCGTCGGGAACGGGTAGAATCG-3´				
OprI-F	5'-ATGAACAACGTTCTGAAATTCTCTGCT-3'	55 (1min)	*opr*I	249	Gholami et al., 2016
OprI-R	5'-CTTGCGGCTGGCTTTTTCCAG-3'				
OprL-F	5'-ATGGAAATGCTGAAATTCGGC-3'	55 (1min)	*opr*L	504	
OprL-R	5'-CTTCTTCAGCTCGACGCGACG-3'				
ToxA-F	5´-GACAACGCCCTCAGCATCACCAGC-3´	66 (1min)	*tox*A	454	Abd El Tawab et al., 2016)
ToxA-R	5´-CGCTGGCCCATTCGCTCCAGCGCT-3´				

2.12 Detection of antibiotics resistance genes

The study investgated 14 different antimicrobial resistance genes among the Gram-negative bacteria including class A β-lactamase resistance (*bla*$_{SHV,}$ *bla*$_{CTX-M}$) (Chanawong et al., 2000; Pagani et al., 2003), carbapenamases resistance *(bla*$_{KPC-1,}$ *bla*$_{SIM-1,}$ *bla*$_{DIM-1,}$ *bla*$_{VIM-1,}$ *bla*$_{NDM-1,}$ *bla*$_{OXA-1,}$ *bla*$_{OXA-48}$), polypeptides resistant (MCR2) along with fluoroquinolone resistance (*qnr*, *qnr*B, *qnr*S, *gyr*B) (Begum & Shamsuzzaman, 2016; Bouchakour et al., 2010; M. I. Jahan et al., 2020; Rakhi et al., 2019; M. Sultana et al., 2019) by gene specific PCR. The expected amplicon size of the *bla*$_{SHV}$, *bla*$_{CTX-M,}$ *bla*$_{KPC-1,}$ *bla*$_{SIM-1,}$ *bla*$_{DIM-1,}$ *bla*$_{VIM-1,}$ *bla*$_{NDM-1,}$ *bla*$_{OXA-1,}$ *bla*$_{OXA-48,}$ MCR2, *qnr*, *qnr*B, *qnr*S, *gyr*B were 861, 593, 900, 551, 688, 801, 465, 882, 438, 219, 200, 264, 400 & 210 bp respectively. Elaborated description of primer sequences of selected antibiotic resistance genes with their anticipated amplicon size are given in **Table 2.6.**

Table 2.6: Sequences of the primers employed for detection of antibiotic resistance genes and annealing temperature of the primers with their expected amplicon size.

Name of primer	Primer Sequence (5′-3′)	Annealing T_a (^{0}C)	Targeted gene	Size (bp)	References
KPC5F	5′-TGTCACTGTATCGCCGTC-3′	58 (30 Sec)	*bla* $_{KPC-1}$	900	Rakhi et al., 2019
KPC10R	5′-CTCAGTGCTCTACAGAAAACC-3′				
SIM1-F	5′-TACAAGGGATTCGGCATCG-3′	56 (30 Sec)	*bla* $_{SIM-1}$	551	
SIM1-R	5′-TAATGGCCTGTTCCCATGTG-3′				
DIM-1A	5′-TCTATTCAGCTTGTCTTCGC-3′	53 (45 Sec)	*bla* $_{DIM-1}$	688	
DIM-1B	5′-TGTTAGAGGCTGTCTCAGCC-3′				
VIM1F	5′-TTATGGAGCAGCAACCGATGT-3′	60 (45 Sec)	*bla* $_{VIM-1}$	801	
VIM1R	5′-CAAAAGTCCCGCTCCAACGA-3′				
NDM-F	5´-CTTCCAACGGTTTGATCGTC-3´	58 (45 Sec)	*bla* $_{NDM-1}$	465	
NDM-R	5´-TAGTGCTCAGTGTCGGCATC-3´				
OXA1F	5´-AGCCGTTAATTAAGCCC-3´	56 (30 Sec)	*bla* $_{OXA-1}$	882	
OXA1R	5´-CTTGATTGAAGGGTTGGGCG-3´				
OXA-F	5´- AAGTCAAAGCAGGGGTTGCCCG-3´	54 (30 Sec)	*bla* $_{OXA-48}$	438	
OXA-R	5´- CATCAAGTTCAACCCAACCG-3´				
SHV-F	5´-CTTTACTCGCTTTATCG-3´	50 (50 Sec)	*bla*$_{SHV}$	861	Chanawong *et al*. 2000
SHV-R	5´-TCCCGCAGATAAATCACCA-3´				
CTXU1	5'-ATGCTGCAGYACCAGTAARGT-3'	55 (40 Sec)	*bla*$_{CTX-M}$	593	Pagani et al, 2003
CTXU2	5'-TGGGTRAARTARGTSACCAGA-3'				
mcr-2_279F	5'-CCTTTTGTGCTGATGGGTTT-3'	51(50sec)	mcr2	279	Liu et al., 2020
mcr-2_279R	5'-ATTTTGGAGCATGGTGGTGT-3'				
*qnr*A -F	5´- ATTTCTCACGCCAGGATTTG-3´	58 (50min)	*qnr*A	516	Salah et al., 2019
*qnr*A -R	5´- GATCGGCAAAGGTTAGGTCA-3´				
QnrB-F	5'-CGMATHGAAATTCGCCACTG-3'	56 (50 Sec)	*qnr*B	264	Saha et al., 2020
QnrB-R	5'-TTTGCYGYYCGCCAGTCGAA-3'				
Qnr-F	5'-GACGTGCTAACTGCGTGAT-3'	58 (1min)	*qnr*S	388	Bouchakour et al, 2010
Qnr-R	5'-AACACCTCGACTTAAGTCTGA-3'				
gyrBP1	5′-AGAGCGAGAAGAAAAAACCT-3′	60 (1min)	*gyr*B	210	Wang et al., 2012
gyrBP2	5′-CTCCCATAAGCATAGAGAAGA-3′				

2.13 Gene sequencing of virulence and resistance genes

The *inv*A, *opr*L, *uid*A, bla_{SHV} genes were confirmed by gene sequencing.

2.14 Assessment of food handler's knowledge and practices

2.14.1 Study design

The study was cross- sectional in nature.

2.14.2 Study population

Willing and available food handlers during data collection of the selected governmental and non-governmental hospital cafeterias of Dhaka city, were included for assessment of knowledge and practices involved.

2.14.3 Sampling method

Purposive sampling method was used in this research.

2.14.4 Data collection techniques

After literature survey, structured questionnaire and checklist **(Appendix-IV)** developed for data collection. The responses of the food handlers were gathered using a structured questionnaire, and an observation checklist was created to gather data on the knowledge and behavior of food handlers. The questionnaire was adapted and modified from previously published studies. There were three different parts in the questionnaire; socio-demographic, knowledge (8 statements) and practices (8 questions). It was prepared in English first. It was translated into Bengali later. Finally the questionnaire was administered in Bengali. Some minor modifications were carried out after the pilot study.

2.14.5 Data analysis

Data analysis was performed using SPSS version 25 statistical package. Data was entered first and cleaned before analysis. For the significant difference among the counts, Mann-Whitney U Test, Independent sample t-test, Shapiro-Wilk test, Levene's test for equality of variance was investigated. To determine the influence of different socio-demographic variables of food handlers on their practice score, Cross tabulations and chi-squared tests (5% significance level) were used. Based on the summation of individual scores of the variables, the score of knowledge and practice was categorized

as: good or poor. The score range for knowledge of food handlers and food handling practices was between 0-8 and the scores were converted to 100 points. The food handlers who earned a score of more than 75% were assumed as good and below it assumed as poor. Eight statements were used to score the food safety knowledge and eight variables were used for scoring overall practice score of the food handlers. Univariate and multivariate analyses were used to identify the Potential influencing factors towards practice. Statistically significant variables in univariate analysis and possesing main interests of the study were selected for multivariate analysis. Appropriate tabulations based on the determined variables, crude or adjusted odds ratio with 95% confidence interval and its corresponding p-values were used for result interpretation and presentation.

Chapter 3:
Results

3.0 Results

Hospital-acquired infections, also known as nosocomial infections is increasing tremendously in the last few decades. As nosocomial infections have been linked to multidrug-resistant infections, hospital-acquired infections have an impact not only on the individual patients but also on the community. Pathogens can be spread in the healthcare setting in a variety of ways, including direct contact with healthcare personnel or exposure to contaminated surroundings, contaminated food, contaminated water etc.

So this present study, carried out at the Institute of nutrition & Food Science and Department of Microbiology, University of Dhaka focused on the prevalence of antibiotic resistant bacteria in hospital cafeteria fast foods in Dhaka, Bangladesh and their association with pathogenicity. The study findings can be featured as-

- Examination of bacterial load and isolation, identification & characterization of the bacterial strains associated with hospital cafeteria fast foods.
- Spectrum of antibiotic resistance among both Gram-positive and Gram-negative bacteria in hospital cafeteria fast foods.
- Phenotypic characterization of both Gram-positive and Gram-negative isolates.
- Molecular characterization of the bacterial strains and phylogenetic diversity of the isolates.
- Phenotypic virulence assays of both Gram-positive and Gram-negative isolates.
- Detection of different virulence and resistance genes among the Gram-negative isolates.
- Assessment of the food handling knowledge and behavior practiced in hospitals through questionnaire.

3.1 Total viable count of bacteria from the fast food samples

The total aerobic bacterial count was assessed by spread plate technique using plate count agar (PCA) media **(Table 3.1)**. For the burger samples, the total viable bacterial count ranges from 2.1×10^2 to 4.0×10^6 (CFU/g). According to the guidelines provided by the Center for Food Safety, 2022 (Center for Food Safety 2022) regarding total bacterial count 37.5% of burger samples was unsatisfactory, 37.5% was in borderline and 25% was in satisfactory condition. The total bacterial count for sandwich samples ranges from 7.0×10^2 to 1.0×10^6 (CFU/g). About 50% of sandwich samples total bacterial count was unsatisfactory, 37.5% was in borderline and only 12.5% was in satisfactory condition.

Table 3.1 Total count of bacteria isolated from Burger and Sandwich samples from governmental and non-governmental hospital cafeterias using PCA

Type of Hospital	Hospital Name	Burger		Sandwich	
		Mean Total Count (CFU/g)*	Remarks	Mean Total Count (CFU/g)*	Remarks
Governmental	H_1	6.0×10^4	Borderline	6.0×10^5	Unsatisfactory
	H_2	7.5×10^3	satisfactory	1.0×10^6	Unsatisfactory
	H_3	4.2×10^5	Unsatisfactory	1.6×10^5	Unsatisfactory
	H_4	6.0×10^4	Borderline	1.3×10^4	Borderline
Non-Governmental	H_5	7.8×10^4	Borderline	2.5×10^4	Borderline
	H_6	6.8×10^5	Unsatisfactory	7.0×10^2	Satisfactory
	H_7	4.0×10^6	Unsatisfactory	4.1×10^4	Borderline
	H_8	2.1×10^2	Satisfactory	4.1×10^5	Unsatisfactory

*$<10^3$ = Satisfactory, 10^3 -$<10^5$ = Borderline, $\geq 10^5$ = Unsatisfactory (Center for Food Safety, 2022.). Average count of 5 duplicates of the same samples for each hospital.

In case of sandwiches, data were checked for normal distribution by Shapiro-Wilk test. The Shapiro-wilk test revealed the mean total count data for Governmental hospitals were normally distributed (P=0.663) but data for Non-Governmental hospitals were not normally distributed (P=0.015). Therefore, the Mann-Whitney U Test, non-parametric tests of Independent sample t-test, was employed to compare the means between government and non-government hospital cafeterias. There is no significant difference

(P=0.343) between the total count of viable bacteria found in sandwiches from different government and non-government hospital cafeterias using PCA as demonstrated by the Mann-Whitney U test result .

In case of burgers, data were checked for normal distribution by Shapiro-Wilk test. The Shapiro-wilk test revealed the mean total count data for both Governmental (P=0,030) and Non-Governmental (P=0.039) hospitals were not normally distributed. Therefore, the Mann-Whitney U Test, non-parametric tests of Independent sample t-test, was employed to compare the means between government and non-government hospital cafeterias. The Mann-Whitney U Test result showed that there is no significant difference (P=0.486) between the total count of viable bacteria found in burgers from different government and non-government hospital cafeterias using PCA.

3.2 Total count of enteric bacteria using MacConkey's agar

Gram-negative and enteric bacilli—which are typically found in the gastrointestinal tract—were isolated using MacConkey's agar, a selective and differentiating bacterial culture medium. A wide variety of enteric pathogens, including those that cause typhoid, paratyphoid, and all forms of dysentery, were found and isolated using this differential plating medium.

In case of sandwich, four hospitals showed unsatisfactory, four showed borderline level of growth. In case of burger fiour hospitals showed unsatisfactory, two hospital showed borderline, one showed satisfactory level of growth and one showed no growth **(Table 3.2)**.

Table 3.2 Total count of enteric bacteria found from Burger and Sandwich samples from different hospital cafeteria using MacConkey's agar.

Type of Hospital	Hospital Name	Burger		Sandwich	
		Mean Total Count (CFU/g)*	**Intensity of Growth**	**Mean Total Count (CFU/g)***	**Intensity of Growth***
Governmental	H_1	2.0×10^1	Satisfactory	5.0×10^2	Borderline
	H_2	1.4×10^3	Borderline	1.1×10^5	Unsatisfactory
	H_3	1.0×10^5	Unsatisfactory	1.2×10^5	Unsatisfactory
	H_4	2.7×10^4	Unsatisfactory	7.3×10^5	Unsatisfactory
Non-Governmental	H_5	2.1×10^3	Borderline	6.0×10^4	Unsatisfactory
	H_6	4.6×10^4	Unsatisfactory	4.1×10^2	Borderline
	H_7	4.0×10^5	Unsatisfactory	2.9×10^2	Borderline
	H_8	-	-	1.4×10^3	Borderline

*$<10^2$ = Satisfactory, 10^2-$\leq 10^4$ = Borderline, $>10^4$ = Unsatisfactory (Microbiological Guidelines for Food, Center for food safety, 2023.). Average of 5 samples for each hospital.

In case of burgers, data were checked for normal distribution by Shapiro-Wilk test. The Shapiro-wilk test revealed the mean total count data for Governmental hospitals were normally distributed (P=0.113) but data for Non-Governmental hospitals were not normally distributed (P=0.020). Therefore, the Mann-Whitney U Test, non-parametric tests of Independent sample t-test, was employed to compare the means between government and non-government hospital cafeterias. The results of the Mann-Whitney U test revealed that the total number of enteric bacteria discovered burgers from various government and non-government hospital cafeterias using MAC did not differ significantly (p=1.00).

In case of sandwiches, data were checked for normal distribution by Shapiro-Wilk test. The Shapiro-wilk test revealed the mean total count data for both Governmental (P=0.058) and Non-Governmental (P=0.312) hospitals were normally distributed. Then, Levene's test for equality of variance was done and data did not follow equal variance

(P=0.001). Therefore, Independent sample t-test, was employed to compare the means between government and non-government hospital cafeterias. The t-test result revealed that there is no significant difference (P=0.227) between the total count of viable bacteria found in sandwiches from different government and non-government hospital cafeterias using PCA.

3.3 Extent of bacterial growth in other differential Media

The degree of growth of bacteria in TCBS, EMB and SS were observed. Presence of fungal growth had been detected by using Potato Dextrose Agar (PDA) medium. Any kind of growth on Aseptic medium was counted as positive fungal growth for that sample. Presence of anaerobic bacteria had been identified by using cooked meat broth medium**.** According to the degree of growth of bacteria, + means low growth; ++ expresses moderate growth and +++ means high growth of organisms. Where no growth of bacterial colonies were observed, it is reported as nil **(Table 3.3).**

Table 3.3 Extent of bacterial growth found in Sandwich and Burger samples from different hospital cafeterias using TCBS, EMB, SS, CM and PDA media.

Hospital	Sandwich					Burger				
	TCBS*	EMB*	SS*	CM*	PDA*	TCBS*	EMB*	SS*	CM*	PDA*
A	-	+	+	+	+++	-	+	++	++	++
B	-	+++	++	+	++	-	++	++	++	++
C	-	++	+	++	+	-	++	+	+	+
D	-	+++	+++	++	++	-	+++	+++	++	+++
E	-	+++	+	+++	+++	-	++	+	+++	++
F	-	+++	+++	++	++	-	+++	+	++	+
G	-	+	+	+	+++	-	+++	+++	++	++
H	-	+	++	+	+++	-	-	-	+	++

*low growth = +, Moderate growth= ++, High growth = +++ and –for no growth (Biva et al., 2019)**.**

In case of growth in EMB and SS, all the hospitals showed growth except the burger sample of hospital H. The burger sample from hospital H had no growth in EMB and SS media. All samples had negative growth in TCBS. All the sandwich and burger samples from all hospitals showed positive growth in CM and PDA.

3.4 Distribution of the isolates

A total of 137 isolates of different bacterial species from the sandwich and burger samples of eight different hospital cafeterias of Dhaka, Bangladesh were isolated of which about 54.74% of the isolates were from sandwich samples and 45.26% isolates were from burger samples **(Figure 3.1.1a).** Eighty seven (63.50%) isolates were Gram-negative and fifty (36.50%) isolates were Gram-positive **(3.1.2a).**

About 52.87 % (46) of the Gram-negative isolates were from sandwich and 47.12% (41) were from burger **(Figure 3.1.1b)**. Among the Gram-positive isolates, about 58% (29) of the isolates were from sandwich and 42% (21) of the isolates from burger **(Figure 3.1.1c)**.

The number of Gram-negative isolates varied greatly between the various bacterial species, with *Klebsiella* spp. accounting for the majority (42.53%), followed by *Leclercia/Enterobacter* spp. (29.89%), *Pseudomonas* spp. (18.39%), *E. coli* (4.59%), *Citrobacter* spp. (2.3%), *Salmonella* spp. (1.15%), and *Acinetobacter* spp. (1.15%) **(Figure 3.1.2b)**. The distribution of Gram-positive isolates demonstrated that *Bacillus* spp. (58%) had the highest percentage of isolates, followed by *Planococcus* spp. (26%), *Micrococcus* spp. (6%), *Streptococcus* spp. (4%), *Clostridium* spp. (4%) and *Staphylococcus* spp. (2%) **(Figure 3.1.2c).**

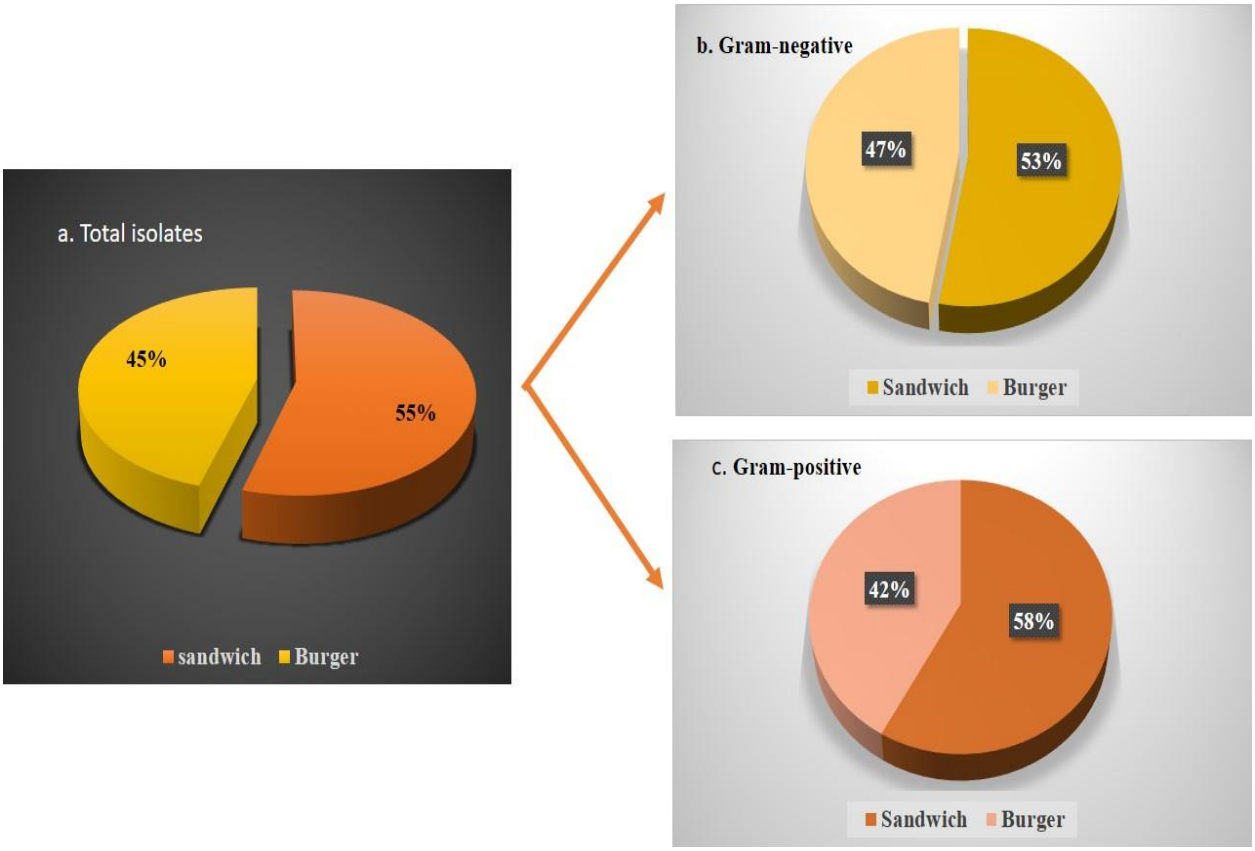


Figure 3.1.1: Prevalence of isolates isolated from different hospital cafeteria fast foods samples from eight different hospital cafeterias in Dhaka, Bangladesh. a) Distribution of total isolates in different types of fast food samples; b) Prevalence of Gram-negative isolates in different types of fast food samples and c) Prevalence of Gram-positive isolates in different types of fast food samples.

