Prevalence of Microorganism in Cell Phone and the behavioral pattern associated with the demographic of Dhaka City (North)

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Declaration

I hereby declare that the thesis project titled **"Prevalence of Microorganisms in Cell Phone and the behavioral pattern associated with the demographic of Dhaka City (North)"** has been written and submitted by me, Nasif Bin Saif and has been carried out under the supervision of Kashmery Khan, Lecturer, Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Abstract

The mobile phone is currently the most used technological device used by the population of the planet. Due to its availability and efficiency of usage, it is very popular amongst the people of both developed and developing Countries. But, alongside its benefits, it also is considered one of the main vectors for transmitting pathogenic organisms and being a reservoir of them. With this in mind, the demography of Dhaka City (North) is selected to observe the prevalence of organism amongst the cellphones of the population and isolate and identify a group of potentially disease-causing organism. Total 100 mobile samples included in this study for isolation of bacteria. 92% of the cellphones were found to be contaminated with bacteria and 187 bacterial samples were isolated from the mobile phones. Out of these colonies, we found *Staphylococcus spp* (50.7%), *Bacillus spp* (13.2%), *Micrococcus spp* (11%), *E. coli* (14.1%) and Fecal coliforms (11%). It is found that the participants who used cellphone while eating, using the cellphone in the washroom, sharing the cellphone with other persons and using the cellphone while being sick had more potentially pathogenic microorganisms than the participants who did not. It is found that the participants who daily cleaned their cellphone with any commercially available cleaning agent such as 70% ethanol, hand sanitizer or even commonly available liquid hand wash had no microorganisms present in them. It is found that personal hand hygiene and cellphone hygiene is very important and also washing of hand before and after handling of food and phone decontamination should be adopted by people of Dhaka City to prevent cross and self-contamination by these bacteria. Regular usage of commercially available solvents are also recommended to be used regularly as they are actively capable of removing bacteria from the surface of cellphone and keeping it clean.

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Chapter 1

Introduction

Microorganisms are living things which are found everywhere including the environment and the human body. They are present in major part of the ecosystem. In these environments they live either freely or as parasites (*Sleigh and Timbury, 1998*)

Human body harbor a number of microbes including several species of bacteria, viruses, fungi and protozoa. The sites where bacteria are found include skin (*staphylococci* and bacteroides), Oropharynx (*streptococci*, anaerobes), large intestine (Enteric bacilli) and vagina (lactobacilli) (*Beaugerie and Petit, 2004*).

1.1 Normal flora of human body:

Normal flora refers to the population of microorganisms that reside in the skin and mucous membranes of a healthy normal person without causing any disease (*Jawetz et al., 2007*). They protect us from disease by competing with invaders for space and nutrients, producing bacteriocins which kill harmful bacteria and lowering the pH so that other bacteria can't grow.

Table 1.1: Classification of Normal flora of the human body (*Eckburg et al., 2005*)

Human body	**Normal flora**
Skin	*Staphylococci*, *micrococci*, diptheroids
Oral and upper respiratory tract	*Neisseria*, *Bordetella*, *Corynebacterium*, and *Streptococcus* spp
Conjunctiva	*Haemophilus* and *Staphylococcus*
Gastrointestinal tract	*Enterococci*, non-haemolytic *streptococcus*, *E.coli*, lactobacillus
Genital Tract	*Corynebacterium*, *Lactobacillus* spp, non-pathogenic *Neisseria* spp,

1.2 Factors associated with microbial flora infection:

Normal microbial flora usually doesn't cause infection in body but can cause infection if the following factors are involved:

Individual susceptibility: Important factors influencing acquisition of the infection by microbial flora include; immune status, age, underlying disease and therapeutic interventions (*Ducel et al,*

2002). If the host immunity is impaired then normal flora can cause disease (*Ann M O'Hara, Fergus Shanahan, 2006*). Malnutrition, irradiation, indiscriminate use of antibiotics can lower the patient's immunity thereby making them more vulnerable to the infection (*Ducel et al., 2002*). **Environmental Factors:** Microbial flora may contaminate objects and materials and subsequent contact by a susceptible individual to these objects may come down with an infection. These contaminated objects can easily be picked by mere contact and transferred by many people who fail to follow the basic infection control such as washing of hands (*Ducel et al., 2002*).