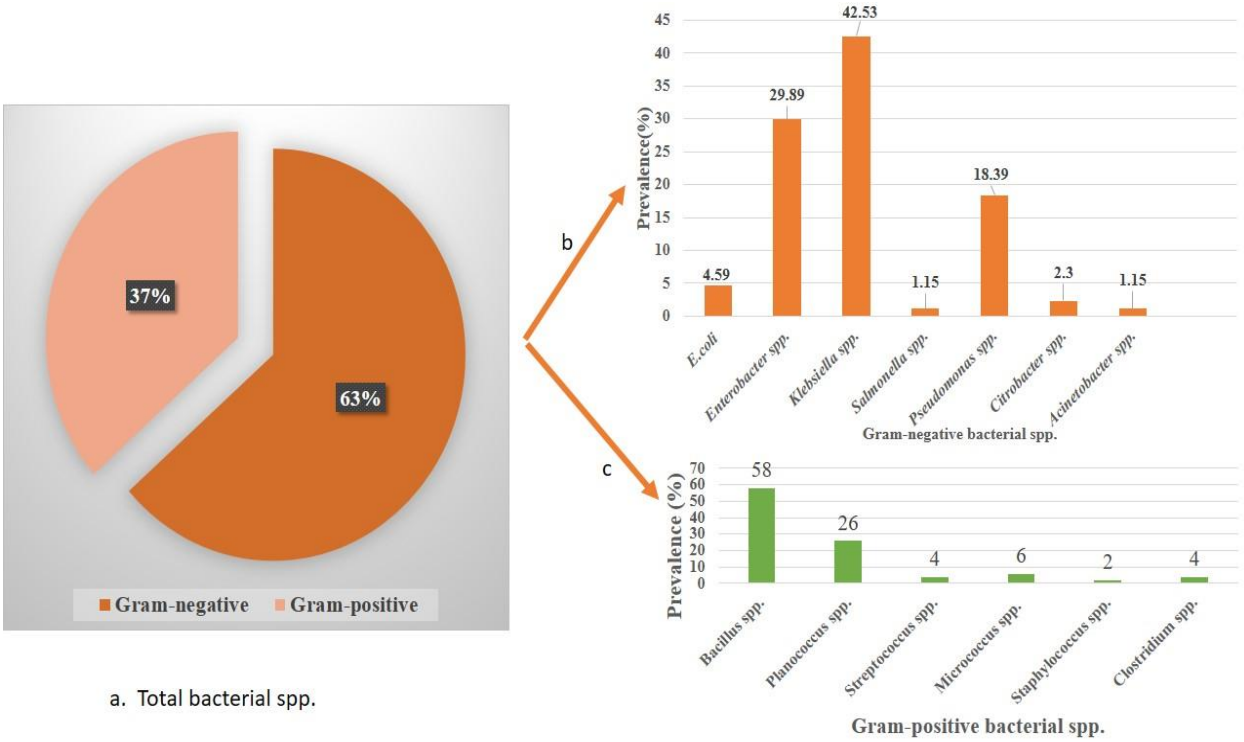
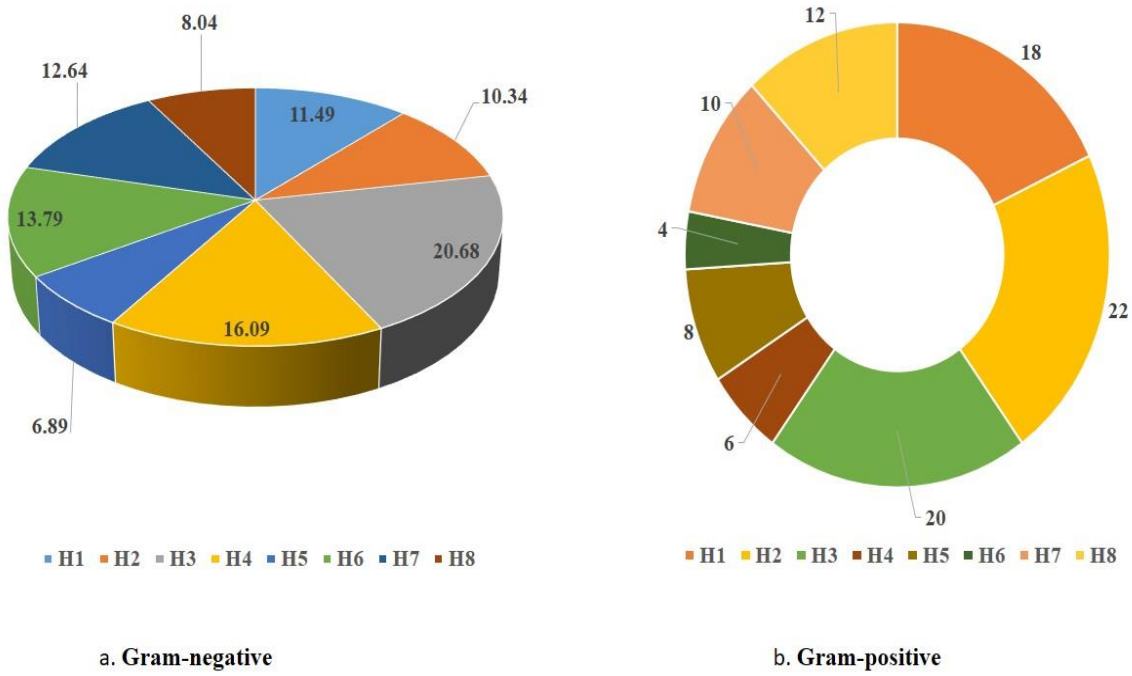


Figure 3.1.2: Distribution of bacterial species isolated from different hospital cafeteria fast foods samples from eight different hospital cafeterias in Dhaka, Bangladesh. a) Prevalence of Gram-negative and Gram-positive isolates; b) Distribution of Gram-negative isolates among different bacterial species and c) Distribution of Gram-positive isolates among different bacterial species.

According to the various hospital cafeterias, different Gram-negative isolate prevalence rates were observed, with H3 having the highest percentage of isolates (20.68%), followed by H4 (16.09%), H6 (13.79%), H7 (12.64%), H1 (11.49%), H2 (10.34%), H8 (8.04%), and H5 (6.89%) **(Figure 3.1.3a).**

Different hospital cafeterias had different rates of prevalence of Gram-positive isolates having the highest number of isolates H2 (22%) followed by H3 (20%), H1 (17%), H8 (12%), H7 (10%), H5 (8%), H4 (6%) and lowest number in H6 (4%) **(Figure 3.1.3b).**



a. **Gram-negative** b. **Gram-positive**

Figure 3.1.3 Prevalence of bacterial isolates among different hospital cafeterias in Dhaka, Bangladesh. a) Distribution of Gram-negative isolates in different hospital cafeterias and

b) Distribution of Gram-positive isolates in different hospital cafeterias. Here, H for hospital cafeteria.

3.5 Phenotypic identification of the isolates

Phenotypic identification of the isolates were done by examining the cultural, morphological and biochemical characteristics of the isolates. Based on different phenotypic tests, the Gram-negative isolates were grouped into seven different presumptive bacterial species including *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Citrobacter* spp. and *Acinetobacter* spp. **(Table 3.4, Figure 3.2).** The presumptive Gram-positive bacteria were grouped into six different presumptive bacterial species including *Bacillus* spp., *Planococcus* spp., *Micrococus* spp., *Streptococcus* spp., *Clostridium* spp. and *Staphylococcus* spp. **(Table 3.5).**

Figure 3.2 Phenotypic characterization of bacterial isolates using culture-based techniques such **A.** Growth in different media SS agar (a), EMB agar (b), BAB agar (c, e), MacConkey agar (d), NA agar (f) and **B**. different biochemical tests such as KIA (a), MIU (b), Citrate (c), MR (d), VP (e), Oxidase (f), Catalase (g) tests were used.

Table 3.4 Cultural, morphological and biochemical characteristics of the Gram-negative isolates
[Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates, Bold isolates are sequenced isolates]

Group (G)	Id of the isolates	Gram reaction	Agar slant culture characteristics	Ferment-ation		KIA				MIU			Nitrate reduction	MR_ VP		Citrate use	Catalase	Oxidase	Presumed organism
				Lactose	Dextrose	Slant	Butt	Gas	H_2S	Motility	Indole	Urea		MR	VP				
G-1	**H2-39B**, H2-20S, H5-87S, H6-106B	Rod (-)	White, moist, glistening growth	+	AG	+	+	+	-	+	+	-	+	+	+	+	-	-	*E. Coli*
G-2	H1-1S, H1-6S, H1-8S, H2-29S, H2-30B, H3-47S, H3-48S, H3-49S, **H3-50S**, H3-57S, **H3-61B**, H4-73S, **H4-77S**, H4-78B **H4-79B,** H4-80B, **H5-86S**, H5-88S, H5-89B, H6-95B, H5-97B, H6-98S, H6-100S, H6-101S, H7-124B	Rod (-)	Abundant, white, thick, glistening growth	+	AG	+	+	+	-	+	-	-	+	-	+	+	+	-	*Enterobacter* spp.
G-3	**H1-7S**, H1-13B, H1-14B, H1-16B, H2-18B, **H2-21S**, **H2-22S,** H2-25S, H2-32B, H3-41S, **H3-59B**, H3-63B, H3-64B, H3-66B, H3-67B, H4-68S, H4-74S, H4-75S **,** **H5-94B**, H6-96B, H6-103B, H6-104B, H6-105B, H6-107B, H6-108B, H7-109S, **H7-113S,** H7-114S, H7-115B, H7-119B, H7-120S, H7-121B, H7-122B, H7-123B, H8-125S, H8-126S, H8-132B, H8-133B	Rod (-)	Slimy, white, somewhat translucent growth	+	AG	+	+	+	-	-	-	+	+	-	+	+	+	-	*Klebsiella* spp.

Table 3.4 Cultural, morphological and biochemical characteristics of the Gram-negative isolates (continued)

Group (G)	Id of the isolates	Gram reaction	Agar slant culture characteristics	Fermentation		KIA				MIU			Nitrate reduction	MR-VP		Citrate use	Catalase	Oxidase	Presumed organism
				Lactose	Dextrose	Slant	Butt	Gas	H_2S	Motility	Indole	Urea		MR	VP				
G-4	**H1-3S**	Rod (-)	Thin, even, grayish growth	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-	*Salmonella* spp.
G-5	H2-24S, H2-36B, H3-44S, H3-52S, H3-58S, **H3-60B**, H3-62B, H4-70S, **H4-81B**, H4-82B, H4-83B, **H7-110S**, H8-127S, H8-128S, H8-130S	Rod (-)	Abundant, thin, white, growth with medium turning green	-	-	-	+	-	-	+	-	d	+	-	-	+	+	+	*Pseudomonas* spp.
G-6	**H1-5S**, H5-85S	Rod (-)	Smooth, low convex, moist, translucent, gray with a shiny surface.	-	+	+	+	+	+	+	-	-				+		-	*Citrobacter* spp.
G-7	**H4-69S**	Rod (-)	Moist, convex, white, translucent	-		-	-	-	-	-	-	d				d	+	-	*Acinetobacter* spp.

Table 3.5 Cultural, morphological and biochemical characteristics of the Gram-positive isolates

[Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates. Bold isolates are the sequenced isolates.]

Group (G)	ID of the isolates	Gram reaction	Agar slant culture characteristics	KIA				MIU			Nitrate reduction	MR-VP		Citrate use	Catalase	Oxidase	Coagulase	Presumed organism
				Slant	Butt	Gas	H_2S	Motility	Indole	Urea		MR	VP					
G-1	H1-2S, H1-4S, H1-10S, H1-11S, H1-12B, H1-15B, H2-23S, H2-26S, H2-31B, H2-33B, H2-35B, H2-37B, H2-38B, H3-42S, H3-43S, **H3-46S**, H3-51S, H3-53S, H3-55S, H3-56S, H4-71S, H4-72S, H5-90B, H5-91B, H7-111S, H7-118B, **H8-131S**, H8-134B, H8-136S.	Rod (Long) (+)	Thin, even, grayish growth	-	+	-	-	+	-	-	+	-	-	-	+	-	*N/D*	*Bacillus* spp.
G-2	H1-9S, H1-17B, H2-27S, H2-34B, H2-40B, H3-54S, **H3-65B**, H5-92B, H6-102S, H7-112S, H7-117B, H8-129S, H8-135S	Coccus (+)	Slimy, white, somewhat translucent growth	-	+	-	-	-	-	+	+	-	-	+	+	-	*N/D*	*Planococcus* spp.
G-3	H6-99S, H8-137S	Coccus (+)	Abundant, white, thick, glistening growth	+	+	-	-	+	-	-	-	+	+	-	-	-	*N/D*	*Streptococcus* spp.

Group (G)	ID of the isolates	Gram reaction	Agar slant culture characteristics	KIA				MIU			Nitrate reduction	MR-VP		Citrate use	Catalase	Oxidase	Coagulase	Presumed organism
				Slant	Butt	Gas	H_2S	Motility	Indole	Urea		MR	VP					
G-4	H2-28S, H5-93B, H7-116B	Coccus (+)	Thin, even, grayish growth	-	+	-	-	-	-	+	+	+	-	-	+	+	*N/D*	*Micrococcus* spp.
G-5	H4-84B	Coccus (+)	Abundant, white, thick, glistening growth	+	+	-	-	-	-	-	+	+	-	-	+	-	-	*Staphylococcus* spp.
G-6	H1-19B, H3-45S	Rod (+)	Smooth, low convex, moist, translucent, gray with a shiny surface.	-	+/-	+/-	+	+	-	-	-	-	-	-	+	-	*N/D*	*Ciostridium* spp.

3.6 Phenotypic virulence assays of the isolates

All the Gram-positive and Gram-negative isolates were assessed for their phenotypic virulence assays. Among the different phenotypic virulence assays, the biofilm formation capability and hemolytic properties of the isolates were examined.

3.6.1 Biofilm formation capacity of the isolates

The cut off optical density (OD) value in this study was set at 0.34, with the average optical density (OD) of the negative control being 0.209 ± 0.043.

3.6.1.1 Biofilm formation capacity of the Gram-negative isolates

According to a comparison of Gram-negative isolates in the biofilm former (BF) category, 34.48%, 33.33%, 16.09%, and 16.09% were non-biofilm formers (NBF), weak biofilm formers (WBF), moderate biofilm formers (MBF), and strong biofilm formers (SBF), respectively **(Figure 3.3a)**. The strong biofilm formers were mainly from *Klebsiella* spp. along with *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp **(Figure 3.4a, Table 3.6).**

3.6.1.2 Biofilm formation capacity of the Gram-positive isolates

Among the Gram-positive isolates, by comparing the category of biofilm former (BF), 66%, 24%, 4%, 6% isolates were non-biofilm formers (NBF), weak biofilm formers (WBF), moderate biofilm formers (MBF) and strong biofilm formers (SBF) respectively **(Figure 3.3b).** The strong biofilm formers were mainly from *Bacillus* spp. and *Planococcus* spp. **(Figure 3.4b, Table 3.6)**.

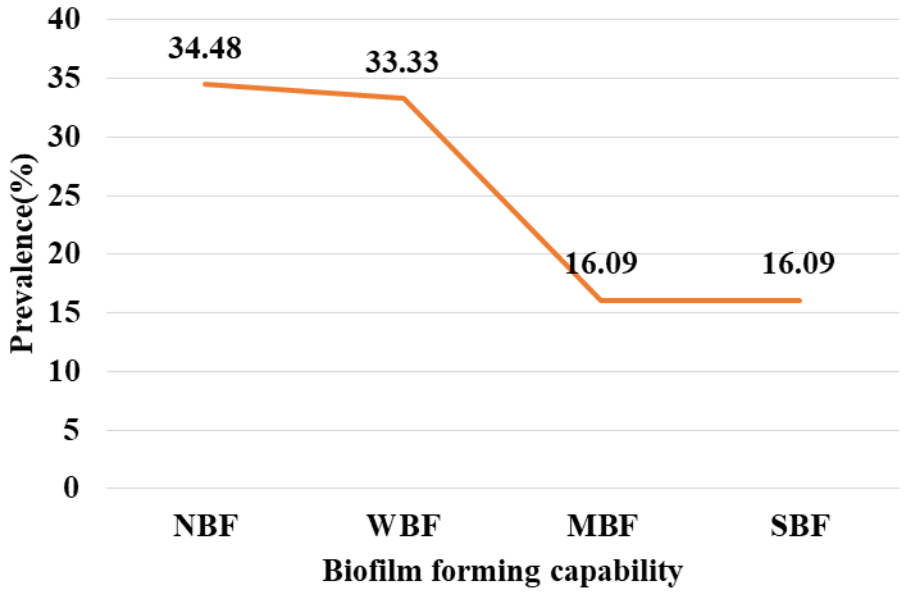


Figure 3.3a Biofilm formation capacity of the Gram-negative isolates

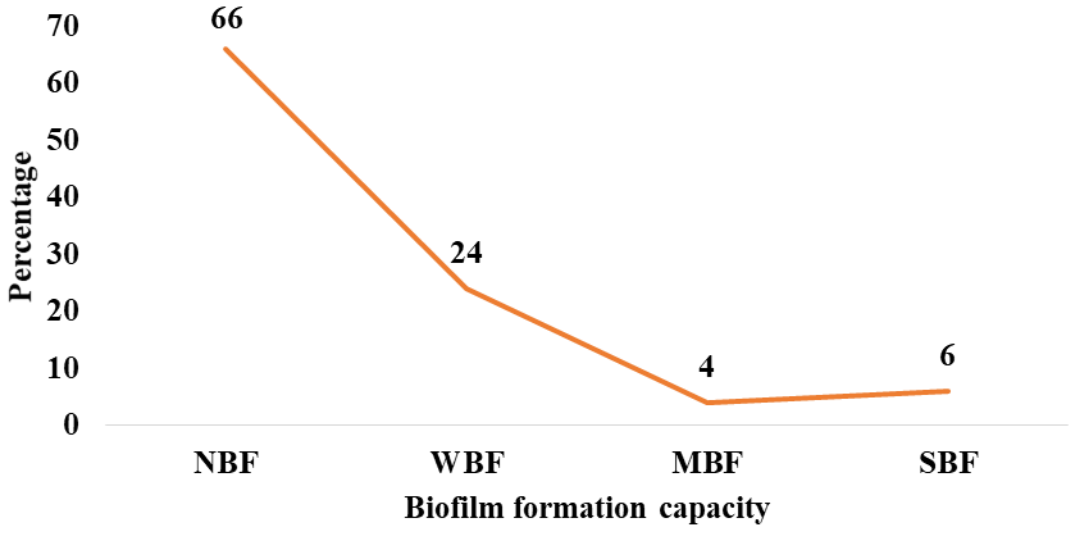


Figure 3.3b Biofilm formation capacity of the Gram-positive isolates

Table 3.6 Distribution of biofilm formation among Gram-negative and Gram-positive isolates

NBF OD range (0.13-0.34)	**WBF (0.35-0.63)**	**MBF (0.76-1.22)**	**SBF (1.38-2.49)**
Gram-negative isolates			
H2-21S, H2-24S, H2-36B, H3-50S, H3-59B, H3-62B, H3-63B, H3-66B, H3-67B, H4-74S, H4-80B, H4-81B, H4-83B, H6-97B, H6-98S, H6-100S, H6-107B, H7-110S, H7-115B, H7-119B, H7-120B, H7-121B, H7-122B,H7-123B, H7-124B, H8-125S, H8-127S, H8-128S, H8-130S, H8-132B	H1-3S, H1-5s, H1-6S, H1-7S, H1-8S, H1-16B, H2-25S, H2-29S, H2-30B, H2-32B, H3-39S, H3-47S, H3-48S, H3-49S, H3-60B, H3-64B, H4-73S, H4-75S, H4-78B, H4-79B, H5-89B, H5-94B, H6-95B, H6-96B, H6-103B, H6-104B, H6-108B, H7-113S, H8-133B	H1-1S, H2-20S, H2-22S, H3-52S, H3-57S, H3-61B, H4-70S, H4-76S, H5-85S, H5-86S, H5-88S, H6-101S, H6-105B, H7-109S,	H1-13B, H1-14B, H1-18B, H3-41S, H3-44S, H3-58S, H4-68S, H4-69S, H4-77S, H4-82B, H5-87S, H6-106B, H7-114S, H8-128S
Gram-positive isolates			
H1-2S, H1-4S, H1-10S, H1-12B, H1-15B, H1-17B, H1-19B, H2-23S, H2-26S, H2-27S, H2-28S, H2-31B, H2-34B, H2-35B, H2-37B, H2-38B, H3-42S, H3-43S, H3-45S ,H3-51S, H3-55S, H3-56S, H4-71S, H4-72S, H4-84B, H5-92B, H5-93B H6-99S, H7-111S, H7-112S, H7-116B H7-117B, H8-137S	H1-9S, H2-33B, H2-40B, H3-53S, H3-54S, H5-90B, H6-102S, H7-118B, H8-129S, H8-134B, H8-135S, H8-136S	H1-11S, H5-91B	H3-46S, H3-65B, H8-131S

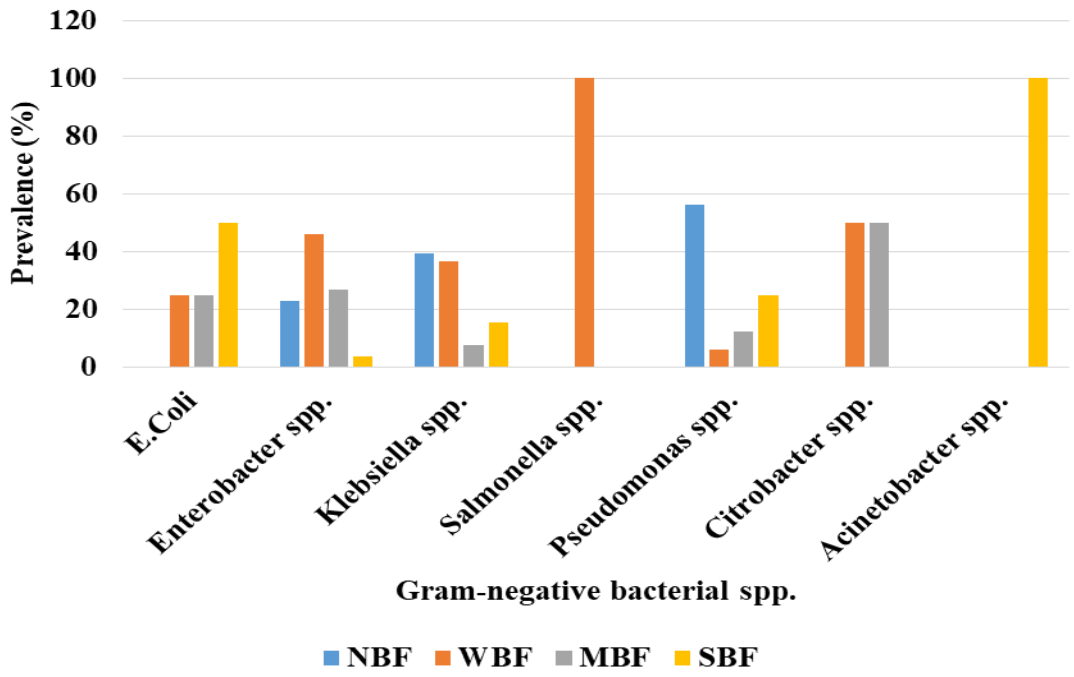


Figure 3.4a Distribution of different Gram-negative bacterial species among their biofilm formation category

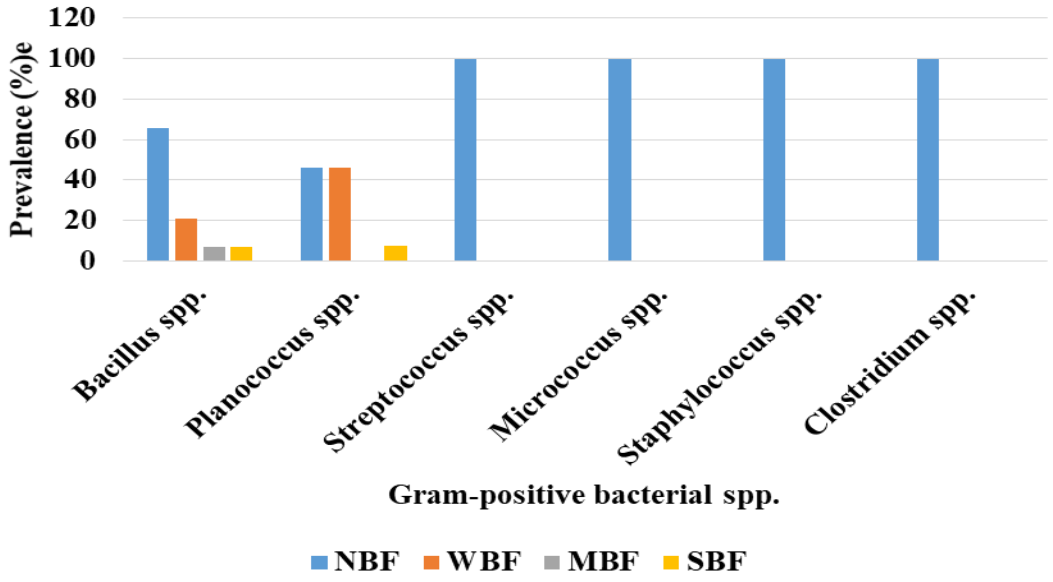


Figure 3.4b Distribution of different Gram-positive bacterial species among their biofilm formation category

3.6.2 Hemolytic properties of the isolates

Blood agar base plates with 5% sheep blood was used to observe the hemolytic properties of the isolates.

3.6.2.1 Hemolytic properties of the Gram-negative isolates

A green-hued zone around the colonies that is partial hemolysis (α-hemolysis) was observed in 40.23% of the isolates, about 13.79% of the isolated displayed complete hemolysis (β-hemolysis) that is clear zones around colonies and 45.98% exhibited no halo around colonies that is no hemolysis (γ-hemolysis). Mainly the presumptive *Pseudomonas* and *Klebsiella* spp. along with *Salmonella* spp. exhibited the β-hemolytic properties **(Figure 3.5a, Figure 3.6, Table 3.7).**

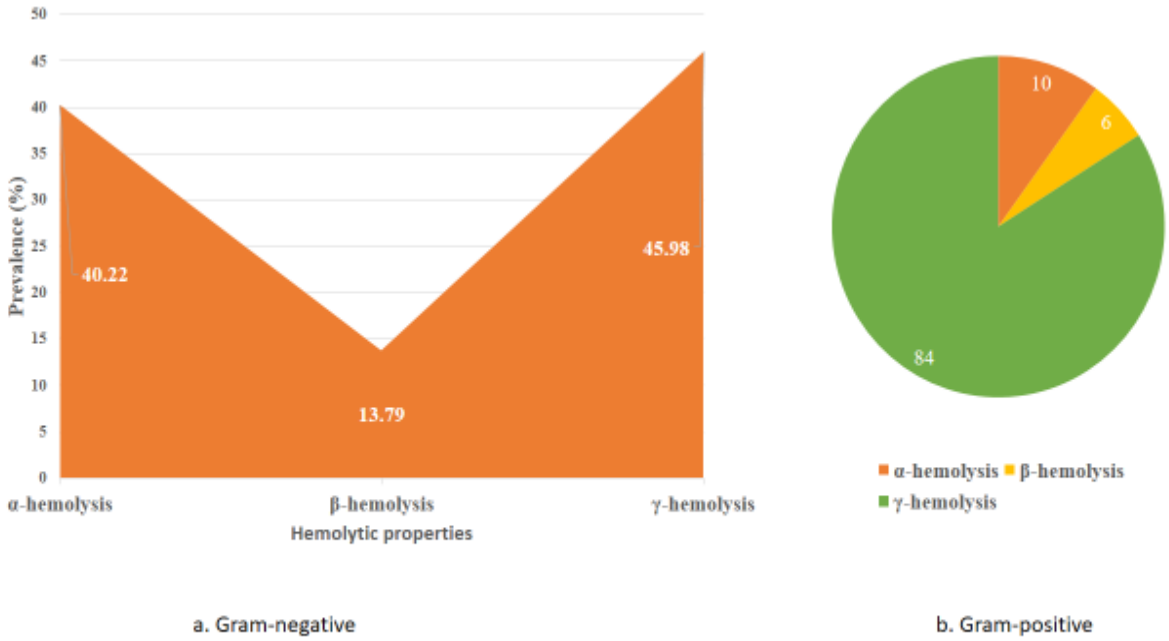
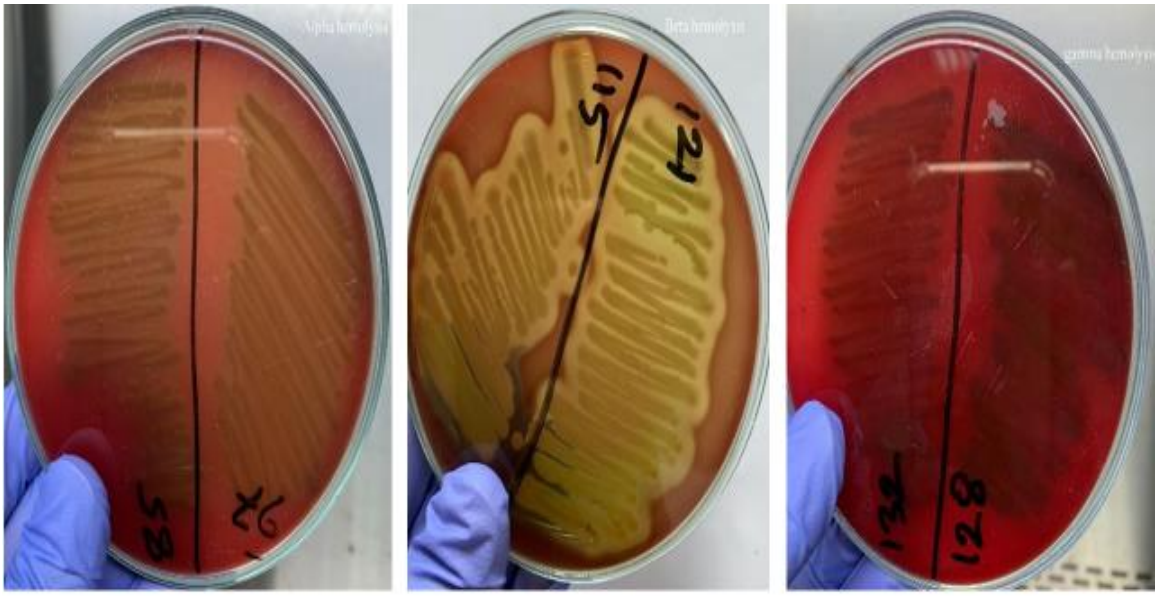


Figure 3.5 Hemolytic properties of the fast food isolates. a) Gram-negative b) Gram-positive



a. α-hemolysis b. β-hemolysis c. γ-hemolysis

Figure 3.6 Hemolytic properties of the fast foods isolates. (a) H5-97B (*Enterobacter* spp.), H5-85S (*Citrobacter* spp.); (b) H7-115B, H7-121B (*Klebsiella* spp.); (c) H8-128S (*Pseudomonas* spp.), H8-132B (*Klebsiella* spp.). Here, H represents hospital cafeteria, S and B represents the sandwich and burger respectively and the number is the isolate number.

Table 3.7 Hemolytic properties of the Gram-negative and Gram-positive isolates

α- hemolysis	**β- hemolysis**	**γ- hemolysis**
Gram-negative isolates		
H1-8S, H1-14B, H1-16B, H1-18B, H2-20S, H2-36B, H2-39B, H3-41S, H3-44S, H3-47S, H3-48S, H3-52S, H3-59B, H3-60B, H3-61B, H3-62B, H3-66B, H4-68S, H4-70S, H4-75S, H4-76S, H4-77S, H4-79B, H5-85S, H5-86S, H6-96B, H6-97B, H6-98S, H6-103B, H6-104B, H6-105B, H7-109S, H7-113S, H7-120B, H7-123B	H1-3S, H2-24S, H4-81B, H4-82B, H4-83B, H7-115B, H7-119B, H7-121B, H7-122B, H8-127S, H8-130S, H8-133B	H1-1S, H1-5S, H1-6S, H1-7S, H1-13B, H2-21S, H2-22S, H2-25S, H2-29S, H2-30B, H2-32B, H2-35B, H3-49S, H3-50S, H3-57S, H3-58S, H3-63B, H3-64B, H3-67B, H4-69S, H4-73S, H4-78B, H4-80B, H5-87S, H5-88S, H5-89B, H5-94B, H4-95B, H6-100S, H6-101S, H6-106B, H6-107B, H6-108B, H7-110S, H7-114S, H7-124B, H8-125S, H8-126S, H8-128S, H8-132B
Gram-positive isolates		
H2-33B, H3-53S, H6-102S, H7-118B, H8-129S	H3-46S, H3-65B, H8-131S	H1-2S, H1-4S, H1-9S, H1-10S, H1-11S, H1-12B, H1-15B, H1-17B, H1-19B, H2-23S, H2-26S, H2-27S, H2-28S, H2-31B, H2-34B, H2-35B, H2-37B, H2-38B, H2-40B, H3-42S, H3-43S, H3-45S, H3-51S, H3-54S, H3-55S, H3-56S, H4-71S, H4-72S, H4-84B, H5-90B, H5-91B, H5-92B, H5-93B, H6-99S, H7-111S, H7-112S, H7-116B, H7-117B, H8-134B, H8-135S, H8-136S, H8-137S.

3.6.2.2 Hemolytic properties of the Gram-positive isolates

About 6% of the isolates showed complete hemolysis (β-hemolysis) that is clear zones around colonies and 10% of the isolates showed partial hemolysis (α-hemolysis) that is a green-hued zone around the colonies. Majority (84%) of the isolates showed no hemolysis (γ-hemolysis) that is no halo around colonies **(Figure 3.5b, Table 3.7).**

3.7 Antibiogram resistance pattern of the isolates

A total of 137 isolates, Gram-negative (87) and Gram-positive (50), were examined against 17 antibiotics from 11 different antibiotics classes. The disk diffusion method (Abbey & Deak, 2019) was employed to examine the antibiogram pattern of the isolates. The results were interpreted by following the CLSI guidelines (Clinical & Laboratory Standards Institute, 2022).

3.7.1 Antibiogram resistance pattern of the Gram-negative isolates

According to the antimicrobial resistance profile, the Gram-negative isolates showed a comparatively higher percentage of resistance against specific antibiotic classes including ampicillin (100%), penicillin (100%) followed by meropenem (95.4%), cephalexin (73.56%), , nitrofurantoin (57.47%), azithromycin (48.27%), tetracycline (43.68%), colistin (20.69%), sulphamethytrimethoprim (32.18%), ciprofloxacin (26.43%), ceftriaxone (18.39%), kanamycin (14.94%), chloramphenicol (13.79%), levofloxacin (11.49%), streptomycin (4.60%), gentamycin (4.59%), imipenem (2.03%) **(Figure 3.7a, Figure 3.7b)**. The study compared the effects of various hospitals and discovered that every isolate from every hospital was resistant to penicillin and ampicillin. Except for the H2, H4, and H6 hospital isolates, all of them displayed 100% meropenem resistance. Aside from penicillin, ampicillin, and meropenem, the isolates from hospital 1 (H1) showed the highest resistance to cephalexin (100%) followed by sulphamethoxytrimethoprim (90%), and tetracycline (80%); whereas, the isolates from hospital 2 (H2) showed the highest resistance to meropenem (88.89%) followed by tetracycline (77.78%), nitrofurantoin (77. The isolates from hospital 3 (H3) demonstrated the highest levels of resistance to azithromycin (55.56%) and cephalexin (88.89%), while those from hospital 4 (H4) demonstrated the highest levels of resistance to meropenem (85.71%), nitrofurantoin (85.71%), and cephalexin (78.57%). In addition, the isolates from Hospital 5 (H5) demonstrated 100% resistance to cephalexin and nitrofurantoin, followed by azithromycin (83.33%), in addition to penicillin, ampicillin, and meropenem. The most resistant strains to cephalexin (100%) and nitrofurantoin (91.67%) were found in hospital 6 (H6) isolates. Tetracycline (36.36%) and colistin (85.71%) were both highly resistant to colistin in isolates from hospital 7, but colistin (85.71%) was the most resistant, followed by tetracycline (57.14%) in isolates from hospital 8 **(Table 3.8).** About 13.79%, 68.35% and 12.64% of the isolates were resistant to <3, 3-7 and > 7 antibiotics class respectively **(Figure 3.8a)**. Multidrug-resistance (MDR) was defined MDR was

described as acquired resistance to at least one agent in three or more antimicrobial categories (Ap et al., 2012). About 80.99% of the Gram-negative isolates were multi-drug resistant **(Table 3.9, Figure 3.8a)**. Antibiotics resistant profile of each of the Gram-negative isolates of hospital cafeterias fast foods were given in **Table 3.9.**

Figure 3.7a Disk diffusion method for antibiogram resistance pattern of fast food isolates. (a) AZM, TE, LE, CIP (b) GEN, C, CL (c) P, K, S, CTR (d) CL, SXT, F and (e) IMI, MER, CT.

3.7.2 Antibiogram resistance pattern of the Gram-positive isolates

Among the Gram-positive isolates, comparatively a larger percentage of resistance against specific antibiotic classes, according to the antimicrobial resistance profile, including ampicillin (100%), penicillin (100%) followed by cephalexin (66%), then nitrofurantoin (64%), meropenem (60%), azithromycin (46%), colistin (24%), ceftriaxone (24%), tetracycline (18%), sulphamethytrimethoprim (10%), ciprofloxacin (10%), levofloxacin (10%), chloramphenicol (8%), gentamycin (8%) , streptomycin (6%), kanamycin (6%) and highly sensitive to imipenem **(Figure 3.7c)**. About 46%, 48% and 6% of the isolates were resistant to <3, 3-7 and > 7 antibiotics class respectively **(Figure 3.8b)**. About 54% of the Gram-positive isolates were multi-drug resistant **(Figure 3.8b).**

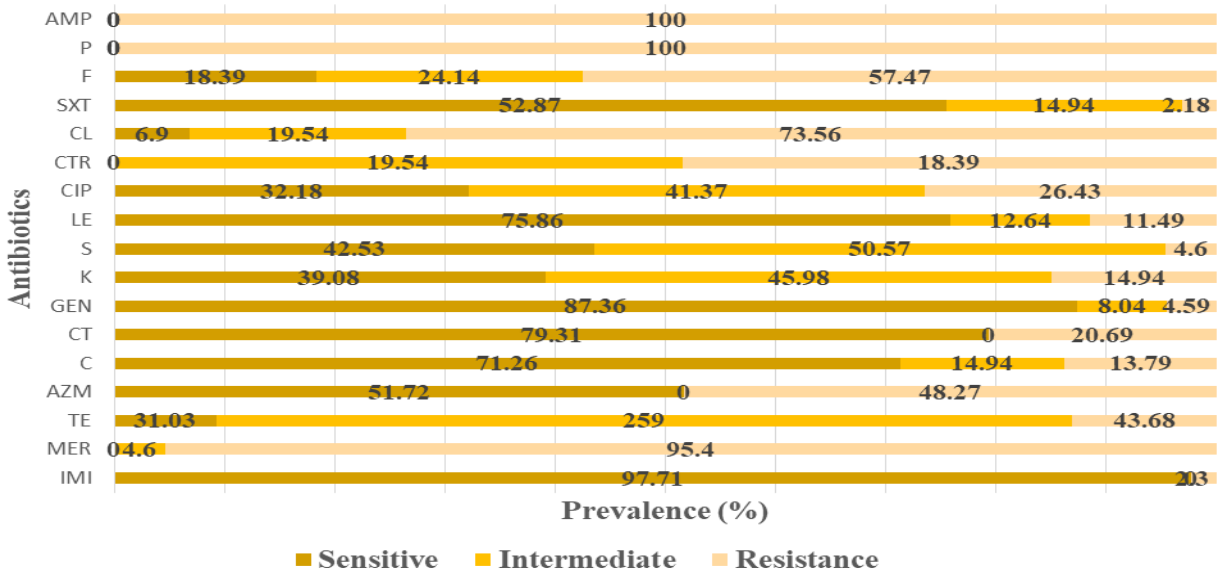


Figure 3.7b Antibiogram resistance pattern of the Gram-negative isolates.

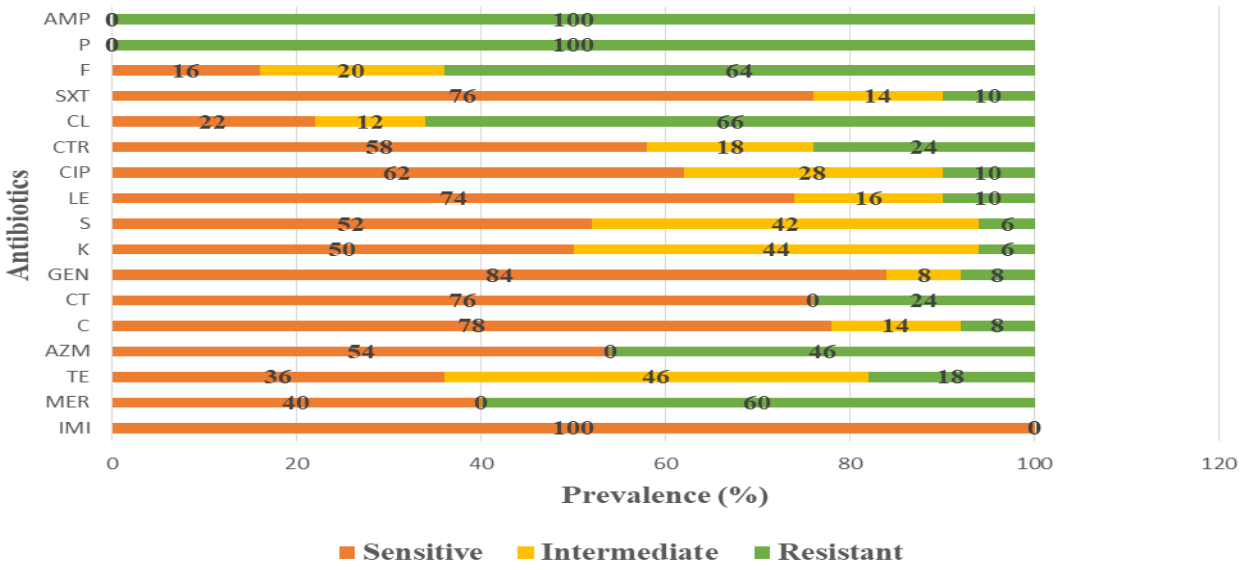


Figure 3.7c Antibiogram resistance pattern of the Gram-positive isolates.

*IMI-Imipenem; MER-Meropenem; TE-Tetracycline; AZM-Azithromycin; C-Chloramphenicol; CT-Colistin; GEN-Gentamycin; K-Kanamycin; S-Streptomycin; LE-Levofloxacin; CIP-Ciprofloxacin; CTR-Ceftriaxone; CL-Cephalexin; SXT-Sulphamethoxytrimethoprim; F-Nitrofurantoin; P-Penicillin and AMP-Ampicillin.

*Categorization of sensitive, intermediate and resistant is based on CLSI guideline (*Clinical & Laboratory Standards Institute*, 2022.).

Table 3.8 Percentage of antibiotic resistance of fast food isolates from each hospital cafeterias [Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital cafeteria]

Antibiotics	**% (Total)**	**H1 (N=10)**	**H2 (N=9)**	**H3 (N=18)**	**H4 (N=14)**	**H5 (N=6)**	**H6 (N=12)**	**H7 (N=11)**	**H8 (N=7)**
IMI	2.29 (2)	0 (0)	0 (0)	5.56 (1)	0 (0)	0 (0)	0 (0)	9.09 (1)	0 (0)
MERO	95.40 (83)	100 (10)	88.89 (8)	100 (18)	85.71 (12)	100 (6)	91.67 (11)	100 (11)	100 (7)
TE	43.68 (38)	80 (8)	77.78 (7)	16.67 (3)	28.57 (4)	50 (3)	41.67 (5)	36.36 (4)	57.14 (4)
AZM	47.12 (41)	40 (4)	55.56 (5)	33.33 (6)	50 (70)	83.33 (5)	83.33 (10)	27.27 (3)	14.29 (1)
C	13.79 (12)	0 (0)	0 (0)	22.22 (4)	21.42 (3)	0 (0)	8.33 (1)	9.09 (1)	42.86 (3)
CT	31.03 (27)	10 (1)	11.11 (1)	22.22 (4)	28.57 (4)	16.67 (1)	8.33 (1)	81.82 (9)	85.71 (6)
GEN	4.59 (4)	0 (0)	0 (0)	5.56 (1)	14.29 (2)	0 (0)	8.33 (1)	0 (0)	0 (0)
K	14.94 (13)	10 (1)	33.33 (3)	16.67 (3)	21.43 (3)	0 (0)	16.67 (2)	9.09 (1)	0 (0)
S	4.59 (4)	0 (0)	0 (0)	5.56 (1)	7.14 (1)	16.67 (1)	0 (0)	9.09 (1)	0 (0)
LE	11.49 (10)	20 (2)	11.11 (1)	5.56 (1)	21.43 (3)	16.67 (1)	8.33 (1)	9.09 (1)	0 (0)
CIP	26.44 (23)	0 (0)	11.11 (1)	27.78 (5)	28.57 (4)	16.67 (1)	83.33 (10)	18.18 (2)	0 (0)
CTR	16.09 (14)	10 (1)	33.33 (3)	33.33 (6)	28.57 (4)	0 (0)	0 (0)	0 (0)	0 (0)
CL	74.71 (65)	100 (10)	77.78 (7)	88.89 (16)	78.57 (11)	100 (6)	100 (12)	27.27 (3)	0 (0)
SXT	26.89(26)	90 (9)	66.67 (6)	27.78 (5)	14.29 (2)	16.67 (1)	33.33 (4)	9.09 (1)	0 (0)
F	57.47 (50)	40 (4)	77.78 (7)	55.56 (10)	85.71 (12)	100 (6)	91.67 (11)	9.09 (1)	0 (0)
P	100 (87)	100 (10)	100 (9)	100 (18)	100 (14)	100 (6)	100 (12)	100 (11)	100 (7)
AMP	100 (87)	100 (10)	100 (9)	100 (18)	100 (14)	100 (6)	100 (12)	100 (11)	100 (7)

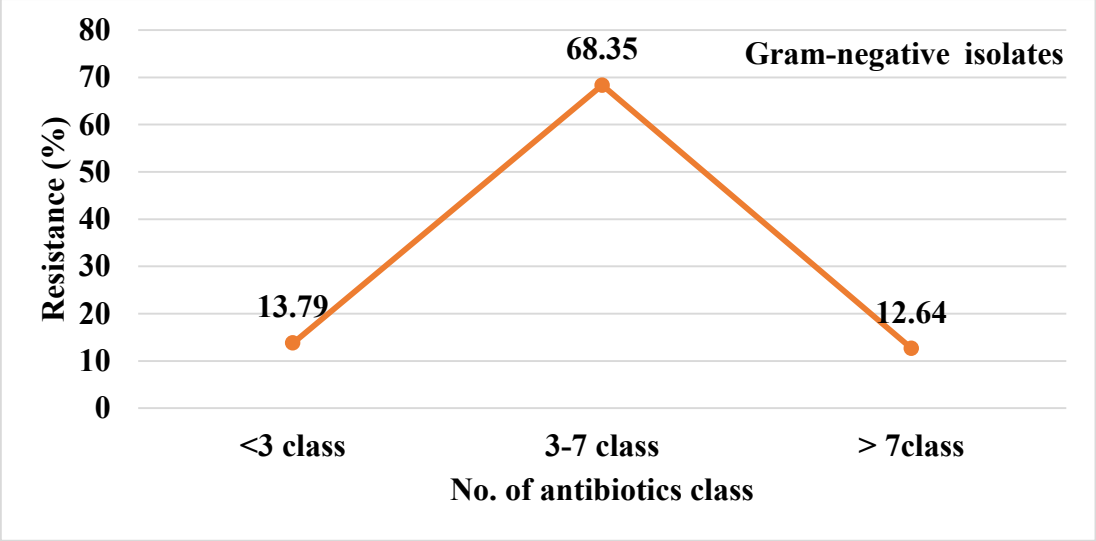


Figure 3.8a Prevalence of Gram-negative resistant isolates among different antibiotics class

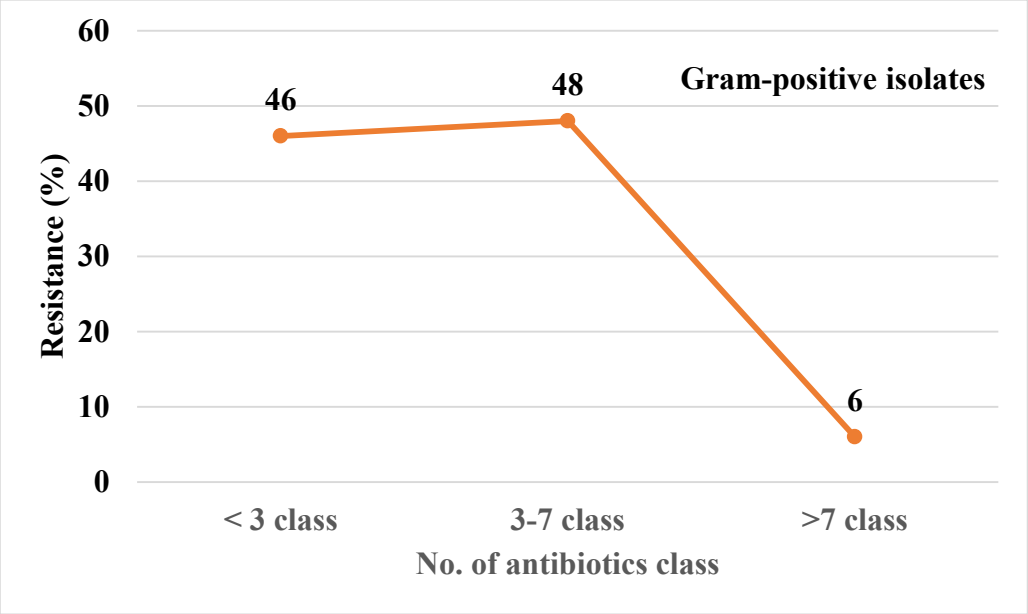


Figure 3.8b Prevalence of Gram-positive resistant isolates among different antibiotics class