1.3 Sources and Mode of transmission of infection:

Microorganisms can be transferred to the host, either directly from the environment or indirectly through an intermediate agent. The reservoir is a site or natural environment in which the pathogen is normally found living and from which infection of the host can occur (*Prescott et al., 1999*). Transmission of infection can be referred to as the movement of pathogens from a source to the appropriate portal of entry. The sources and modes of transmission can be of different ways which include:

a. **Direct contact**: Person-to-person transmission is a form of direct contact transmission. Here the agent is transmitted by physical contact between two individuals. For example, shaking hands. Direct contact can be categorized as vertical, horizontal, or droplet transmission.
b. **Indirect contact**: Indirect contact transmission involves inanimate objects called fomites that become contaminated by pathogens from an infected individual or reservoir. For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or a towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections.

The United State (US) center for disease control (CDC) and prevention stated that contaminated public surfaces most of which are of microorganisms are perhaps the most widespread problem

in the contemporary world and is responsible for about one-third of death worldwide through infections, with adverse effects which can reduce economic productivity (WHO, 2002).

1.4 Transmission of pathogens by hands:

Human hands serve as vectors for the transmission of microorganisms from place to place and from person to person. Human hands usually constitute microorganisms both as part of the body normal flora as well as transient microbes contracted from the environment (*Dodrill et al., 2011*). Although it is nearly impossible for the hand to be free of microorganisms but the presence of pathogenic bacteria on hands may lead to chronic or acute illness. Curtis *et al.* (2003) and Lorna *et al.,* (2005) reported that hands often act as a medium that carries disease-causing pathogens including bacteria and viruses from person to person either through direct contact or indirectly via surfaces. *Al- Ghamdi et al*., (2011) stated that 80% of infections are spread through hands contact with hands or other objects. *Staphylococcus epidermis* is present on almost every hand (*Larson et al., 1992*). Some members of Enterobacteriaceae family also found on hands (Leyden *et al*., 1991; Scott and Bloomfield, 1990). Pathogens that may be present on the hand include *Escherichia coli*, *Salmonella typhi*, *Shigella* spp, *Clostridium perfringes*, *Giardia lamblia*, Norwalk virus and Hepatitis A virus, *Pseudomonas aeruginosa*, *S. aureus*, *Proteus mirabilis, Citrobacter freundii, Enterobacter spp*; *Streptococcus spp, Klebsiella spp*. (*Orskov et al., 1997*).

1.5 Fomites:

Fomites refer to the porous or non-porous surfaces or nonliving objects that when contaminated with pathogenic organisms can transfer them to the new host and act as a medium in transmitting infection (Greene 2009; Cramer, 2013). The fomites include handheld devices like cellphones, tablets, showers, toilet, especially those found in public offices, hospitals, hotels, restaurants and restrooms (*Bright et al., 2010*). These surfaces constitute a major source of spread of infectious diseases. *Presscott et al., 1993* stated that the major source of spread of community-acquired infections are fomites.

1.5.1 Transfer rate of bacteria from fomites to hands:

Transfer rates of microbes to hands are more significant from hard, nonporous surfaces such as stainless steel (*Rheinbaben, Schunermann, Gross, & Wolff, 2000; Rusin et al., 2002*). 40% transfer

rate was evaluated for *Escherichia coli* from a nonporous surface to hands in one study (Scott & Bloomfield, 1990). *Rusin et al.* (2002) observed bacterial transfer rates of 38.5% to 41.8% from an average cellphone and rates of 27.6% to 40.0% from a plastic bodied cellphone to a person's hand with minimal contact times.