Table 3.9 Antibiotics resistant profile of each of the Gram-negative isolates of hospital cafeterias fast foods

Isolates ID	**Presumed organism**	**Antibiotics resistant profile**
H1-1S	*Enterobacter sp.*	MER+TE+AZM+CL+SXT+F+P+AMP
H1-3S	*Salmonella sp.*	MER+LE+CL+SXT+P+AMP
H1-5S	*Citrobacter sp.*	MER+TE+CL+SXT+P+AMP
H1-6S	*Enterobacter sp.*	MER+CL+P+AMP
H1-7S	*Klebsiella sp.*	MER+TE+LE+CL+SXT+F+P+AMP
H1-8S	*Enterobacter sp.*	MER+TE+CL+SXT+F+P+AMP
H1-13B	*Klebsiella sp.*	MER+TE+LE+CL+SXT+F+P+AMP
H1-14**B**	*Klebsiella sp.*	MER+TE+AZM+CL+SXT+F+P+AMP
H1-16B	*Klebsiella sp.*	MER+TE+AZM +CL+SXT+P+AMP
H1-18B	*Klebsiella sp.*	MER+TE+K+CTR+CL+SXT+P+AMP
H1-20S	*E. coli.*	MER+TE+CL+SXT+F+P+AMP
H1-21S	*Klebsiella sp.*	MER+TE+CL+SXT+P+AMP
H2-22S	*Klebsiella sp.*	MER+TE+SXT+F+P+AMP
H2-24S	*Pseudomonas sp.*	MER+TE+AZM+K+CTR+CL+SXT+F+P+AMP
H2-25S	*Klebsiella sp.*	MER+TE+AZM+K+LE +CL+SXT+CIP+P+AMP
H2-29S	*Enterobacter sp.*	MER+AZM+K+CTR+CL+F+P+AMP
H2-30B	*Enterobacter sp.*	MER+TE+AZM+CT+CL+F+P+AMP
H2-32B	*Klebsiella sp.*	TE+AZM+CL+F+P+AMP
H2-36B	*Pseudomonas sp.*	MER+CTR+SXT+F+P+AMP
H2-39B	*E. coli.*	MER+TE+K+CIP+CTR+CL+SXT+P+AMP
H3-41S	*Klebsiella sp.*	MER+CTR+CL+P+AMP
H3-44S	*Pseudomonas sp.*	MER+CT+CTR+CL+F+P+AMP
H3-47S	*Enterobacter sp.*	MER+AZM+CL+F+P+AMP
H3-48S	*Enterobacter sp.*	MER+AZM+CT+CL+F+P+AMP
H3-49S	*Enterobacter sp.*	MER+CL+P+AMP
H3-50S	*Enterobacter sp.*	MER+TE+CIP+CL+SXT+P+AMP
H3-52S	*Pseudomonas sp.*	MER+C+CTR+CL+F+P+AMP

H3-57S	*Enterobacter sp.*	MER+ AZM+ K+CTR+CL+F+P+AMP
H3-58S	*Pseudomonas sp.*	MER+C+CTR+CL+F+P+AMP
H3-59B	*Klebsiella sp.*	MER+AZM+CT+CIP+CL+F+P+AMP
H3-60B	*Pseudomonas sp.*	MER+TE+AZM+C+GEN+LE+CIP+CL+SXT+F+P+AMP
H3-61B	*Enterobacter sp.*	MER+IMI+ CTR+CL+S+SXT+F+P+AMP
H3-62B	*Pseudomonas sp.*	MER+P+AMP
H3-63B	*Klebsiella sp.*	MER+P+AMP
H3-64B	*Klebsiella sp.*	MER+CL+P+AMP
H3-66B	*Klebsiella sp.*	MER+C+CT+K+S+CL+SXT+P+AMP
H3-67B	*Klebsiella sp.*	MER+AZM+CIP+F+P+AMP
H4-68S	*Klebsiella sp.*	MER +CIP+F+P+AMP
H4-69S	*Acinetobacter sp.*	MER+AZM+CTR+CL+F+P+AMP
H4-70S	*Pseudomonas sp.*	MER+C+CTR+CL+F+P+AMP
H4-73S	*Enterobacter sp.*	MER+AZM+K+LE+CIP+CTR+CL+P+AMP
H4-74S	*Klebsiella sp.*	F+P+AMP
H4-75S	*Klebsiella sp.*	MER+CT+F+P+AMP
H4-76S	*Enterobacter sp.*	MER+AZM+K+CL+F+P+AMP
H4-77S	*Enterobacter sp.*	MER+TE+CT+CTR+CL+SXT+F+P+AMP
H4-78B	*Enterobacter sp.*	MER+AZM+CL+P+AMP
H4-79B	*Enterobacter sp.*	MER+CL+F+P+AMP
H4-80B	*Enterobacter sp.*	AZM+CL+F+P+AMP
H4-81B	*Pseudomonas sp.*	MER+TE+AZM+C+K+S+LE+CIP+CL+SXT+F+P+AMP
H4-82B	*Pseudomonas sp.*	MER+TE+AZM+CT++C+GEN+LE+CIP+CL+F+P+AMP
H4-83B	*Pseudomonas sp.*	MER+TE+CT+CL+F+P+AMP
H5-85S	*Citrobacter sp.*	MER+TE+AZM+LE+CIP+CL+SXT+F+P+AMP
H5-86S	*Enterobacter sp.*	MER+TE+AZM+CT+S+CL+F+P+AMP
H5-87S	*E. coli.*	MER+TE+AZM+C +F+P+AMP
H5-88S	*Enterobacter sp.*	MER +AZM +CL +F+P+AMP
H5-89B	*Enterobacter sp.*	MER +AZM +CL +F+P+AMP
H5-94B	*Klebsiella sp.*	MER+CL+F+P+AMP

H6-95B	*Enterobacter sp*.	MER+TE+AZM+CIP+CL+F+P+AMP
H6-96B	*Klebsiella sp*.	MER +AZM+C+LE+CIP+CL+SXT+F+P+AMP
H6-97B	*Enterobacter sp*.	MER+AZM+K+CIP+CL+F+P+AMP
H6-98S	*Enterobacter sp*.	MER +AZM+GEN+K+CIP+CL+P+AMP
H6-100S	*Enterobacter sp*.	MER+TE+AZM+CIP+CL+SXT+F+P+AMP
H6-101S	*Enterobacter sp*.	AZM+CL+F+P+AMP
H6-103B	*Klebsiella sp*.	MER+TE+CIP +CL+F+P+AMP
H6-104B	*Klebsiella sp*.	MER+TE+AZM+CL+F+P+AMP
H6-105B	*Klebsiella sp*.	MER+TE+AZM+CT+CIP+CL+SXT+F+P+AMP
H6-106B	*E. coli.*	MER +AZM+CIP+CL +F+P+AMP
H6-107B	*Klebsiella sp*.	MER +AZM+CIP+CL+SXT+F+P+AMP
H6-108B	*Klebsiella sp*.	MER +CIP+CL+F+P+AMP
H7-109S	*Klebsiella sp*.	MER +AZM+CIP+CL +F+P+AMP
H7-110S	*Pseudomonas sp*.	IMI+MER+TE+AZM+CTR+S+LE+CIP+CL+SXT+F+P+AMP
H7-113S	*Klebsiella sp*.	MER+TE+AZM +CL+P+AMP
H7-114S	*Klebsiella sp.*	MER +P+AMP
H7-115B	*Klebsiella sp*.	MER+P+AMP
H7-119B	*Klebsiella sp*.	MER +P+AMP
H7-120B	*Klebsiella sp*.	MER+P+AMP
H7-121B	*Klebsiella sp*.	MER+P+AMP
H7-122B	*Klebsiella sp*.	MER+TE+C+CT+CL+P+AMP
H7-123**B**	*Klebsiella sp*.	MER+TE+CT+P+AMP
H7-124B	*Enterobacter sp*.	MER+P+AMP
H8-125S	*Klebsiella sp*.	MER+P+AMP
H8-126S	*Klebsiella sp*.	MER+TE+P+AMP
H8-127S	*Pseudomonas sp*.	MER+TE+C +CT+P+AMP
H8-128S	*Pseudomonas sp*.	MER+TE+AZM+C+CT+P+AMP
H8-130S	*Pseudomonas sp*.	MER+TE+C +CT+P+AMP
H8-132B	*Klebsiella sp*.	MER+P+AMP
H8-133B	*Klebsiella sp*.	MER+P+AMP

3.8 Molecular fingerprinting of the Gram-negative isolates

Diversity among the Gram-negative isolates were identified by employing Random Amplification of Polymorphic DNA (RAPD)-PCR. A low annealing temperature was used for amplification to allow numerous incompatible sequences to hybridize. Ten distinctive patterns were observed among the gram-negative isolates based on RAPD typing. So, according to RAPD-PCR, the Gram-negative isolates were divided into 10 different genotypes **(Figure 3.9, Table 3.10)**.

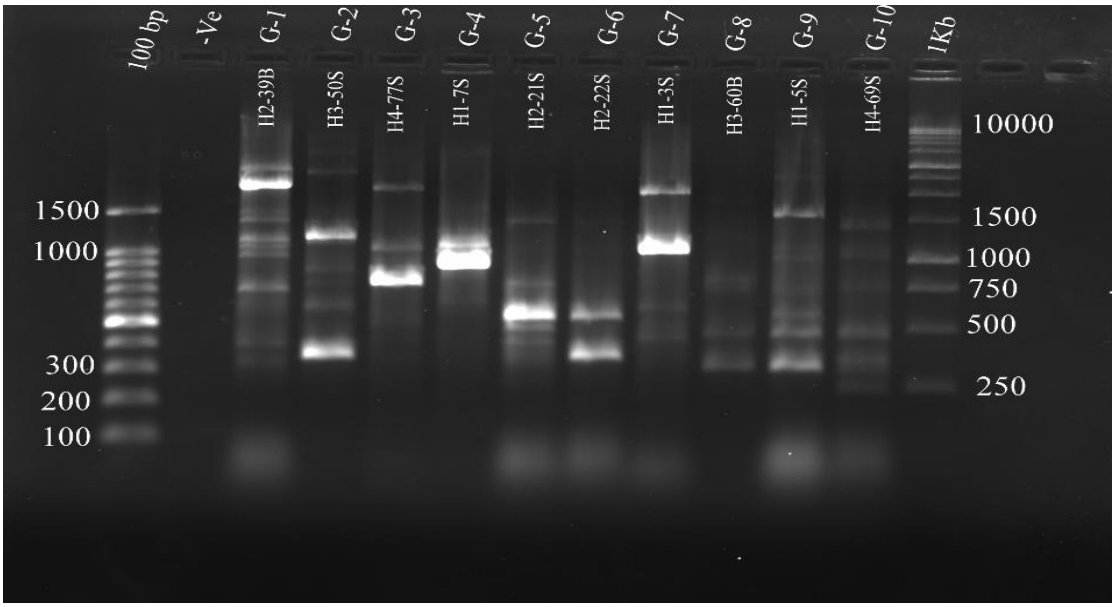


Figure 3.9 RAPD analysis of representative bacterial strains. Lane 1 & 13 represents molecular markers of 100 bp and 1Kb (Promega, USA) used as control. Lane 3-12 is selected bacterial strains of RAPD group 1 to 10. Negative control is placed in lane 2.

Among the 10 different genotypes, the genotype -1 had four isolates of *Escherichia* spp., genotype-2 and 3 had thirteen isolates each of *Enterobacter* spp., genotype-4, 5 and 6 had twelve, thirteen and thirteen isolates respectively of *Klebsiella* spp., genotype-7 had one isolate of *Salmonella* spp., genotype-8 had fifteen isolates of *Pseudomonas* spp., genotype-9 had two isolates of *Citrobacter* spp. and genotype-10 had one isolate of *Acinetobacter* spp. The RAPD figure of all isolates of all genotypes are provided in **appendix V.**

3.9 16S rRNA gene (Ribosomal gene) sequence analysis of bacterial isolates

Each isolate's isolated template DNA was employed for amplification of the 16S rRNA gene using the standard primers 27F and 1492R. Every single isolate underwent a successful PCR reaction, yielding a result of roughly 1400–1450 bp **(Figure 3.10).**

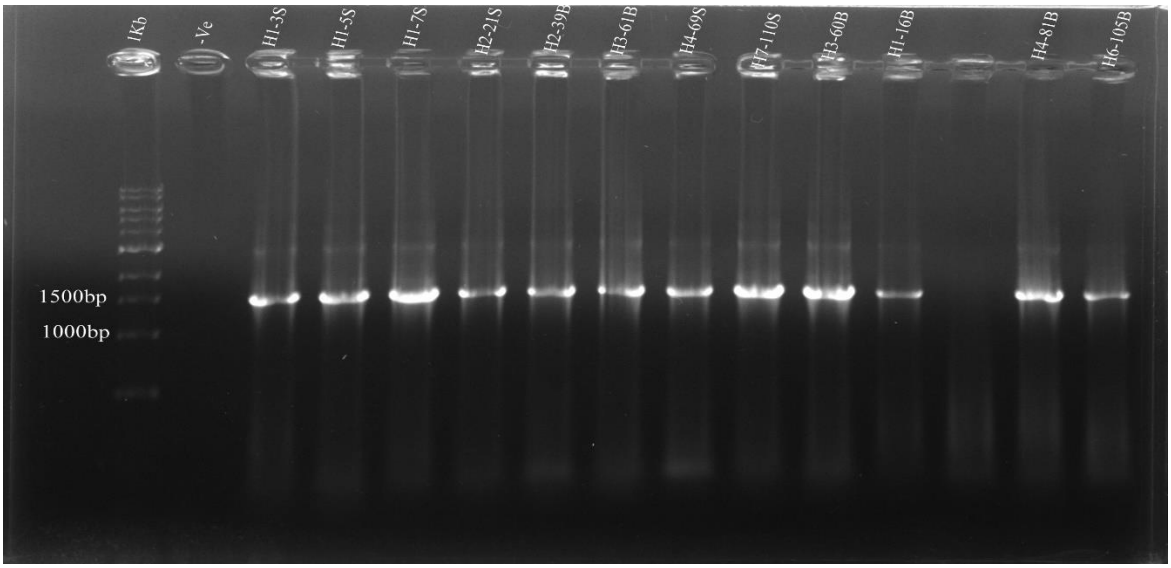


Figure 3.10 Representative figure of 16S rRNA PCR. Lane 1 is 1 Kb ladder (Promega, USA), negative control is in lane 2, and lane 3-15 is representative isolates (Product size-1465 bp).

3.9.1 16S rRNA gene (Ribosomal gene) sequence analysis of Gram-negative bacterial isolates

Genotyping of the Gram-negative bacteria from hospital cafeteria fast foods was done by RAPD-PCR. RAPD analysis revealed 10 different genotypes of bacterial isolates. The analysis of the phylogenetic correlation to the closest species level was done on a total of 18 isolates, one or more isolates from each genotype being chosen as representatives. The isolates selected were H2-39B (G-1), H3-50S (G-2), H3-61B (G-2), H4-77S (G-3), H4-79B (G-2), H5-86S (G-3), H1-7S (G-4), H2-21S (G-5), H3-59B (G-4), H5-94B (G-5), H2-22S (G-6), H7-113S (G-6), H1-3S (G-7), H3-60B (G-8), H7-110S (G-8), H4-81B (G-8), H1-5S (G-9) and H4-69S (G-10).

Sequencing of the 16S rRNA amplicons were done and identified by BLAST analysis. The BLAST results are shown in **Table 3.10** along with the accession number of the NCBI-submitted sequences, which ranges from OQ092741 to OQ092758. The sequences are given in the **appendix VI.**

All of the isolates from genotype 1 (G-1) to genotype 10 (G-10) were gram-negative bacteria classified as gamma (γ)-proteobacteria, according to BLAST search and phylogenetic analysis (**Table 3.10)**. It was discovered that the isolates from 10 genotypes cluster with 9 different bacterial genera in phylogeny. Isolate H2-39B of genotype-1 (G-1) was phylogenetically closely related to *Escherichia coli*. with 96% 16S rRNA gene sequence identity. Isolates under genotype-2 (G-2) (H3- 61B, H4-79B) were closely related to *Enterobacter* spp. and H3-50S was 99% identical to *Leclarcia* spp. *Leclarcia* spp. and *Enterobacter* spp. fell under the same genotype in RAPD and their 16SrRNA sequences were closely related to each other. This isolate (H3-50S) requires genome wide analysis for confirmed identification. Isolates under genotype-3 (G-3) (H4-77S, H5-86S) were closely related to *Enterobacter* spp. Isolates from genotype-4 (G-4) (H1-7S, H3-59B), genotype-5 (G-5) (H2-21S, H5-94B) and genotype-6 (G-6) (H2-22S, H7-113S) demonstrated close relation to *Klebsiella* sp. and H1-3S of genotype-7 (G-7) was related to *Salmonella* sp. The genotype-8 (G-8) isolate, H3-60B shared 98.73% similarity to *Stutzerimonas stutzeri* and other two isolates (H7-110S, H4-81B) showed close relatedness to *Pseudomonas* spp. Isolates from genotype 9 (G-9), H1-5S and genotype-10 (G-10), H4-69S were phylogenetically closely related to *Citrobacter* spp. and *Acinetobacter baumannii* respectively **(Figure 3.11).**

Table 3.10 Profile of the isolates from 10 RAPD genotypes and maximum identity profile of the sequences of 16S rRNA gene of representative isolates of 10 genotypes in accordance with BLAST identification. [Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, Bold isolates are sequenced isolates. B & S= Burger and Sandwich respectively, the number represents the number of the isolates.]

Genotypes (RAPD)	Isolate ID	Close similarity to (accession number)	Identity (%)	Phylogenetic affiliation
G-1	**H2-39B** H2-20S, H5-87S, H6-106B	*Escherichia coli* strain GER14 (OM403061.1)	95.68%	γ Proteobacteria
G-2	H1-1S, H1-8S, H2-29S, H2-30B, **H3-50S**, H3-57S, **H3-61B**, **H4-79B,** H4-80B, H6-95B, H6-98S, H6-100S, H6-101S	*Leclercia* sp. strain S2 (MT188710.1)	99%	γ Proteobacteria
		Enterobacter hormaechei strain SLB11 (MZ707902.1)	99.53%	γ Proteobacteria
		Enterobacter ludwigii strain MBPSL -189 (MT355912.1)	97.15%	γ Proteobacteria
G-3	H1-6S, H3-47S, H3-48S, H3-49S, H4-73S, **H4-77S**, H4-78B, **H5-86S**, H5-88S, H5-89B, H5-97B, H7-124B	*Enterobacter cloacae* strain IIPRAJCP-2 (MT436392.1)	100%	γ Proteobacteria
		Enterobacter hormaechei strain AUH-ENM30 (CP045611.1	99.34%	γ Proteobacteria
G-4	**H1-7S**, H1-13B, H1-14B, H2-25S, **H3-59B**, H3-63B, H3-64B, H6-103B, H6-104B, H6-105B, H6-108B, H8-126S	*Klebsiella quasipneumoniae subsp. similipneumoniae* strain 2437 (MT604862.1)	99.27%	γ Proteobacteria
		Klebsiella pneumoniae strain F16KP0070 (CP052585.1)	100%	γ Proteobacteria
G-5	**H2-21S**, H1-16B, H2-18B, H3-66B, H3-67B, H4-74S, **H5-94B**, H6-96B, H6-107B, H7-109S, H7-120S, H7-122B, H8-133B	*Klebsiella pneumoniae* strain B17KP0020 (CP052503.1)	99.78%	γ Proteobacteria
		Klebsiella pneumoniae strain Bckp186 (CP050843.1)	99.57%	γ Proteobacteria
G-6	**H2-22S,** H2-32B, H3-41S, H4-68S, H4-75S, **H7-113S**, H7-114S, H7-115B, H7-119B H7-121B, H7-123B, H8-125S, H8-132B,	*Klebsiella pneumoniae* strain 2020CK-00096 (CP104659.1)	99.09%	γ Proteobacteria
		Klebsiella pneumoniae strain MBR11 (JX966429.1)	100%	γ Proteobacteria
G-7	**H1-3S**	*Salmonella enterica* SESen3709 (AP020332.1)	99.78%	γ Proteobacteria
G-8	H2-24S, H2-36B, H3-44S, H3-52S, H3-58S, **H3-60B**, H3-62B, H4-70S, **H4-81B**, H4-82B, H4-83B, **H7-110S**, H8-127S, H8-128S, H8-130S	*Stutzerimonas stutzeri* strain WS8 (MF106208.1)	98.73%	γ Proteobacteria
		Pseudomonas sp. sampath10 (HM749063.1)	99.93%	γ Proteobacteria
		Pseudomonas sp. JM14 (JX484804.1)	100%	γ Proteobacteria
G-9	**H1-5S**, H5-85S	*Citrobacter freundii* strain CX-98 (MH368414.1)	99.85%	γ Proteobacteria
G-10	**H4-69S**	*Acinetobacter baumannii* strain JCM 6841 (MK351230.1)	99.92%	γ Proteobacteria

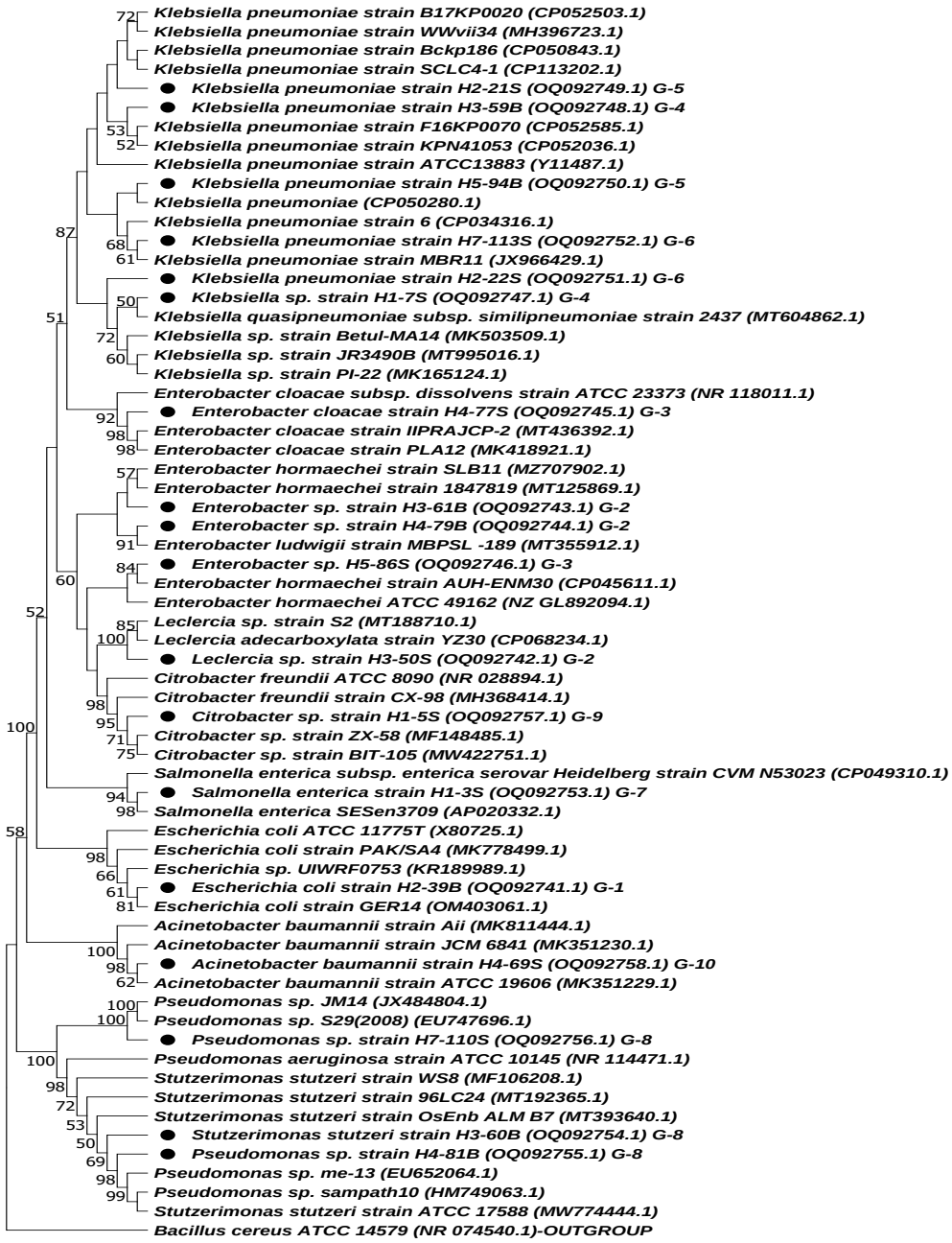


Figure 3.11 Phylogenetic tree of 16S rRNA gene sequences of gram-negative isolates from hospital burger and sandwiches and close relative reference isolates retrieved from database with accession numbers. MEGA11 used to generate the tree using the Neighbour-Joining algorithm with the *Bacillus cereus ATCC 14579* as outgroup. Isolates in this study are indicated with symbol in the beginning. Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates.

3.9.2 16S rRNA gene (Ribosomal gene) sequence analysis of Gram-positive bacterial isolates

Three isolates (from the most abundant groups, resistant to 12 or more antibiotics, strong biofilm formers, and β-hemolysis pattern) were chosen as representatives to analyze their phylogenetic correlation to the closest species level by detailed 16S rRNA gene sequence analysis. This was done based on the abundance of the isolates, their antibiotic resistance pattern, and phenotypic virulence assays, which include biofilm formation capacity and hemolysis pattern. The isolates selected were H3-46S (G-1), H3-65B (G-2) and H8-131S (G-1).

The 16S rRNA amplicons were sequenced and identified using BLAST analysis. The accession number of NCBI submitted sequences are from OR486967 to OR486969 (Appendix VII) and the BLAST results were in **Table 3.11.**

All of three isolates were Gram-positive bacteria under firmicutes according to BLAST search and phylogenetic analysis. Isolate H3-46S of G-1 was phylogenetically closely related to *Chryseomicrobium* spp. with 99.62% 16S rRNA gene sequence identity. H3-65B of G-2 shared 99.85% identity to *Sporosarcina psychrophila* and H8-131S of G-1 were closely related to *Bacillus licheniformis* with 99.93% identity **(Figure 3.12).**

Table 3.11 Highest similarity profile of the sequences of 16s rRNA gene of selected isolates in accordance with BLAST identification

Isolate ID	Close similarity to (accession no.)	Identity(%)	Phylogenetic affiliation
H3-46S (G-1)	*Chryseomicrobium* sp. strain YL2 (KY855388.1)	99.62%	Firmicutes
H3-65B (G-2)	*Sporosarcina psychrophila* strain DSM 6497 (KU569324.1)	99.85%	Firmicutes
H8-131S (G-1)	*Bacillus licheniformis* strain YS65 (KU551256.1)	99.93%	Firmicutes

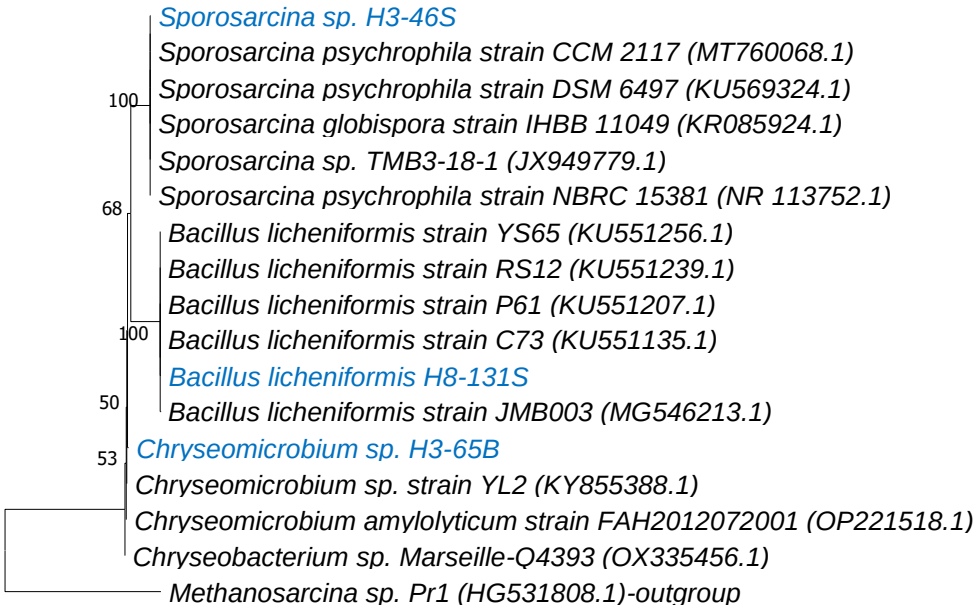


Figure 3.12: Evolutionary tree of 16S rRNA gene sequences of gram-positive isolates from hospital cafeteria fast foods and close relative reference isolates retrieved from database with accession numbers. MEGA11 was used to generate the tree using the Neighbour-Joining algorithm with the *Methanosarcina sp.* sequence as outgroup. Bootstrap values (n =1000 replicates) are shown at branch nodes. Isolates in this study are indicated with symbol in the beginning. Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates.

3.10 Genetic diversity of Gram-negative bacteria

To investigate the genetic diversity, the Gram-negative isolates were subjected to different virulence and resistance gene specific PCR using primers specific for those virulence and resistance genes in previously described PCR condition along with definite annealing temperature **(Table 2.5 and 2.6).**

3.10.1 Detection of virulence genes among Gram-negative bacteria

11 different virulence genes for different bacterial species were investigated in this study including *uid*A, *ial*, *stx*1, *stx*2 for group 1; *iut*A, *uid*A for group 2 and 3; *iro*N, *hly*A, *iut*Afor group 4, 5 and 6; *inv*A for group 7 and *opr*I, *opr*L, *tox*A for group 8. Genotyping for virulence revealed that none of the isolates harbored genes encoding for *iro*N, *hly*A, *iut*A, *ial*, *stx*1, *stx*2, *tox*A. Among the harbored virulence genes; all *Pseudomonas* spp. were *opr*I positive and 12.5% were *opr*L positive. One isolate was *inv*A positive which was of *Salmonella* sp. Among the *E. coli*, 50% were *uid*A positive **(Table 3.12, Figure 3.15).**

3.10.2 Detection of antibiotic resistance genes among Gram-negative bacteria

14 different resistance genes for different classes of antibiotics were screened in this study including *gyr*B, *qnr*, *qnr*B, *qnr*S, $bla_{\text{DIM-1}}$, bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-48}}$, $bla_{\text{KPC-1}}$, $bla_{\text{VIM-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{SIM-1}}$, MCR2. Genotyping for resistance revealed that none of the isolates harbored genes encoding for *qnr*, *qnr*B, $bla_{\text{VIM-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{SIM-1}}$, MCR2. Among the resistance genes, *gyr*B showed the highest prevalence (50%) followed by *qnr*S (41.67%), $bla_{\text{DIM-1}}$ (31.03%), bla_{SHV} (20%), $bla_{\text{OXA-48}}$ (16.67%), $bla_{\text{OXA-1}}$ (2.22%), $bla_{\text{KPC-1}}$ (2.22%) and $bla_{\text{CTX-M}}$ (1.11%) **(Table 3.12, Figure 3.13, Figure 3.14, Figure 3.15).**

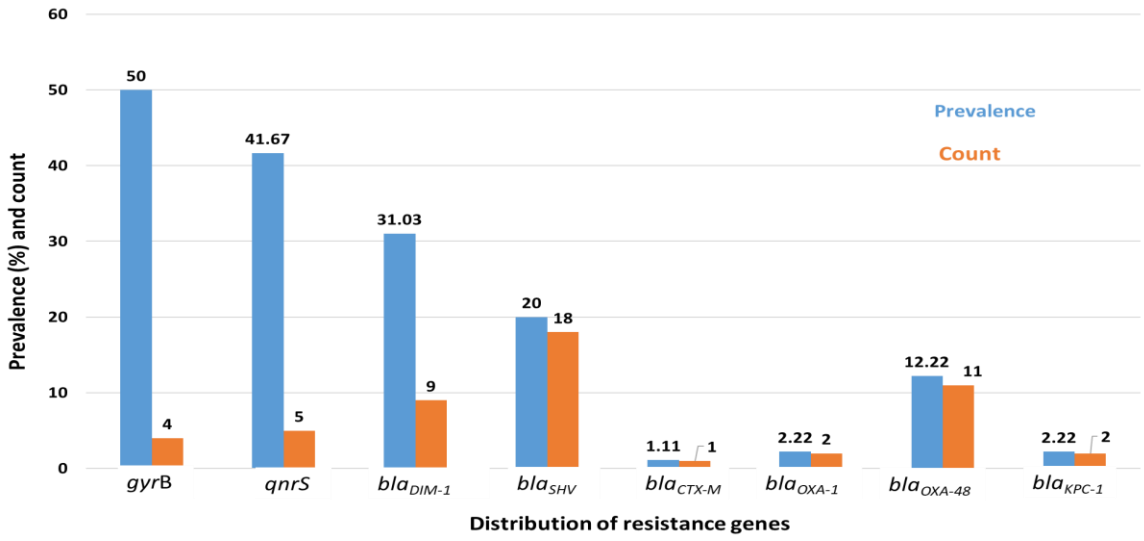


Figure 3.13 Diversity of antibiotic resistance genes among different Gram-negative bacterial isolates

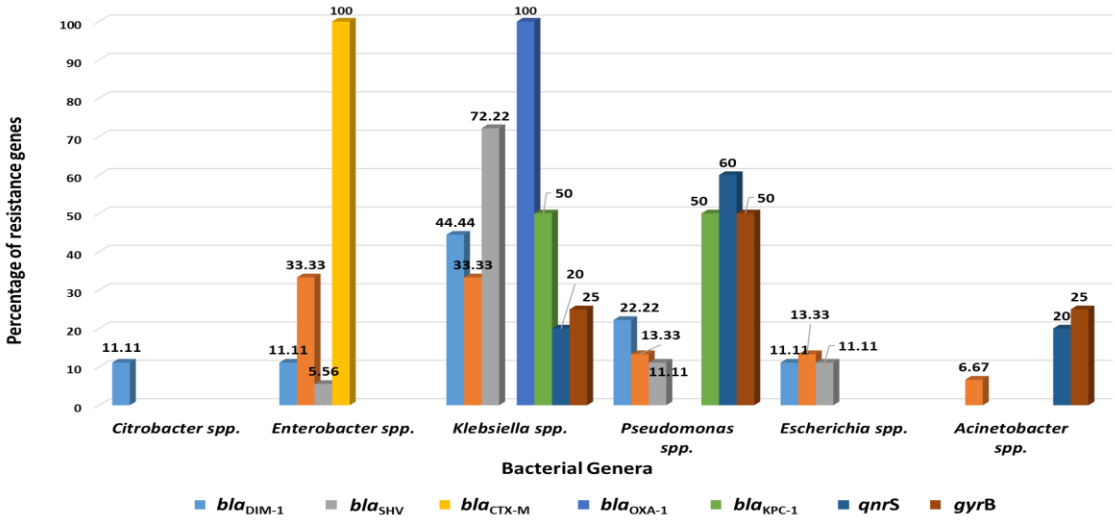


Figure 3.14 Distribution of antibiotic resistance genes among different bacterial species

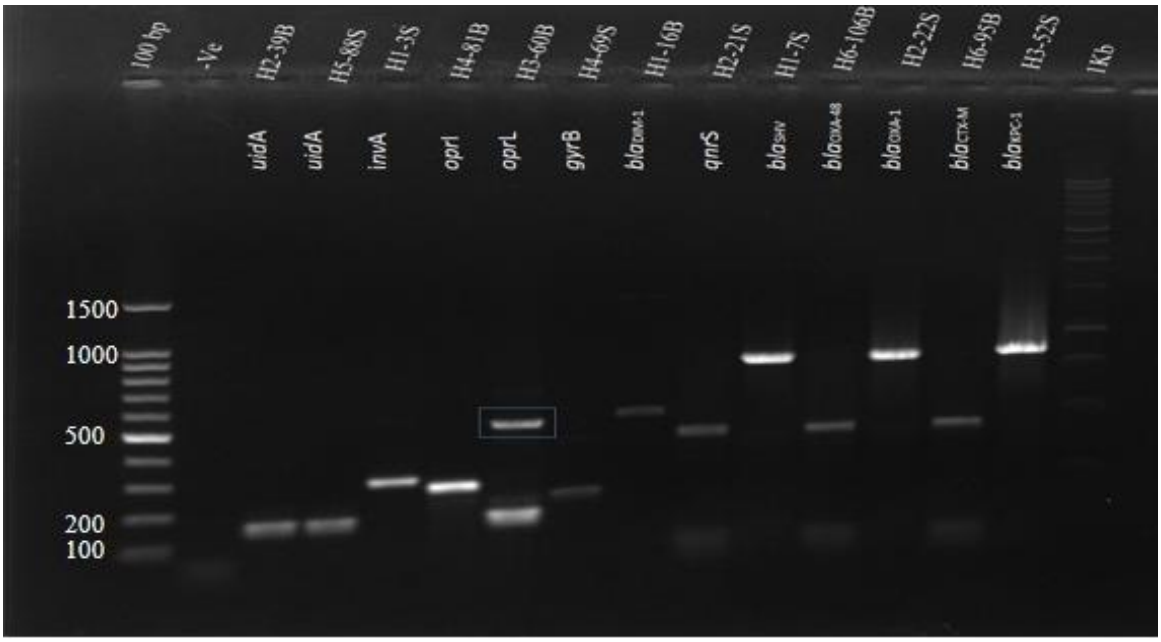


Figure 3.15 Representative figure of different virulence and antibiotic resistance genes detected from the isolates of hospital cafeterias fast foods. Lane 1 and lane 16 are molecular ladders of 100bp and 1kb (Promega, USA), respectively. Lane 2 is negative control. Lane 3 and 4 are *uid*A (147bp) for *E. coli* and *Enterobacter* spp., respectively. Lane 5 is *inv*A, (284 bp); lane 6 is oprI, (249 bp); lane 7 is *opr*L, (504 bp); lane 8 is *gyr*B, (210 bp); lane 9 is $bla_{\text{DIM-1}}$, (688 bp); lane 10 is *qnr*S, (400 bp); lane 11 is bla_{SHV}, (861 bp); lane 12 is $bla_{\text{OXA-48}}$, (438 bp); lane 13 is $bla_{\text{OXA-1}}$, (882 bp); lane 14 is $bla_{\text{CTX-M}}$, (593 bp); lane 15 is $bla_{\text{KPC-1}}$, (900 bp). All virulence and antibiotic resistance genes gel figure is given in **appendix VIII.**

3.10.3 Sequencing of virulence and antibiotic resistance genes

The *inv*A, *opr*L, *uid*A, bla_{SHV} genes were confirmed by gene sequencing and these gene sequences were deposited in NCBI databases under accession no. OQ108511, OQ108512, OQ108513, OQ108509 respectively. The gene sequences are given in **appendix IX.**

Table 3.12 Diversity of virulence and antibiotic resistance genes among the Gram-negative isolates with their phenotypic virulence assay.

The positive isolates of at least one gene, either virulence or resistant, were included here. [Bold isolates are sequenced isolates, ND=Not done, G-1= *Escherichia* spp., G-2 &3= *Enterobacter* spp., G-4, 5 &6= *Klebsiella* spp., G-7= *Salmonella sp.*, G-8= *Pseudomonas* spp. ,G-9= *Citrobacter sp.* and G-10= *Acinetobacter sp.*]

Isolate Id	**Virulence gene**	**Resistance gene**	**Phenotypic virulence assays**		
			Biofilm formation properties	**Hemolytic properties**	**Antibiotic resistance profile**
(G-7)					
H1-3S *(Salmonella enterica)*	*inv*A	-	WBF	β	MER+LE+CL+SXT+P+AMP
(G-9)					
H1-5S *(Citrobacter freundii)*	ND	$bla_{\text{DIM-1}}$	WBF	γ	MER+TE+CL+SXT+P+AMP
(G-2 & G-3)					
H1-6S	-	$bla_{\text{OXA-48}}$	WBF	γ	MER+CL+P+AMP
H3-48S	-	$bla_{\text{OXA-48}}$	WBF	α	MER+AZM+CT+CL+F+P+AMP
H3-49S	-	$bla_{\text{OXA-48}}$	WBF	γ	MER+CL+P+AMP
H3-50S (*Leclercia* sp.)	-	$bla_{\text{DIM-1}}$	NBF	γ	MER+TE+CIP+CL+SXT+P+AMP
H4-76S	-	bla_{SHV}	MBF	α	MER+AZM+K+CL+F+P+AMP
H4-77S (*Enterobacter cloacae*)	-	$bla_{\text{OXA-48}}$	SBF	α	MER+TE+CT+CTR+CL+SXT+F+P+AMP
H5-88S	*uid*A	-	MBF	γ	MER +AZM +CL +F+P+AMP
H6-95B	*uid*A	$bla_{\text{CTX-M}}$ + $bla_{\text{OXA-48}}$	WBF	γ	MER+TE+AZM+CIP+CL+F+P+AMP
(G-4, G-5 & G-6)					
H1-7S (*Klebsiella quasipneumoniae subsp. Similipneumoniae)*	-	$bla_{\text{DIM-1}}$+ bla_{SHV} + $bla_{\text{OXA-1}}$	WBF	γ	MER+TE+LE+CL+SXT+F+P+AMP
H1-13B	-	bla_{SHV}	SBF	γ	MER+TE+AZM+CT+CL+SXT+F+P+AMP
H1-14B	-	$bla_{\text{DIM-1}}$+ bla_{SHV}	SBF	α	MER+TE+AZM+CL+SXT+F+P+AMP
H1-16B	-	$bla_{\text{DIM-1}}$+ bla_{SHV}	WBF	α	MER+TE+AZM+CL+SXT+P+AMP
H1-18B	-	$bla_{\text{OXA-48}}$	SBF	α	MER+TE+K+CTR+CL+SXT+P+AMP
H2-21S (*Klebsiella pneumonia)*	-	bla_{SHV} + $bla_{\text{OXA-48}}$	NBF	γ	MER+TE+CL+SXT+P+AMP
H2-22S (*Klebsiella pneumonia)*	-	$bla_{\text{OXA-1}}$	MBF	γ	MER+TE+SXT+F+P+AMP
H3-41S	-	bla_{SHV}	SBF	α	MER+CTR+CL+P+AMP
H3-59B (*Klebsiella pneumoniae)*	-	bla_{SHV}	NBF	α	MER+AZM+CT+CIP+CL+F+P+AMP
H3-66B	-	$bla_{\text{KPC-1}}$	NBF	α	MER+C+CT+K+S+CL+SXT+P+AMP
H3-67B	-	$bla_{\text{OXA-48}}$	NBF	γ	MER+AZM+CIP+F+P+AMP
H5-94B (*Klebsiella*	-	bla_{SHV} +	WBF	γ	MER+CL+F+P+AMP

pneumonia)		*bla*$_{OXA-48}$			
H6-103B	-	*bla*$_{SHV}$	WBF	α	MER+TE+CIP +CL+F+P+AMP
H6-105B	-	*bla*$_{DIM-1}$+ *bla*$_{SHV}$	MBF	α	MER+TE+AZM+CT+CIP+CL+SXT+F+P+AMP
H6-107B	-	*bla*$_{SHV}$	NBF	γ	MER+AZM+CIP+CL+SXT+F+P+AMP
H7-113S (*Klebsiella pneumonia)*	-	*bla*$_{SHV}$	WBF	α	MER+TE+AZM +CL+P+AMP
H7-122B	-	*bla*$_{OXA-48}$+ *qnr*S + *gyr*B	NBF	β	MER+TE+C+CT+CL+P+AMP
H8-126S	-	*bla*$_{SHV}$	SBF	γ	MER+TE+P+AMP
(G-8)					
H2-24S	*opr*I	-	NBF	β	MER+TE+AZM+K+CTR+CL+SXT+F+P+AMP
H2-36B	*opr*I	-	NBF	α	MER+CTR+SXT+F+P+AMP
H3-52S	*opr*I	*bla*$_{KPC-1}$	MBF	α	MER+C+CTR+CL+F+P+AMP
H3-58S	*opr*I	*bla*$_{SHV}$	SBF	γ	MER+C+CTR+CL+F+P+AMP
H3-60B (*Stutzerimonas stutzeri*)	*opr*L + *opr*I	*bla*$_{DIM-1}$+ *bla*$_{SHV}$	WBF	α	MER+TE+AZM+C+ GEN+LE+CIP+CL+SXT+F+P+AMP
H3-62B	*opr*I	-	NBF	α	MER+P+AMP
H4-70S	*opr*I	-	MBF	α	MER+C+CTR+CL+F+P+AMP
H4-81B (*Pseudomonas sp.*)	*opr*L + *opr*I	*bla*$_{DIM-1}$+ *bla*$_{OXA-48}$+ *qnr*S	NBF	β	MER+TE+AZM+C+K+S+LE+CIP +CL+SXT+F+P+AMP
H4-82B		*qnr*S	SBF	β	MER+TE+AZM+CT++C+GEN+LE+CIP+CL+F+P+AMP
H4-83B	*opr*I	-	NBF	β	MER+TE+CT+CL+F+P+AMP
H7-110S (*Pseudomonas sp.*)	*opr*I	*qnr*S + *gyr*B	NBF	γ	IMI+MER+TE+AZM+CTK+S+LE +CIP+CL+SXT+F+P+AMP
H8-127S	*opr*I	*gyr*B	WBF	β	MER+TE+C +CT+P+AMP
H8-128S	*opr*I	*bla*$_{OXA-48}$	NBF	γ	MER+TE+AZM+C+CT+P+AMP
H8-130S	*opr*I	-	NBF	γ	MER+TE+C+CT+P+AMP
(G-1)					
H2-20S	-	*bla*$_{SHV}$	MBF	α	MER+TE+CL+SXT+F+P+AMP
H3-39B (*Escherichia coli)*	*uid*A	*bla*$_{DIM-1}$+ *bla*$_{OXA-48}$	WBF	α	MER+TE+K+CIP+CTR+CL+SXT +P+AMP
H5-87S	*uid*A	*bla*$_{SHV}$	SBF	γ	MER+TE+AZM+C +F+P+AMP
H6-106B	-	*bla*$_{OXA-48}$	SBF	γ	MER +AZM+CIP+CL +F+P+AMP
(G-10)					
H4-69S (*Acinetobacter baumannii*)	ND	*bla*$_{OXA-48}$+ *qnr*S + *gyr*B	SBF	γ	MER+AZM+CTR+CL+F+P+AMP

3.11 Socio-demographic profile of the food handlers

A total of 120 food handlers participated in this study. Nearly half of the food handlers (48.4%) were under twenty years old, all (100%) were male, most (70.0) had educational backgrounds below the secondary level, nearly half (48.3%) made between Tk. 6,000 and Tk. 10,000 per month, 66.7% were single, and most (70.0%) had less than six years of work experience. (Liu et al., 2020)

Table 3.13 Socio-demographic profile of Dhaka city's hospitals cafeterias food handlers

Socio-demographic variable	**Frequency**	**Percentage**
Age (Years)		
<20	58	48.4
21-30	36	30.0
31-40	12	10.0
41-50	10	8.3
>51	4	3.3
Sex		
Male	120	100
Educational Level		
Illiterate	2	1.7
Class1-8	84	70.0
S.S.C	22	18.3
H.S.C	10	8.3
>H.S.C	2	1.7
Monthly Income (Tk.)		
5,000 and less	40	33.3
6,000-10,000	58	48.3
11,000-15,000	8	6.7
16,000-20,000	8	6.7
21,000 and above	6	5.0
Marital Status		
Unmarried	80	66.7
Married	40	33.3
Work Experience (Years)		
<6	84	70.0
6-10	18	15.0
11-15	10	8.3
>15	8	6.7
Overall	**120**	**100**

3.12 Knowledge of the food handler's

First, general questions about food borne illnesses, food contamination causes, disease transmission routes, and information sources were posed to the food handlers in order to gauge their level of knowledge. The knowledge score was then calculated using eight statements.