1.5.2 Factors associated with bacterial transfer between environmental surfaces:

The factors involved in bacterial transfer between surfaces include:

- The relative humidity or moisture levels
- Bacterial species involved
- The temperature
- The surface materials and properties
- Pressure and friction between the contact surfaces
- Inoculums size on surfaces

1.6 Diseases transmitted by environmental surfaces:

Diseases commonly spread by means of environmental surfaces such as computers, classroom walls, cellphones, chairs, and so on include the common cold, cold sores, conjunctivitis, giardiasis, impetigo, meningitis, pinworm disease, diarrhea and pneumonia (WHO, 1980). Bacteria such as *Escherichia coli*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Staphylococcus aureus* as well as *Corynebacterium diphtheriae* cause diarrhoea, dysentery, pneumonia, skin infections, food poisoning and intoxication as well as whooping cough respectively (FAO, 1989; WHO, 1980). The organism and the diseases that can be transmitted through the use and sharing of cellphones include boil and food borne diseases (*Staphylococcus aureus* and *Escherichia coli),* and diarrhoea *(Escherichia coli, Pseudomonas aeruginosa)* and sore throat (*Streptococcus pyogenes)* (Peleg and Hooper 2010; Schmidt and Brubaker 2004).

1.7 Cellphone and Bacterial Contamination

A mobile phone is a long-range, portable electronic device for personal telecommunication. Aside from the standard voice function of a mobile phone, a mobile phone can support many additional services such as SMS for text messaging, email, pocket switching for access to the internet, and MMS for sending and receiving photos and video. At present, Bangladesh has one of the fastest growth rates of mobile phone subscribers from different parts of the world. The use of mobile phones by individuals may serve as a potential vehicle for the spread of pathogenic microorganisms (*Brady et al., 2006*). A mobile phone can spread infectious diseases by its frequent contact with hands (*Kilic et al., 2009*). Mobile phones are increasingly becoming an important means of communication. The vast majority of mobile phones are handheld (Al-Abdalall, 2010). Today mobiles have become one of the most indispensable accessories of professional and social life. Although they are usually stored in bags or pockets, mobile phones are handled frequently and held close to the face station. Thus, the present study was conducted to determine whether mobile phones play a vital role in the spread of bacterial pathogens and to proffer possible control or preventive measures that could be instituted to avoid this likely vehicle of infections. It is also focused to show the necessity of cleanliness in handling personal objectives like cell phones carefully with either proper cover which would prevent the multiplication of microorganisms both pathogenic and non-pathogenic (Suganya and Judia Harriet Sumathy, 2012) or through the cleaning of the surface using ethanol.

Research has shown that the combination of regular handling and the heat generated by the phones creates a prime breeding ground for all sorts of microorganisms that are normally found in our skin and environment. The human body surface is constantly in contact with environmental microorganisms and becomes readily colonized by certain microbial species. Because of the achievements and benefits of the mobile phones, it is easy to overlook its hazard to health; this is against the background that many users may have to regard for personal hygiene and the number of people who may use the same phone. This constant handling of the phone by different users exposes it to an array of microorganisms and makes it good carrier for microbes living on each square inch of the phone (*Ibrahim et al., 2013*). In hospitals, laboratories or while

in intensive care, mobile phone use often occurs. Although, patients do not have direct contact with these phones, colonized bacteria on the devices may be transmitted to them by healthcare staff. This may cause nosocomial infections if patients' immune system is weak (*Brady et al.,2006; Karabay et al., 2007*). This study was aimed at isolation and characterization of bacteria associated with mobile phones. Most of the time people go to hotels and cafeterias and order food to the waiter for their meal of interest. Then they wash their hand and waiting for foods. Until food come they try to play games, chatting with somebody, calling and picking up calls on their mobile phones. Then as soon as the food comes, they try to eat while assuming mobile phones as a neat thing. Even if during dining time they pick up calls, which is a major condition to contaminate themselves with pathogenic bacteria from mobile phone. Also, some medical laboratory workers who work with those pathogenic organisms; touch their mobile phone with gloves during working and when they finish work, they touch their mobile phone on bare hand. We used to carry mobile phones in our palm, these comes into a direct contact with human body and thus microbes prompting transfer from the skin and hands to face, ears or hair. Therefore, appropriate hand and body hygiene is very important. In Dhaka's closely-knit hovels, accessibility of water is a huge problem. This shows that hand washing and drying could be difficult in different parts of the city. These situations and living conditions lead to the contamination of mobile phones. This pilot study is designed to access the presence of bacteria on the mobile phones of Dhaka city of Bangladesh based on the 21 thanas. Currently, mobile phones are the most popular mobile communication devices for business and personal use. It has become a necessity of everyday life and an indispensable attribute of the modern society which imposes a change in human behavior. This study is carried out to gain insight into the isolation and characterization of bacteria which is found in mobile phone due to poor personal hand hygiene and could be of potential health risk of our society. As this type of research has never been conducted in Bangladesh, this is a very important avenue to gather valuable information regarding how people's behavior and their cellphone results in increased health problems and how to most effectively reduce this problem.