Approximately 63.3% of people who handle food have heard of at least one food-borne illness. 30.0% of food handlers who were aware of food-borne illness learned about it from the media. According to the food handlers, there are three main ways that food-borne diseases are spread: through contaminated food (35.0%), contaminated water (36.7%), and vectors (28.3%). The

majority of food handlers (66.7%) believed that a dirty workplace was the main contributor to food contamination.

Table 3.14 Knowledge of foodborne illness, their modes of transmission, and sources of food contamination among food handlers

Variables	**Frequency**	**Percentage**
Food handlers knowledge of food borne illness		
Yes	76	63.3
No	44	36.7
Source of information about FBD		
Health Center	2	1.7
Training	14	11.7
Media	36	30.0
School education	24	20.0
Do not know	44	36.6
Knowledge regarding route of transmission		
Tainted food	42	35.0
Tainted water	44	36.7
Vector	34	28.3
Knowledge about cause of food contamination		
Dirty utensils	6	5.0
Dirty work environment	80	66.7
Dirty hands	20	16.7
Diseased food handler	6	5.0
Do not know	8	6.6
Overall	**120**	**100**

The majority of food handlers (56.7%) agreed that wearing gloves when handling raw foods lowers the risk of food contamination, while almost half (48.3%) believed that healthy people can carry germs to food and 53.3% stated that refrigeration and freezing kills most bacteria. More than half (55.0%) of food handlers disagreed that reheating foods increases the risk of bacterial food contamination; 58.3% disagreed that contact between raw and cooked foods increases the risk of bacterial food contamination; and 65.0% were unaware that sanitizing and cleaning utensils lowers the risk of food contamination.

Table 3.15 Knowledge statements regarding the knowledge of the food handling personnel

Knowledge statements	**Frequency**	**percentage**
Prior knowledge (Heard about) of food borne illness		
Yes	**76**	63.3%
No	**44**	36.7%
Healthy people can cause illness by carrying germs to food		
Yes	58	48.3%
No	62	51.7%
Reheating foods contributes to bacterial food contamination		
Yes	**54**	45.0%
No	**66**	55.0%
Contact between raw and cooked foods contributes to food contamination		
Yes	**50**	41.7%
No	**70**	58.3%
Cleaning and sanitizing utensils reduces the risk of food contamination		
Yes	**42**	35.0%
No	**78**	65.0%
Refrigeration and freezing do not destroy most bacteria		
Yes	**56**	46.7%
No	**64**	53.3%%
Using gloves to handle raw foods reduces the risk of food contamination		
Yes	**68**	56.7%
No	**52**	43.3%
Use of jewels such as rings, watches, wearing in food handling cause food contamination		
Yes	**48**	40%
No	**72**	60%
Overall	120	100

3.13 Knowledge score of the food handlers

Most food handlers (62.50%) had low knowledge scores based on the average of their individual knowledge statement scores. Those with good knowledge scores were those who received six or more correct answers out of a possible eight.

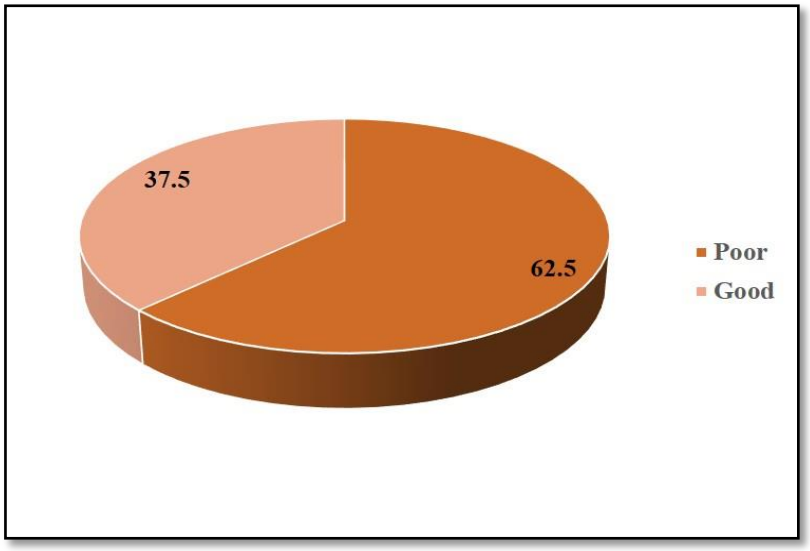


Figure 3.16 Food handler's knowledge score of selected hospitals cafeterias of Dhaka city

3.14 Food handlers practices regarding food handling

28.3% of those who handled food worn gloves, 35% had their hair covered, 48.3% were dressed neatly, 73.3% checked the expiration dates of the ingredients, 80.0% washed their hands before beginning any activity, and 73.3% had short, clean nails when being inspected. All (100%) of the food handlers did not worn apron, 61.7% did not worn any jewelry during inspection. Most of them (61.7%) had no training regarding food preparation and handling.

Table 3.16 Food handling practices of the food handlers

Food handling practices	**Frequency**	**Percent**
Food handlers worn gloves during inspection		
Yes	34	28.3
No	86	71.7
Food handlers worn apron during inspection		
Yes	0	0
No	120	100
Food handlers hair covered		
Yes	42	35.0
No	78	65.0
Food handlers dress		
Clean	58	48.3
Not Clean	62	51.7
Food handlers checking of expiry date of ingredients		
Yes	88	73.3
No	32	26.7
Wash hands before any event		
Yes	96	80.0
No	24	20.0
Finger nail cut short and clean		
Yes	88	73.3
No	32	26.7
Wear jewelry during inspection		
Yes	46	38.3
No	74	61.7
Training acceptance regarding food preparation and handling		
Yes	46	38.3
No	74	61.7
Overall	120	100

3.15 Food handling personnel practice score of food handling

71.67% of them scored poorly on food handling practices, according to the average of their individual scores. People who received scores of 6 or higher on all eight practice questions were classified as following good food handling procedures.

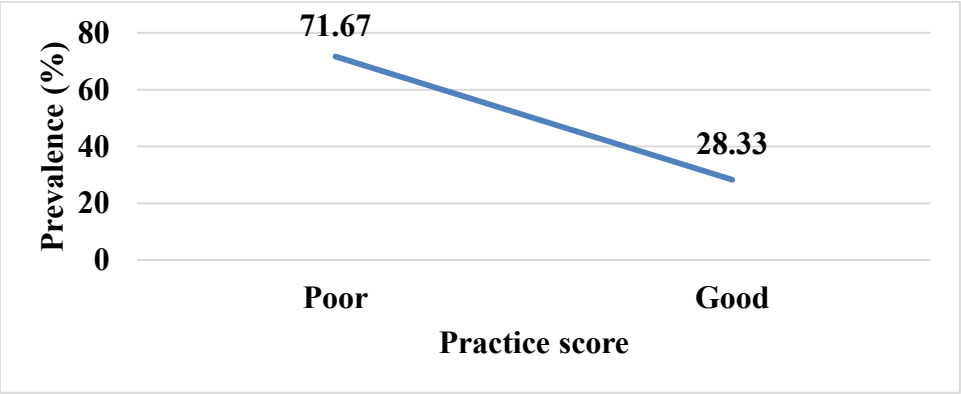


Figure 3.17 Food handling practice score of food handlers

3.16 Relationship between food handlers sociodemographic profile and food handling practice score

3.16.1 Univariate analysis between food handling personnel socio-demographic profile and food handling practice score

With regard to training status, educational attainment, and monthly income, univariate analysis revealed a statistically significant difference between the good and poor food handling practice scores. The food handlers' age and work history had no impact on their practice score.

Table 3.17 Univariate analysis between food handling personnel socio-demographic characteristics and food handling practice score

Socio-economic variables	**Overall practice score**		**P-value**
	Good	Poor	
Age (Years)			0.067
<20	16	42	
21-30	12	24	
31-40	6	6	
41-50	0	10	
>51	0	4	
Training status			<0.005
Yes	8	66	
NO	26	20	
Educational Level			<0.005
Illiterate	0	2	
Class1-8	14	70	
S.S.C	16	6	
H.S.C	4	6	
>H.S.C	0	2	
Monthly Income (Tk.)			0.031
5,000 and less	14	26	
6,000-10,000	10	48	
11,000-15,000	2	6	
16,000-20,000	4	4	
21,000 and above	4	2	
Work Experience (Years)			0.771
<6	22	62	
6-10	6	12	
11-15	4	6	
>15	2	6	
Overall	34	86	

3.16.2 Multivariate analysis between food handlers socio-demographic characteristics and food handling practice score

Training acceptance was identified as a potential influencing factor by multivariate analysis with AOR=0.049%, 95% CI=0.011, 0.225, and P=<0.005. Also, education level (P<0.006) was discovered to have a significant impact on the food handlers' score on food handling practice**.**

Table 3.18 Multivariate analysis between socio-demographic profile of food handlers and food handling practice score

Socio-economic variables	**Coefficient**	**95% CI**	**P-value**
No training acceptance	RC		
Training acceptance	0.049	(0.011, 0.225)	**0.000**
Educational level	0.06	(0.003, 0.127)	**0.006**

3.17 Relationship between food handling personnel knowledge scores and scores related to food handling

The food handlers' knowledge score and food handling score are significantly correlated

(P <0.05) in the selected hospital cafeterias in the city of Dhaka.

Table 3.19 Relationship between food handlers' knowledge scores and scores related to food handling

Knowledge score	**Food handling practice score**		**Total**	**P-value**
	Poor	Good		**0.002**
Poor	61	14	75	
Good	25	20	45	
Total	86	34	60	

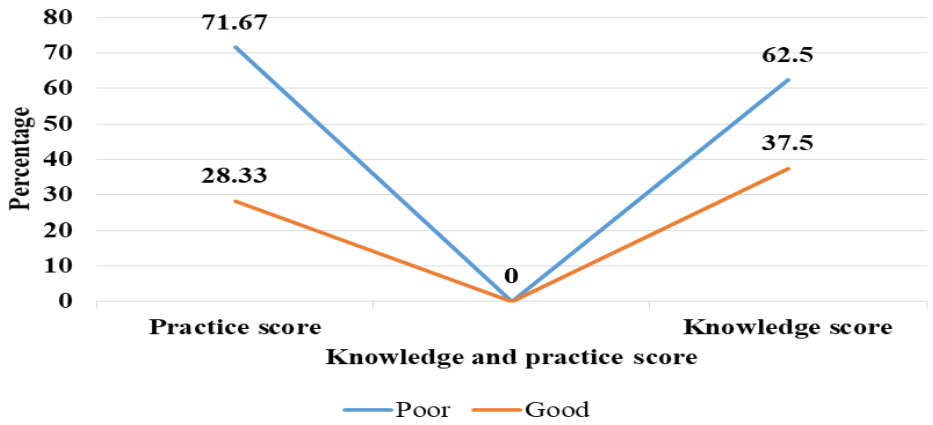


Figure 3.18 Relation between Knowledge and practice score of the food handler's

Chapter 4: Discussion

4.0 Discussion

A universal blueprint for a marvelous, more just, and more sustainable way of life on our universe was released by the United Nations in 2015 as the Sustainable Development Goals (SDGs). Many SDGs may not be achieved due to the AMR's ongoing emergence especially the SDG 3 (Gajdács et al., 2021). Without the availability of powerful antibiotics, the achievement of many of the adopted targets in the SDG 3 on health will be retarded. The development of AMR currently jeopardizes the advancements made in reducing maternal mortality over the previous 15 years, particularly in low- and middle-income countries (LMICs), where the maternal mortality rate is up to 19 times higher than in the rest of the world (Jasovský et al., 2016). Considering public health threat that antibiotic resistance pose and the rapid transmission of virulence and antibiotic resistance genes in bacteria, the search for the presence of antibiotic resistant bacteria with their virulence and antibiotic resistance genes in hospital cafeteria fast foods in Bangladesh seems worthy. To date, there is no extensive study on the pathogenicity of the antibiotic resistant bacteria among hospital cafeteria fast foods in Bangladesh.

4.1 Total count of bacteria in the hospital cafeteria fast foods revealed the unsatisfactory condition of most of the fast food samples

The majority of the food samples from various hospital cafeterias were highly loaded with various bacterial strains, according to the total count of bacteria from the sandwich and burger samples **(Table 3.1)**. Most of the food samples had a total bacterial count that was significantly higher than what was acceptable, and most of the samples were in poor condition (Center for Food Safety, 2022.). Unsatisfactory levels of bacteria were present in the most of the sandwiches from the governmental hospital cafeterias **(Table 3.1).** The study findings regarding total bacterial count are in accordance to studies in Bangladesh (Sabuj et al., 2020); (N. Hoque et al., 2012); (Hasan, 2014); Thailand (Rattanasena & Somboonwatthanakul, 1899) , Egypt (Shaltout et al., 2016).

Most of the sandwich and burger samples showed unsatisfactory level of count of enteric bacteria **(Table 3.2).** A study conducted on enteric bacterial contamination of ready-to-eat food with enteric bacteria in India found different types of enteric bacteria including *E.coli, Klebsiella spp.,Salmonella spp., Enterobacter spp. , Pseudomonas spp., Shigella spp. , Proteus spp.* (Kaur & Walia, 2014). High prevalence of enteric bacteria also observed in a study in Ethiopia (Getie et al., 2019). Determining the magnitude of incidence and prevalence of specific enteric pathogens in the globe is a very challenging

issue. The majority of enteric infections are influenced by their environment, with specific geographic and seasonal patterns related to the level of hygienic practices and access to clean drinking water (Petri et al., 2008)**.**

4.2 Distribution of the isolates demonstrated a large number of Gram-negative and Gram-positive bacteria possessing antibiotic resistance

A diverse number of Gram-positive and Gram-negative bacteria were present in the hospital cafeteria fast foods. Gram-negative isolates made up 63.50% of the total, while Gram-positive isolates accounted for 36.50% of the total. The likelihood that they contained bacteria is increased by the use of mayonnaise, fresh salad ingredients, and the moist interior of sandwiches and hamburgers. A significant amount of *coliforms, E. coli, Enterobacter, Bacillus cereus, Staphylococcus aureus,* and other bacterial spp. were found in freshly cut vegetables and lightly processed ready-to-eat food samples. (Kaneko et al., 1999). By examining their morphological, cultural, biochemical, and Random Amplification of Polymorphic DNA (RAPD) (for Gram-negative isolates) characteristics, the Gram-positive isolates from this study were divided into six different groups and the Gram-negative isolates were divided into ten different groups. The Gram-negative isolates found from this study distributed significantly among different bacterial species keeping the highest number of isolates in *Klebsiella* spp. followed by *Enterobacter* spp., and less abundant species were *Salmonella* spp. and *Acinetobacter* spp. **(Table 3.4, Table 3.10, Figure 3.1.2b and Figure 3.11).** These Gram-negative isolates resistance to different classes of antibiotics **(Table 3.9).** There were many differences in the distribution of Gram-positive isolates, with *Bacillus spp*. accounting for the majority of isolates and *Staphylococcus spp.* for the minority **(Table 3.5, Figure 3.1.2c).** Numerous studies have shown that sandwiches and hamburgers contain a significant number of food borne pathogens (Easa, 2011; Sabuj et al., 2018; Shaltout et al., 2016; University et al., 2015). Numerous researchers have found that inadequate education, lack of training, and inadequate food handler knowledge and practices all contributed to the unsatisfactory condition of the food (Abdi et al., 2020; Ghosh & Alim, 2022; Meleko et al., 2015; Smith et al., 2010).

4.3 Antibiotics resistance belonged to both Gram-negative and Gram-positive isolates possessing multi-drug resistant properties

In nations like Bangladesh, there is a strong selective pressure for antibiotic resistance due to the ongoing overuse or misuse of drugs in hospitals, veterinary clinics, and the poultry industry (Faiz & Basher, 2011; Mooljuntee et al., 2010). The occurrence of drug-resistant microorganisms as a result of improper use and continued use of antibiotics has gradually increased mortality rates (Kumar et al., 2020). Food contamination by drug-resistant bacteria poses a serious risk to public health due to the potential for the determinants of antibiotic resistance to spread to other bacteria that have therapeutic relevance for humans (Van et al., 2007). About 80.99% of the Gram-negative isolates **(Table 3.9, Figure 3.8a)** and 54% of the Gram-positive isolates **(Figure 3.8b)** were resistant to at least one agent in three or more antimicrobial categories, so displayed characteristics of multidrug resistance (MDR) (Ap et al., 2012).

The antimicrobial resistance profile revealed that the study isolates had significantly higher percentages of resistance to particular antibiotic classes. For example, in case of Gram-negative isolates, Penicillin group resistance is 100%; meropenem, a carbapenem, resistance is 95.4%. Imipenem was highly effective against all isolates (97.71%) **(Figure 3.7a).** Among the carbapenem, imipenem is highly sensitive, whereas meropenem is highly resistant. By comparing of sampling areas' relative resistance, the amount of resistance for a specific pattern is identical but various drugs. Furthermore, no single antibiotic from the group was universally effective against all of the isolates from a particular sampling area (hospitals) **(Table 3.8)**. The pattern of antibiotic resistance can be linked to prior findings from Bangladesh, where higher resistance to various antibiotics, including ampicillin, azithromycin, ceftriaxone, ciprofloxacin, nitrofurantoin, tetracycline, and gentamycin, was reported (Abanish, 2015; I. Ahmed et al., 2019; Sohel et al., 2022; Urmi et al., 2021) (Sohel et al., 2022), (Urmi et al., 2021), (Abanish, 2015), (I. Ahmed et al., 2019) along with international studies.

4.4 MDR Gram-negative isolates including WHO enlisted priority 1 organisms

In accordance with the urgency of new antibiotics, the WHO list is broken down into three categories: critical, high, and medium priority. Bacteria that are resistant to multiple antibiotics, are a major threat to patients in healthcare facilities like hospitals and nursing homes along with those whose care requires equipment like ventilators and

blood catheters, are among the most dangerous of all. *Acinetobacter, Pseudomonas*, and various Enterobacteriaceae *(Klebsiella, E. coli, Serratia, and Proteus*) are among them. They can cause serious infections that are frequently fatal, like pneumonia and bloodstream infections (WHO, 2023).

These study revealed the presence of a large number of WHO enlisted priority 1 organisms. Most of the *Pseudomonas, enterobacter and klebsiella* isolates of this study were MDR and many of them possessed different virulence and antibiotic resistance genes **(Table 3.12).** In addition to different carbapenamases resistance *(bla*$_{\text{KPC-1,}}$ *bla*$_{\text{SIM-1,}}$ *bla*$_{\text{DIM-1,}}$ *bla*$_{\text{VIM-1,}}$ *bla*$_{\text{NDM-1,}}$ *bla*$_{\text{OXA-1,}}$ *bla*$_{\text{OXA-48}}$) and class A β-lactamase resistance (*bla*$_{\text{SHV,}}$ *bla*$_{\text{CTX-M}}$); some of the *Klebsiella* spp. (H7-122B) and *Pseudomonas* spp. (H4-81B, H4-82B, H7-110S, H8-127S) harbored fluoroquinolone resistance (*qnr*S, *gyr*B) genes. In addition to different antibiotic resistance genes, some of the *Klebsiella* spp. (H1-13B, H1-14B, H1-18B, H3-41S, H8-126S) and *Pseudomonas* spp. (H3-58S, H4-82) showed strong biofilm formation capacity **(Table 3.12)**. The multi-drug resistant *Acinetobacter baumannii* (H4-69S) was strong biofilm former and harbored *bla*$_{\text{OXA-48}}$, *qnr*S and *gyr*B genes **(Table 3.12)**. The *Acinetobacter baumannii* and *Pseudomonas* spp. are highly involved with nosocomial infections revealed by different studies (Ayoub Moubareck & Hammoudi Halat, 2020; Nocera et al., 2021; Reynolds & Kollef, 2021; Safeyah et al., 2020; Spagnolo et al., 2021).

4.5 Different types of virulence properties of the isolates demonstrated their potential association with pathogenicity

A wide range of virulence traits were frequently present in the food-borne isolates, which may have contributed to their pathogenicity in humans (Camellini et al., 2021; Osman & Waheed, 2017). These isolates have undergone a variety of phenotypic tests, including hemolytic tests and biofilm formation capacity, to evaluate their virulence factors. The development of biofilms and hemolysins is a crucial component of pathogenicity and increases resistance to several kinds of antimicrobials (M. N. Hoque et al., 2020; Jovanović et al., 2023; Mogrovejo et al., 2020).

Among the Gram-negative isolates, hemolytic and biofilm formation capabilities were present in 54.02% and 65.52% of the isolates, respectively, according to the results of phenotypic virulence assays. About 16.09% isolates were strong biofilm formers (SBF**) (Figure 3.3a).** The strong biofilm formers were mainly from *Klebsiella* spp. along with *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp **(Figure 3.4a, Table 3.6).** The

findings are consistent with the results of different studies (Ramos-Vivas et al., 2019; Seifi et al., 2016; Yang et al., 2019**;** Li et al., 2023)**.** 13.79% isolates showed complete hemolysis and 40.23% isolated showed partial hemolysis **(Figure 3.5a, Figure 3.6)**. Mainly the presumptive *Pseudomonas* and *Klebsiella* spp. along with *Salmonella* spp. exhibited the β-hemolytic properties **(Table 3.7).** Among Gram-positive isolates approximately 6% of the isolates were strong biofilm formers, while 4%, 24%, and 66% of the isolates were moderate biofilm formers, weak biofilm formers, and non-biofilm formers, respectively **(Figure 3.3b)**. The strong biofilm formers were mainly from *Bacillus* spp. and *Planococcus* spp. **(Figure 3.3b, Table 3.6)**. Approximately 6% of the isolates exhibited β-hemolytic characteristics **(Figure 3.5b)**. Mainly the *Bacillus* spp. and *Planococcus* spp. exhibited the β-hemolytic properties **(Table 3.7)**.

In this study, 11 different virulence genes were examined for Gram-negative bacterial strains. These include production of toxins, siderophores, lipoproteins, iron transport systems, invasins etc. The *inv*A gene was prevalent in the Salmonella isolate **(Table 3.12)**. The *inv*A genes have been demonstrated to be suitable for particular targets in various diagnostic and research laboratories. The *inv*A genes contain sequences that are specific to *Salmonella* spp. which is responsible for invasion into host epithelial cells (Somda et al., 2018; K. Sultana et al., 2021). The *uid*A gene, which is the *E. coli* genus-specific gene (Alsanjary & Sheet, 2022) and is present in the majority of *E. coli* (Feng et al., 1991) was one of the objectives of the current study. It was investigated in *E. coli* and *Enterobacter* spp. The glucuronidation process, which limits the excretion of substances from the body, is reversed by the beta-glucuronidase enzyme, which is encoded by the *uid*A gene. In this study, the *uid*A gene was present in about 50% of the *E. coli* isolates and 7.69% of the *Enterobacter* isolates **(Table 3.12)**. For the *Pseudomonas* spp. , *opr*I and *opr*L genes were investigated (Matthijs et al., 2013). All of the *Pseudomonas* isolates demonstrated the prevalence of *opr*I gene **(Table 3.12)**, which is a genus specific gene for *Pseudomonas* spp. (Gholami et al., 2016b). About 12.5% of the *Pseudomonas* spp. showed the presence of *opr*L gene **(Table 3.12)**, which is used for the detection of *P. aeruginosa* species (Gholami et al., 2016b) that are associated with nosocomial infection. These findings are consistent with other studies that found *Pseudomonas* species to be widespread in hospitals and among hospitalized patients, and that they can spread to people, particularly those with immunological depression (Al-Dujaili & Harris, 1975; Jami Al-Ahmadi & Zahmatkesh Roodsari, 2016).

14 different antibiotic resistance genes from different classes of antibiotics were studied among Gram-negative isolates in this study. The prevalence of the class A β-lactamase resistant genes bla_{SHV} and bla_{CTX-M} was 20% and 1.1%, respectively **(Figure 3.13, Table 3.12)**. While the prevalence of bla_{CTX-M} was lower than in other studies, the results of bla_{SHV} were somewhat consistent across studies (Gundran et al., 2019), (Ejaz et al., 2021), (Peymani et al., 2017). *gyr*B and *qnr*S, two fluoroquinolone resistance genes, showed prevalence rates of 50% and 41.67%, respectively **(Figure 3.13, Table 3.12)**. Studies in Bangladesh (Mahmud et al., 2018), as well as in other nations like Germany (Juraschek et al., 2021) , and China (Cheng et al., 2020), reported the presence of these genes in various food samples. The prevalence of the carbapenemase resistance genes, $bla_{KPC-1,}$ $bla_{DIM-1,}$ $bla_{OXA-1,}$ bla_{OXA-48} was 2.22%, 31.03%, 2.22%, and 12.22%, respectively **(Figure 3.13, Table 3.12)**. These results were consistent with findings from various studies using various samples (Begum & Shamsuzzaman, 2016; Shrestha et al., 2019; Taggar et al., 2020).

4.6 Poor food handling knowledge and practices of the food handlers may contribute to the development of large number of food borne illness

Inappropriate food handling by the food handlers contributes a large number of food borne diseases**.** In line with studies done in northern Ethiopia (Tessema et al., 2014), eastern Ethiopia (Tamiru et al., 2022) and India (Kubde et al., 2016), only 37.5% of the food handlers in this study had good knowledge of food borne diseases, food handling, and food safety **(Figure 3.16)**. This is significantly lower than the study done by Zain (Zain & Naing, 2002) . Only 28.33% of the food handlers used good food handling techniques **(Figure 3.17)**, which is comparable to studies carried out in Gondar, Ethiopia (30.3%) (Gizaw et al., 2014) and Arba-Minch (32.6%) (Reta et al., 2021). But compared to studies done in Malaysia (Ab. Hamid et al., 2014) , Jordan (Habiballah et al., 2017), and Northwest Ethiopia (Tessema et al., 2014) , the finding is less significant. Lack of awareness and personal hygiene of the food handlers can cause infections with intestinal pathogens (Kubde et al., 2016). During food preparation maintenance of personal hygiene can reduce the food borne diseases by 67-85% (Augustin et al., 2020). In this study most of the food handlers had poor food handling knowledge and practices that is a potential threat to the development of large number of food borne illness.

It is a serious matter of concern for human health that there is such a high concentration of MDR bacteria present in hospital cafeteria fast foods along with poor food handling knowledge and practices. MDR infections could result in higher rates of morbidity and mortality, the need for expensive medications, and the requirement for lengthy hospital stays. The accomplishment of the Sustainable Development Goals (SDGs) may be jeopardized by the existence of these microbiological food hazards in hospital cafeteria fast foods.

Chapter 5: Conclusion

5.0 Conclusion

The present study demonstrated the prevalence of different multi-drug resistant bacterial species from fast foods of selected hospital cafeterias of Dhaka in Bangladesh. A higher level of diversity among the isolates from fast food samples was observed through RAPD-PCR and gene sequencing results. A huge proportion of isolates were multi-drug resistant (MDR) as revealed by antibiotic resistance profile. Presence of different virulence and antibiotic resistance genes displayed the pathogenic properties of the isolates. A sign of unnecessary and inappropriate use of antibiotics in hospitals is evidenced by the prevalence of MDR isolates along with the prevalence of various antibiotic resistance genes. The cafeterias ought to be separated from the primary treatment structure, and food handlers need to receive the proper training in food handling. The present study provides valuable insights into the characteristics of microbial flora associated with hospital cafeteria fast foods, their multi-drug resistance pattern, different potential virulence and antibiotic resistance genes and thereby facilitate adopting effective measures to minimize foodborne pathogen transmission in Bangladesh and thus contribute the achievement of Sustainable Development Goals (SDGs) especially SDG 3. However, further research with more hospital cafeterias along with different geographical region should be done to understand better the antimicrobial resistance, prevalence of different virulence and resistance genes as well as the pathogenic properties of the isolates from hospital cafeteria fast foods.

Chapter 6:
Reference

6.0 References

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Chapter 7:
Appendices

Appendix-I

Unless otherwise mentioned, all media were sterilized by autoclaving at 121° C for 15 minutes at 15 lbs pressure. Distilled water was used for preparation of all media. The media used in this thesis have been given below:

1. **Plate Count Agar (M091A) (Himedia, India)**

Ingredients	**Amount(g/L)**
Casein enzyme rich hydrolysate	5.000
Yeast extract	2.500
Dextrose	1.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

2. **MacKonkey Agar (M081B) (Himedia, India)**

Ingredients	**Amount(g/L)**
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Agar	13.500
Crystal Violet	0.001
pH after sterilization(at 25°C)	7.1±0.2

3. **Eosin Methylene Blue (EMB) Agar (M317) (Himedia, India)**

Ingredients	**Amount(g/L)**
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin-Y	0.400
Methylene blue	0.065
Agar	13.500
Final P^H (25°C)	7.2±0.2

4. Salmonella-Shigella (SS) Agar (Modified M1032) (Himedia, India)

Ingredients	**Amount(g/L)**
Beef extract	5.000
Peptic digest of animal tissue	5.000
Lactose	10.000
Bile salts mixture	5.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0/025
Agar	12.000
Final P^H (25°C)	7.2±0.2

- SS was not autoclaved or overheated

5. Thiosulphate Citrate Bile-salt Sucrose (TCBS Agar) (M189) (Himedia, India)

Ingredients	**Amount(g/L)**
Proteose peptone	10.000
Yeast extract	5.000
Sodium thiosulphate	10.000
Sodium citrate	10.000
Oxgall	8.000
Sucrose	20.000
Sodium Chloride	10.000
Ferric citrate	1.000
Bromothymol blue	0/040
Thymol blue	0.040
Agar	15.000
Final P^H (25°C)	8.6±0.2

- TCBS was not autoclaved or overheated

6. Cooked Meat Medium (R.C. Medium) M149

Ingredients	**Amount(g/L)**
Beef heart, solids	98.000
Proteose peptone	20.000
Dextrose	2.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

7. Nutrient Agar (M001) (Himedia, India)

Ingredients	**Amount (g/L)**
Peptic digest of animal tissue	5.0000
Sodium Chloride	5.0000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
pH	7.4±0.2

8. Nutrient Broth (OXOID)

Ingredients	**Amount (g/L)**
"Lab lemco" powder	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0
pH	7.4±0.2

9. Muller Hinton Agar (MHA) (OXOID)

Ingredients	**Amount (g/L)**
Beef dehydrated infusion form	300.0
Caseinin hydrolysate	17.5
Starch	1.5
Agar	17.0
pH	7.3±0.1 at 25˚C

10. Muller Hinton Broth (MHB) (OXOID)

Ingredients	**Amount (g/L)**
Beef dehydrated infusion form	300.0
Casein hydrolysate	17.5
Starch	1.5
pH (at 25˚C)	7.3±0.1

11. Simmon's Citrate Agar (Simmons', 1926, Modified)

Ingredients	**Amount (g/L)**
NaCl	5.0
$MgSO_4$	0.2
NH_4PO_4	1.0
K_2HPO_4	1.0
Sodium citrate	2.0
Agar	15.0
pH	6.8±0.2

12. Mannitol Salt Agar (MSA) (OXOID)

Ingredients	**Amount (g/L)**
Sodium Chloride	**75.0**
D-Mannitol	**10.0**
Phenol Red	**0.025**
Agar	**15.0**

13. Blood Agar Base (OXOID)

Ingredients	**Amount (g/L)**
Peptone	**10.0**
Tryptose	**10.0**
Sodium Chloride	**5.0**
Agar	**15.0**

14. Voges-Proskaur (VP), Methyl Red (MR) broth

Ingredients	**Amount (g/L)**
Buffered peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0
pH	6.9±0.2

15. Kligler Iron Agar (Oxoid)

Ingredients	Gram / Litre
Beef extract	3.000
Yeast extract	3.000
Peptic digest of animal tissue	15.000
Sodium chloride	5.000
Lactose	10.000
Proteose peptone	5.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH (at 25°C)	7.4±0.2
Lactose	10.00
Dextrose	1.00
Sodium Thiosulfate	0.50
Bacteriological Agar	15.00

16. Motility Indole Urease (MIU)

Peptone (rich in tryptophan)	3.0 g
KH2PO4	1.0 g
Agar	5.0 g
Phenol red (alcoholic solution)	2.0 g
Distilled water	1000 ml

Appendix-II

Solutions and Reagents used

Preparations of the stock solutions used in this work are given below: (all the working solutions used in this work were prepared from the stock solutions).

Peptone Water

Ingredients	**Amount (g/L)**
Total Nitrogen	≥10.0%
Undigested Protein	Negative
Ash	≤15.0%
Proteases	Slightly positive
Loss of drying	≤5.0%
pH (2% solution)	6.5- 7.5
Nitrites	Negative

TAE buffer

242 g of tris-base, 57.1 ml of glacial acetic acid, 100 ml of 0.5 M EDTA (pH 8.0) was taken and distilled water was added to the mixture to make 1L. 1X concentrated TAE buffer was made by adding 10 ml 50X TAE buffer with 490 ml distilled water and stored at RT.

Ethidium bromide solution

10 µl of ethidium bromide was dissolved in 100 ml TAE buffer to make a final concentration of 20 mg/ml and stored at 4°C in the dark.

0.5 M EDTA

186.1 g of $Na_2EDTA.2H_2O$ and 20.0 g of NaOH pellets were added and dissolved by stirring to 800 ml distilled water on a magnetic stirrer. The pH was adjusted to 8.0 with a few drops of 10 M NaOH and final volume was made up to 1L with distilled water. The solution was sterilized by autoclaving and stored at RT.

Indole Reagent

Ingredients	**Gram/Litre**
Paradimethyl amino benzaldehyde	5.0 ml
Isoamyl alcohol	75.0 ml
Hydrochloric acid (concentrated)	25.0 ml

Kovac's reagent

Ingredients	Amount
p-dimethaylamino benzaldehyde	0.5 g/L
Amyl alcohol	75 ml/L
Hydrochloric acid, concentrated	25 ml/L
Distilled water	Up to 1 L

Methyl Red indicator

Ingredients	Amount (g/L)
Methyl Red	0.1 g
Ethyl alcohol	300 ml
Distilled water	500 g

Gel loading buffer

Ingredients	Amount (g/L)
Sucrose	6.7
Bromophenol blue	0.04
Distilled water	Up to 1 L

*Stored at 4°C

Appendix III

Instruments & Apparatus

The important instrument and apparatus used through the study are listed below:

ABI Prism 3130 Genetic Analyzer	Applied Biosystem, USA
AlphaImager HP System Versatile Gel Imaging	Cell Bioscience, USA
Autoclave, Model no: HL-42AE	Hirayama corp, Japan
Microcentrifuge (temperature controlled)	Sigma, USA
Class II Microbiological safety cabinet	Nuaire, USA
Electric balance, Scout, SC4010	Shimadzu, Japan
Freezer (-30°C)	Liebherr, Germany
Horizontal gel elctrophoresis apparatus Hl-SET	CBS Scientific, UK
Incubator	Japan
Microcentrifuge	Mikro20, Germany
Microcentrifuge tube	Eppendorf, Germany
Micropipettes	Eppendorf, Germany
Microwave oven, Model: D90N30 ATP	Butterfly, China
NanoDrop 2000	Thermo Scientific, USA
PCR Thermocycler	Biometra , Germany; Veritin96 well Thermal Cycler, USA
pH meter, Model no: MP220	Eppendorf, Germany
Power pack	Toledo, Germany
Refrigerator (4°C)	Vest frost
Room temperature horizontal shaker	Gerhardt, Germany
-80^{0} C Freezer	Nuaire, USA
Maxwell^{R}16 Instrument	Promega, USA
Sterilizer, Model no: NDS-600D	Japan
Water bath, Model: SUM	England

Appendix-IV

Questionnaire:

Assessment of food safety knowledge and food handling practices of food handlers of selected hospital cafeterias in Dhaka city, Bangladesh

Name of the hospital:
Type: Governmental/Non-governmental

Respondent's information:

Name:	Age: Sex:
Educational level:	Monthly income:
Marital status:	Work Experience:
Training acceptance:	

Food Preparation and serving

1. When do you prepare the food? ----------am/pm
2. When you start serving the food & when you close?
3. What do you do with the left over? ------Throw out----------Serve next day
4. Do you preheat food before serving? ----------Yes----------No. If yes what is the procedure for reheating?
5. How long leftover foods are heated? _____ reheating is not necessary _____ just until they are hot _____at room temperature

Kitchen condition:

6. Is the floor clean at time of visit? _____ Yes _____No
7. Floor status: _____In good condition _____Average condition _____Bad condition
8. Is the wall free from visible dust, soot, dirt or spider web? ____Yes ____No
9. Does the kitchen space serve for other additional purpose? _____Yes (specify) _____No

Lighting and ventilation

10. Is the kitchen provided with adequate lighting systems? ____Yes ____No
11. Is the kitchen provided with adequate ventilation systems? ____Yes ____No
12. Is the serving space well ventilated or with AC?

Kitchen equipment's and food handlers

13. Are the equipment's kept clean and free from visible dirt and filth? ____Yes ____No
14. Are equipment's easily cleanable? ____Yes ____No
15. Is there basin for washing utensils used for food preparation? ____Yes ____No
16. Cleanness of the basin and its surrounding area: _____kept _____not kept
17. Modes of cleaning and sanitizing of utensils:

_____ Hot and cold water and detergent used for cleaning
_____Only cold water with detergent used
_____ Only hot and cold water used

_____ Only cold water used
_____Only local soap and cold water used
_____Sanitization of equipment's & utensils socked in sedex

Knowledge statements regarding the knowledge of the food handlers

18. Have you heard about food borne disease before? ----------Yes-------No
19. Source of information about food borne disease----Health center-----Training------Media----School education
20. Knowledge about route of transmission of food borne disease------Contaminated food--------Contaminated water------Vector
21. Knowledge about cause of food contamination of food borne disease-----Dirty utensils-------Dirty work environment------Dirty hands---------Diseased food handler----Do not know
22. Healthy people can cause illness by carrying germs to food-----Yes------No
23. Reheating foods contributes to bacterial food contamination-------Yes--------No
24. Contact between raw and cooked foods contributes to food contamination----Yes-----No
25. Cleaning and sanitizing utensils reduces the risk of food contamination-----Yes------No
26. Refrigeration and freezing do not destroy most bacteria-------Yes-------No
27. Using gloves to handle raw foods reduces the risk of food contamination----yes-----No
28. Use of jewels such as rings, watches wearing in food handling cause food contamination-----Yes------No

Food handling practices of the food handlers

29. Do all food handlers wear appropriate clothes, caps, gloves? ____Yes ____No
30. Do all food handlers wear apron? ------Yes------No
31. Are food handlers' clothing clean? ____Yes ____No
32. Are food handlers' nails short trimmed and clean? ____Yes ____No
33. Do food handlers have discharges from nose and eye and cough during visit? ____Yes ____No
34. Is any kind of visible skin rash, boil, cut and wound observed at time of visit? ____Yes ____No
35. If any visible cut and wound has been observed, is it:
_____Plastered with water impermeable bandage _____ openly left _____Other (specify)
36. Do handlers wear any type of jewelry at time of visit? ____Yes ____No
37. Do the handlers wash their hands before any event? ---------Yes------No
38. Do the food handlers check the expiry date of the ingredients? ------Yes----No
39. Do managers supervise workers on their normal work? ____Yes ____No
40. Is cooked food handled properly in kitchen/ kept in sealed conditions to prevent access to insect and environment? ____Yes ____No

Waste management

41. Are appropriate refuse receptacles present in the kitchen? ____Yes ____No
42. Are the receptacles properly covered and tight? ____Yes ____No
43. Are the receptacles overfilled at the time of visit? ____Yes ____No
44. Are the refuse transported to final disposal before over filing? ____Yes ____No
45. Final disposal of the refuse is: _____ Supplied to municipal service _____Burnt at site (open burn)
_____Disposed on street or in o rivers _____Other (specify)
46. Is there a drainage system for collection and handling of liquid waste? ____Yes ____No

Storage and refrigeration

47. Is a refrigerator available for storage of perishable foods? ____Yes ____No
48. Are highly perishable and non-perishable foods stored together? ____Yes ____No
49. Is the refrigerator over filled in such a way that it limits circulation of air? ____Yes ____No
50. Storage of cooked foods and raw foods:
_____Separate refrigerators for raw and cooked foods.
_____Same refrigerator (cooked food in different comportment).
_____Same refrigerator (raw and cooked side by side).
_____Other (specify)
51. What is the reading of temperature at time of visit? _____
52. Is there a separate storage room? ____Yes ____No
53. If yes, type of floor: _____ Concrete/ cement _____ Plastered _____Bricks _____Wooden _____ Earthen _____ other (specify)
54. Is the storage room free from moisture and dust? ____Yes ____No

Sanitary facilities and water supply

55. Source of the water: _____ privately installed from municipal supply
_____from communal distribution _____buy from privately installed pipe
_____others (specify)
56. Type of toilet: _____ flush type _____dry pit latrine _____other (specify) _____no latrine
57. Is the latrine clean &comfortable to use at time of visit? ____Yes ____No
58. Fly infestation at time of visit? ____Yes ____No
59. Is hand wash basin provided to use after toilet near toilet? ____Yes ____No
60. Location of the toilet ----------Near----------Far.

Cloak room
61. Is there separate room for clothing, resting and placing of clothes for workers? __Yes ___No

CONSENT FORM

Purpose of the research:

Institute of Nutrition and Food Science (INFS), University of Dhaka is going to do a research work by collecting cafeteria fast foods from government and non-government hospitals and test them in laboratory to check their quality load to increase the awareness of health issues as a public health concern.

Importance of the research:

Many patients come to the hospitals along with their relatives, friends, attendants in hospitals for different biological tests. These people are dependent to uptake foods from the hospital cafeteria, so checking the quality of the foods of the cafeteria is important. That's why this research is going on.

Why participate in this study?:

By this research, everyone can know about the quality level of served fast foods that are consumed from this cafeteria are whether healthy or not!

Expectation from the respondent:

If you are agreed to our proposal of enrolling in this study, I will ask you some questions through questionnaire and take the information, for which approximately ten minutes are needed.

Risk and benefits of the research:

There is no significant risk to participate in this study. I will only collect some information through questionnaire for which I need some time from you.

Privacy, anonymity and confidentiality:

Your given information will be kept strictly confidential. Later on all information will be preserved in computer by password protection. None other than the research staff of this study will not be able to use any information for the protection of the participated respondents. The information collected from this study can be shared with other researchers if needed. But the information taken from you will be maintained by strict confidentiality.

Right not to participate and withdraw:

Your participation in this study is completely voluntary. You have the right to withdraw yourself from this study at any time.

If you are willing to enroll in this study, please give your signature at the specified space below.

Thank you for your cooperation.

_____

_____

Signature of participant Date

_____

_____

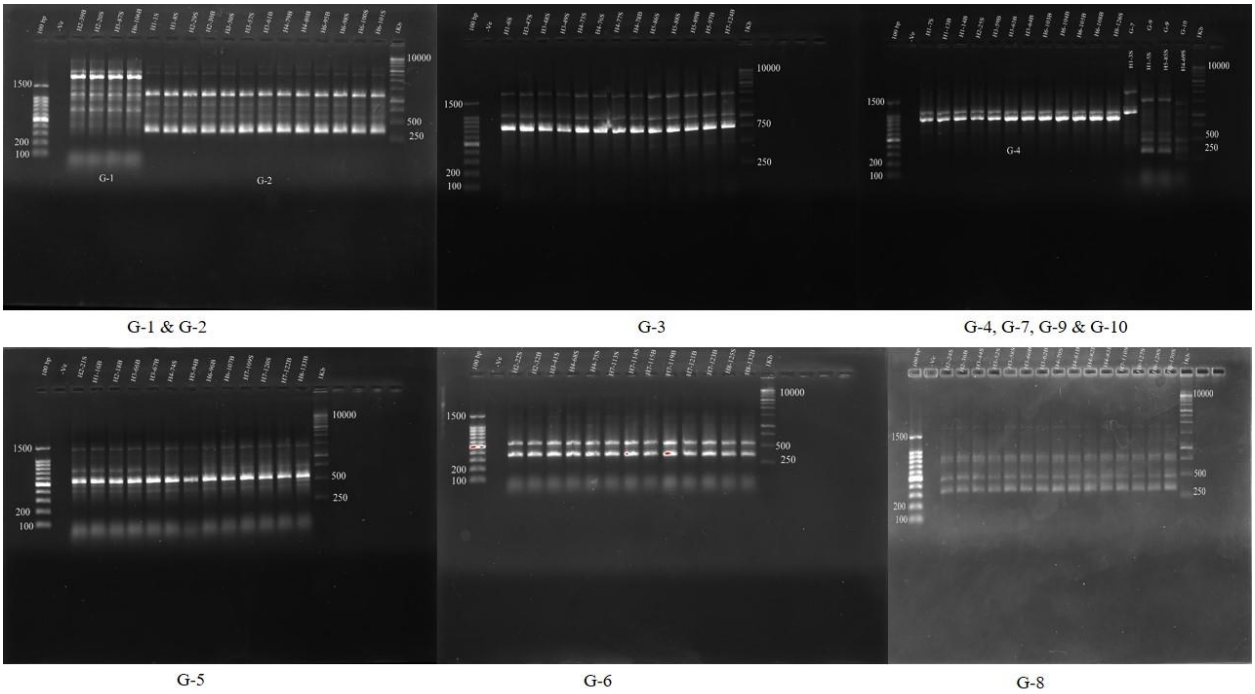
Signature of the interviewer Date

_____

_____

Signature of the PI or his/her representative Date

Appendix V



RAPD gel figure of all Gram-negative isolates. G-1: *Escherichia* spp.*;* G-2, 3*: Enterobacter* spp.*;* G-4, 5,6*: Klebsiella* spp.*;* G-7*: Salmonella sp.;* G-8*: Pseudomonas* spp.*;* G-9*: Citrobacter sp.* and G-10*: Acinetobacter sp.*

Appendix-VI

16S rRNA gene sequences: All the 16S rRNA gene sequences have been submitted to NCBI under accession no. OQ092741 to OQ092758.

***Escherichia coli* strain H2-39B (Accession no. OQ092741)**

GGGTTTAGGTTTTGTTGGTGGGGGGAACCGGGTTCACCAAAGGGGAAGGATCCCTAAGGTGG
TTCTGGAGGGGAAGAACCAGCCCACAACGGAAACTGAGACATCGGTTCCAGAATTCCTACCG
GGAGGGCAGCAGTGGGGAATTTTTGCCCCAAGGGGGGCAAGCCCTGATGCAAGCCATGCCGG
GGGTATGGAAGAAGGCCTTTCGGGTTGTTAAGTACTTTTCAGCGGGGGAGGAAGGGAGTAAA
GTTAATACCTTTGTTCATTGACGTTACCCGCAGAAGAAGCAACGGGCTAACTCCGTGCCAGCA
GCCGCGGTTATTACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAG
GCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGC
AAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCT
GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAG
GTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACG
GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAAGTTTCCAGAGATG
GAAAGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGG
GAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA
TGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCG
CGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCC
ATGAAGTCGGAATCGGTTGTAGTCGTGG

***Leclercia* sp. strain H3-50S (Accession no. OQ092742)**

TCGAAGGTACACAGAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGG
GAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCA
AGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAG
TAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACT
TTCAGCGAGGAGGAAGGCGTTGAGGTTAATAACCGCAGTGATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAAT
TACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTCAAC
CTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTG
TAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAA
AGACTGACGCTCAGGTGCGAAAGCGTGTGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCTAATGACTTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTGATTTCTATGCAACGCGCAGAACCTTACCTACTCTTGACA
TCCAGAGAACTTGCCAGAGATGGCTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGG
CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTGTATCC
TTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGG
TGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCG
CATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGG
ATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCT
ACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTGCAAA
AGAAGTAGGTAGCTTAACCTTCGCGAGGGCGC

Enterobacter sp. strain H3-61B (accession no. OQ092743)

GCAGCCCATGCCGCGTGTATGAAGAAGGCCTTTCGGGTTGTAAAGTACTTTCAGCGGGGAGG
AAGGCGATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACT
CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
TCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
TCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGG
TTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAA
GCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGTGTGCAAAAGAAGTATTAGCTT
A

Enterobacter sp. strain H4-79B (Accession no. OQ092744)

TATGCCCCAATGGGCCGCAAGCCCCAACACACCCATGTCGGTGTATAAGAAGAGGGCTTTAC
GGATTGAAAATTACTCTCAGCGGGGAGGAAGGCGGTGAGGTTTAATAACCTTAAGCAATTGA
CGTTACCCGCAGAAGAAGCACTGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGCGTG
CAAGCGTTAATCGGAATTACTGAGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTG
ACATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGC
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCTCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGC
TTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAA
TGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGA
ACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCT
GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGT
GATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACA
CACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAA
AGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAAT
CGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT
GGGAGTGGGTTGCAAAAGAAGTAGGTAG

Enterobacter cloacae strain H4-77S (Accession no. OQ092745)

ACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGG
AAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGC
CATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGAT
CCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGT
ATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAA
CCGCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGT
CAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTGGA
GTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCC
CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAG
GTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
TGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGC
CTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAA
GGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC

GACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAA
GCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCG
GAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACACCATGGGAGT

***Enterobacter sp*. strain H5-86S (Accession no. OQ092746)**

GCTTGATGCTTAGCTGACTAGTGGCGGACGGGTGAGTAATGACTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGG
ACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGG
CTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGAT
GCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA
GGCGATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC
GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGC
GCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTC
GAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTACAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGT
GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCG
ACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGC
AGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTA
GGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAG
TCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCG
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTC
GACTCCATGAAGTCGGAATCGCTTGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGTTGTAGCAAAAGAAG

***Klebsiella sp*. strain H1-7S (Accession no. OQ092747)**

GGGAATATTTGCCCAATGGGCGCAAGCCTGAATGCAGCCCAGCCGGCGTGTATGAAGAAGGC
CTTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATT
GACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGG
TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAG
GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGAT
TAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTG
GCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCA
AATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAA
GAACCTTACCTGGTCTTGACATCCACAGAACTTAGCAGAGATGGTTTGGTGCCTTCGGGAACT
GTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCA
GTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTA
CACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATA
AAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA
ATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ATGGGAGTGGGTGCAAAAGAAGTAGGTAGCT

***Klebsiella pneumoniae* strain H3-59B (accession no. OQ092748)**

TGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATAC
CGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAG
AGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTC
GGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGACG
TTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA

AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAA
ATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC
GGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTC
CGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACC
TTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAG
ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGAT
AAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACAC
GTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGT
ATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGT
AGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT

***Klebsiella pneumoniae* strain H2-21S (accession no. OQ092749)**

TCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCT
GGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCG
CAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCT
AGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGC
CACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCA
CTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCA
ACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGG
TGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
CGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGT
TAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGA
CATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC
CTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGATCTGCCAGTGATAAACTGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGG
CATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCG
GATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGC
TACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGTGTGCAA
AAGAAGTAGGTAGCTTAAC

***Klebsiella pneumoniae* strain H5-94B (Accession no. OQ092750)**

GTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTC
TGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTC
GCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGC
TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAG
CCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGC
ACTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGG
AATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTC
AACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAG
GTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGAC
AAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACTATGTCGATTTGGAGGTTGTGCCCATGAGGCGTGGCTTCCGGAGCTAACGC
GTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTT
GACATCCACAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGC
ATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTA
TCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGA
AGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATG

GCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCC
GGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATG
CTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGTTTGCAA
AATAAGTAGGTAGCTAACCT

***Klebsiella pneumoniae* strain H2-22S (Accession no. OQ092751)**

ACCTTCGGGCCTCCAGCCCTTCAGAATTGCCCCAGATGGGATTAGGTAGTAGGTGGGGTAAC
GGCTCACCTAGGCGACGATCCCTAGTTGGTTTGAGAGGATGACCAGCCACACTGGAACTGAG
ACACGGTCCAGATTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
ATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGG
AAGGCGTTAAGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACT
CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
TCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
TCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGG
TTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAA
GCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTC
CCGGGCCTTGTACACACCGCCCGTCACG

***Klebsiella pneumoniae* strain H7-113S (accession no. OQ092752)**

GAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGT
AGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTG
CCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAA
AGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGG
GGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGA
ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
GGGAGGAAGGCGGTGAGGTTAATAACCTCATCGATTGACGTTACCCGCAGAAGAAGCACCGG
CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAA
CTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGA
CGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA
ACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAG
AACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCC
AGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATG
ACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATACAAA
GAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTC
TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAG
GTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAAC
AAGGTAACC

***Salmonella enterica* strain H1-3S (Accession no. OQ092753)**

CTTGCGGCTTAGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGA
CCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTTGTTGGTGAGGTAACGGCT
CACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC
AGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGG
TGTTGTGGTTAATAACCACAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGT
GCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGC
ACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGA
AACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTA
GAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTG
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTA
CTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCC
AGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATT
AGGTCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGC
GACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACT
CGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTGCAAAAGAAGTAG

***Stutzerimonas stutzeri* strain H3-60B (Accession no. OQ092754)**

CCAAATTCCTACGGGGAGGCAGCAGTGGGGAAATATTGGACAATGGGCGAAAAGCCTGATTC
CCAGCCATGCCGGCGTGTGTGAAGAAAGTCTTTCGGATTGTAAAGCACTTTAAGTTGGGAGG
AAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTT
CGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG
CGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATC
CAAAACTGGCGAGCTAGAGTATGGCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGC
GTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAATACTGACACTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGT
CGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTG
GGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCAC
GTTAAGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
CAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGT
TGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCA
ACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGTTT

***Pseudomonas sp*. strain H4-81B (Accession no. OQ092755)**

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCG
GATGAGTGGAGCTTGCTCCATGATTCAGCGGCGGACGGGTGAGTAATACCTAGGAATCTGCC
TGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAG
TGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGT
AAAGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG
CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGG
AGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTA
ACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTG
CATCCAAAACTGGCGAGCTAGAGTATGGCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAA
ATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAATACTGACACT
GAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
ATGTCGACTAGCCGTTGGGATCCTTGAGATCTTA

GTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAA
ATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG
AACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
TGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAA
CGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTAAGGTGGGCACTCTAAGGAGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTA
CACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATA
AAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTA
ATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCTTCGGGGGGACGGTTACCACGGAGT
GATTCATGACTGGGGTGAAGTCGTAACAAGG

Pseudomonas sp. strain H7-110S (Accession no. OQ092756)

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGG
TAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCT
GGTAGTGGGGGATAACGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGC
AGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGT
AATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG
CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGG
AGGAAGGGTTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTA
ACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTG
CATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAA
ATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTAATACTGACACT
GAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
ATGTCAACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTAACGCATTAAGTTGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAA
CTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAG
CACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
CGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAGAG
GGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCT
GCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGC
TAGTCTAACCTTCGGGAGGACGGTTACCACGGTGTGATTCATGACTGGGGTGAAGTCGTAAC
AAGG

Citrobacter sp. strain H1-5S (Accession no. OQ092757)

AGTGGCGGACGGGTGATAATGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT
CGGATGTGCCCAGATGGGATTAGCTTGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCC
TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATG
AAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGTGGTTAATAACCG
CAGCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAA
GTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTGGCAGGCTAGAGTC
TTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTG
AGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTA
AAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCA
ACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTC
GGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAG
TCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAG
ACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGT
AGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGG
ACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATC

GCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGGGAGTG

Acinetobacter baumannii strain H4-69S (Accession no. OQ092758)

GGACAACATCTCGAAAGGGATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCT
TCGGACCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTA
CCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAG
CCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTA
CTTTAGTTAATACCTAGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGC
CAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGTGC
GTAGGCGGCTTATTAAGTCGGATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATAC
TGGTGAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAG
ATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAATACTGACGCTGAGGTACGAA
AGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAG
CCGTTGGAGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGT
ACGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATACTAGAAACTTTCCAGAG
ATGGATTGGTGCCTTCGGGAATCTAGATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTACTTGCCAGCATTTCGGAT
GGGAACTTTAAGGATACTGCCAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCAT
CATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCTACAC
AGCGATGTGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGGAGTCTGCAACTCGACT
CCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATTCGTTCCCGGGC
CTTGTACACACCGCCCGTCACACCA

Appendix-VII

16S rRNA gene sequences of Gram-positive bacteria: All the 16S rRNA gene sequences have been submitted to NCBI under accession no. OR486967 to OR486969.

***Sporosarcina sp*. strain H3-46S (Accession no. OR486967)**

AGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCTACAGATGGGGATAACTCCGGGAAACCGGG
GCTAATACCGAATAATCAGTTTGTCCGCATGGACAAACTCTGAAAGACGGTTTCGGCTGTCACTGTAGGA
TGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCCTACCAAGGCAACGATGCGTAGCCGACCTG
AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
CTTCCACAATGGACGAAAGTCTGATGGAGCAATGCCGCGTGAGCGAAGAAGGTTTTCGGATCGTAAAGC
TCTGTTGTAAGGGAAGAACACGTACGGGAGTAACTGCCCGTGCCATGACGGTACCTTATTAGAAAGCCA
CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTA
AAGCGCGCGCAGGCGGTTCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAA
ACTGGAGAACTTGAGTACAGAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGT
GGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAAGGGGGTTTCC
GCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCA
AAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTT
ACCAGGTCTTGACATCCCACTGACCGGTGTAGAGATACGCCTTTCCCTTCGGGGACAGTGGTGACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATC
TTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGATACAGAGGGTTGCC
AACCCGCGAGGGGGAGCCAATCCCATAAAATCATTCCCAGTTCGGATTGGAGGCTGCAACTCGCCTCCA
TGAAGCCGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCTGGTCTTGTACACAC
CGCCCGTCACACCACGAGAG

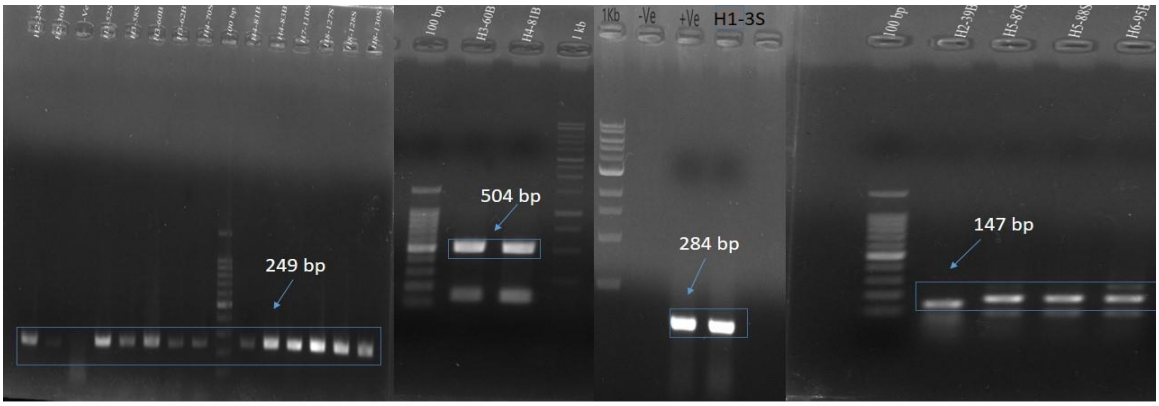
***Chryseomicrobium sp.* strain H3-65B (Accession no. OR486968)**

CGAAAGTTCGATGGAGCAACGCCGCGTGAGGGAAGAAGGTTCTCGGATCGTAAAACTCTGTTGTGAGGG
AAGAACAAGTACCGGAGTAACTGCCGGTACCTTGACGGTACCTCATTAGAAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATCATTGGGCGTAAAGCGCGCGCAGG
CGGTCCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTG
AGTGCAGAAGAGGAAAGCGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAG
TGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCA
GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC
CCGCCGACCGCCCAGGAGACTGGGCCTTCCCTTCGGGGACGGCGGTGACAGGTGGTGCATGGTTGTCGT
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATT
CAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCAT
GCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCGAACCCGCGAGGGGG
AGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGC
TAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAC
G

Bacillus licheniformis strain H8-131S 16S (Accession no. OR486969)

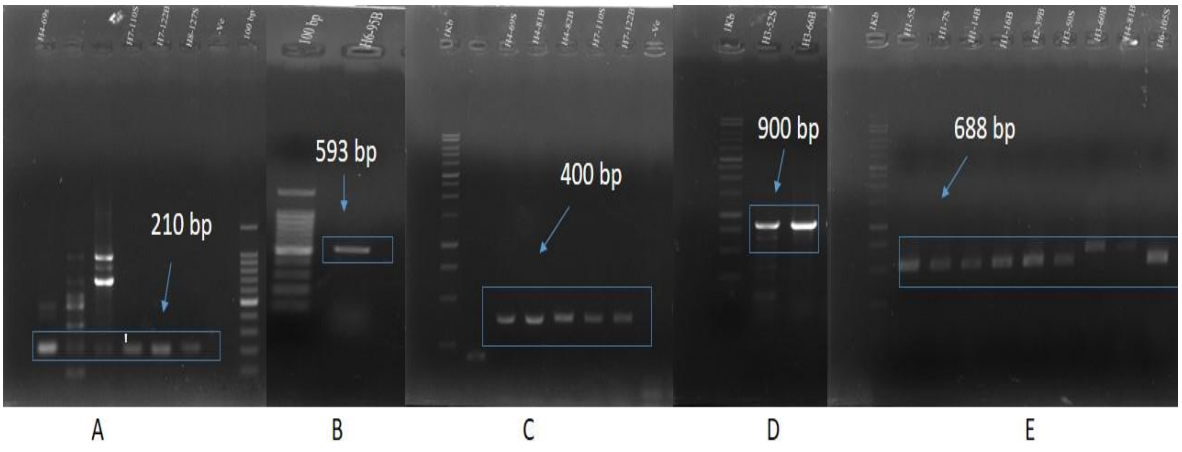
AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACCGACG
GGAGCTTGCTCCCTTAGGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGG
ATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATCATAAAAGGTGG
CTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC
GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGA
AGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGCACCTTGA
CGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCA
ACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGC
GGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG
AGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGC
TAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGG
TCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGGCAACCCTAGAGATAGGGCTTCCCCTTCGG
GGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG
GCAGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCA
GTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACC
TTTTGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACC

Appendix-VIII

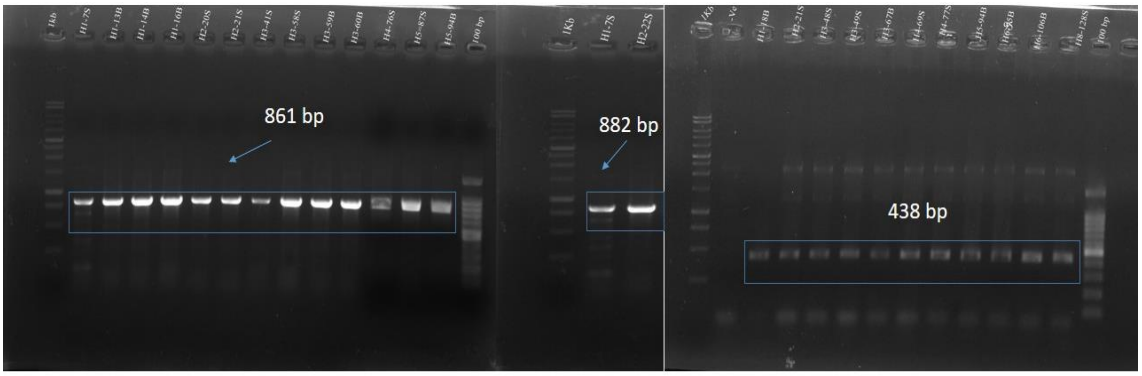


Virulence gene specific PCR results among different bacterial species from fast foods of hospital cafeterias of Dhaka, Bangladesh. Here, (A): *opr*I, 249 bp for *Pseudomonas spp,* lane 3 is negative control, lane 10 is 100bp molecular ladder(Promega, USA).; (B):*opr*L, 504 bp for *Pseudomonas spp.*, lane 1 is 100bp and lane 4 is 1 Kb molecular ladder(Promega, USA).; ; (C): *inv*A, 284 bp for *Salmonella spp.*, lane 2 is negative control,lane 3 is positive control, lane 1 is 1 Kb molecular ladder(Promega, USA).;and (D) *uid*A, 147 bp for *E. Coli* and *Enterobacter spp.*, lane 1 is 100bp molecular ladder(Promega, USA), lane 2 & 3 represents isolates from *E. Coli spp.* and lane 4 and 5 represents isolates from *Enterobacter spp.* .

Appendix-IX



Antibiotic esistance gene specific PCR results among different bacterial species from fast foods of hospital cafeterias of Dhaka, Bangladesh. Here, (A): *gyr*B, 210 bp, lane 8 is 100 bp ladder (Promega, USA), lane 7 is negative control; (B): $bla_{\text{CTX-M}}$, 593 bp, lane 1 is 100 bp ladder (Promega, USA); (C): *qnr*S, 400 bp, lane 1 is 1 Kb ladder (Promega, USA); (D): $bla_{\text{KPC-1}}$, 900 bp, lane 1 is 1 Kb ladder (Promega, USA); (E): $bla_{\text{DIM-1}}$, 688 bp, lane 1 is 1 Kb ladder (Promega, USA);



Antibiotic esistance gene specific PCR results among different bacterial species from fast foods of hospital cafeterias of Dhaka, Bangladesh. Here, (F): *bla*$_{SHV}$, 861 bp , lane 1 is 1 Kb ladder (Promega, USA); (G): *bla*$_{OXA-1}$, 882 bp , lane 1 is 1 Kb ladder (Promega, USA) and (H): *bla*$_{OXA-48}$, 438 bp , lane 1 is 1 Kb ladder (Promega, USA), lane 2 is negative control.

Appendix-X

Virulence and antibiotic resistance gene sequences:

*inv*A, *opr*L, *uid*A, *bla*$_{SHV}$ genes sequences have been submitted to NCBI under accession no. OQ108511, OQ108512, OQ108513, OQ108509 respectively.

Accession no. OQ108511 *Salmonella enterica* strain H1-3S (*inv*A) gene

ATTGGCGATAGCCTGGCGGTGGGTTTTGTTGTCTTCTCTATTGTCACCGTGGTCCAGTTTATCGTT
ATTACCAAAGGTTCAGAACGCGTCGCGGAAGTCGCGGCCCGATTTTCTCTGGATGGTATGCCCGG
TAAACAGATGAGTATTGATGCCGATTTGAAGGCCGGTATTATTGATGCGGATGCCGCGCGCGAAC
GGCGAAGCGTACTGGAAAGGGAAAGCCAGCTTTACGGTTCCTTTGACGGTGCGATGAA

Accession no. OQ108512 *Stutzerimonas stutzeri* strain H3-60B (*opr*L) gene

CTGGCCATGGCTGTTGCTGTCGGTTGTTCGTCCAAAGGCGGCGACGATTCGGGCGAAGGTTCGGG
CGCGATCGATCCGAATGCCGGTTACGGTGCTGATTCGGGCGCCATCGACGGCAGCATGAGCGAAG
AAGCCGCTCTGCGCGCCATCACCACCTTCTACTTCGAGTACGACAGCTCCGACCTGAAGCCGGAA
GCCATGCGCGCTCTGGACGTTCACGCCAAGGACCTGAAAGGCAACGGCGCTCGCGTCGTTCTGGA
AGGCCACACCGACGAGCGTGGTACCCGTGAGTACAACATGGCTCTGGGCGAGCGTCGTTCCAAGG
CCGTTCAGCGCTACCTGGTTCTGCAGGGCGTTTCCCCGGCTCAGCTGGAACTGGTTTCCTACGGC
GAAGAGCGTCCGGTTGCCATGGGCAACGACGAGCAGTCCTGGGCTCAGAACCGTCGCGTC

Accession no. OQ108513 *Escherichia coli* strain H2-39B (*uid*A) gene

TTCTTTAACTATGCCGGGATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTGGAC
GATATCACCGTGGTGACGCATGTCGCGCAAGACTGT

Accession no. OQ108509 *Klebsiella pneumoniae* strain H6-105B (*bla*$_{SHV}$) gene

ATGCGTTATATTCGCCTGTGTATTATCTCCCTGTTAGCCGCCCTGCCGCTGGCGGTACACGCCAGC
CCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTCGGGCCGCGTAGGCATGATAGA
AATGGATCTGGCCAGCGGCCGCACGCTGACCGCCTGGCGCGCCGATGAACGCTTTCCCATGATGA
GCACCTTTAAAGTAGTGCTCTGCGGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTG
GAGCGAAAGATCCACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCT
TGCCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATAACAGCGCCG
CCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCCTTTTTGCGCCAGATCGGC
GACAACGTCACCCGCCTTGACCGCTGGGAAACGGAACTGAATGAGGCGCTTCCCGGCGACGCCCG
CGACACCACTACCCCGGCCAGCATGGCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGA
GCGCCCGTTCGCAACGGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATC
CGCTCCGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGGTGCGC
GCGGCATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTGTGTTAT

Publications during candidature

1. Supriya Ghosh, Sharmin Rumi Alim. Food handler's understanding regarding food handling knowledge and practices and contributing factors to those practices of selected hospital cafeterias in Dhaka, Bangladesh. Int J Health Sci Res. 2022; 12(9):233-243. DOI: https://doi.org/10.52403/ijhsr.20220930.

2. Supriya Ghosh, Humaira Anjume, M. Anwar Hossain, Munawar Sultana and Sharmin Rumi Alim. Prevalence of Gram-Positive Bacteria in Hospital Cafeteria Fast Foods in Dhaka, Bangladesh and their Resistance to Current Antibacterial Agents. Bioresearch Communications, Volume 10, Issue 1, January 2023. (Accepted).

3. Supriya Ghosh, Humaira Anjume, Abu Hasib Lipu, Sharmin Rumi Alim, M. Anwar Hossain, Munawar Sultana. Prevalence and characterization of multi-drug resistant bacteria in hospital cafeteria fast foods of Dhaka, Bangladesh. (Submitted).