1.8 Hand hygiene programs:

Hand Hygiene applies to hand washing, antiseptic hand wash, antiseptic hand rub, or surgical hand antisepsis (Jasmine, A. and Iyer. H. R., 2013). Hand washing is a fundamental cautionary measure to protect against the transmission of diseases and is one of the primary practices to reduce the transfer of bacteria from person to person, or from person to food contact surfaces (Chinakwe *et al*., 2012). It is established that unwashed hands can transmit pathogens, especially fecal pathogens, to food product after a visit to the toilet. Investigation of foodborne illness showed that poor personal hygiene, primarily ineffective hand washing is an important contributor to foodborne illness (Lambrechts *et al*., 2014).

Contamination by hands or environmental objects due to human involvement harbor microorganisms that increase the risk of illness among students. In order to reduce the risk of bacterial infection from the toilets, regular hand washing and cleaning of toilets with disinfectants are particularly recommended for infection control programs. In view of the problems associated with the level of hygiene in most of the areas in Dhaka city, there was a need to determine the type of microorganisms that are associated with the contact surfaces of the city. This study is expected to highlight the problem of Cellphone contamination and to raise awareness about Phone cleaning programs among the people of Dhaka City.

1.9 Literature Review:

Zakai *et al.,* (2015) ran an investigation to identify both pathogenic and nonpathogenic bacteria on cell phones of 105 medical students at King Abdulaziz University, Jeddah, Saudi Arabia, using standard microbiological methods. Out of 105 cell phones screened, 101 (96.2%) were contaminated with bacteria. Coagulase-negative staphylococci were the most abundant isolates (68%). Seventeen (16.2%) cell phones were found to harbor *Staphylococcus aureus*. Gram-positive bacilli were isolated from 20 (19%) samples. Viridans streptococci and Pantoea species were also isolated but at lower levels. Although most cell phones tested were contaminated with one or more microorganisms, contamination with S. aureus was found in 17 cell phones. The findings indicated that cell phones could act as reservoirs of both pathogenic and nonpathogenic organisms.

Gashaw *et al*., (2014) carried out a search to find out the prevalence of bacteria isolated from mobile phones of health care professionals working in different health centers in Gondar Town,

Ethiopia. A total of 58 health care professional's mobile phones were swabbed before and after decontamination with 70% alcohol and assessed for contamination with bacteria. Among them, about 98.3% of the mobile phones were found to be contaminated with bacteria. A total of 59 bacterial isolates were identified from these mobile phones and from the isolates Gram-positive bacteria accounted for 77.9%, coagulase-negative staphylococci being the most frequently (47.5%) isolated bacteria followed by *Staphylococcus aureus* (27.1%) and *Streptococcus pyogenes* (3.4%). *E. coli* (6.8%) was the most frequently isolated Gram-negative bacteria followed by *Providencia stuartii* (5%). *Enterobacter cloacae, Klebsiella pneumoniae,* and *Citrobacter* species each accounted for 3.4% of the isolates. Decontamination with 70% alcohol significantly decreased the rate of contamination from 98.3% to 55.2%.

Selim *et al*., (2015) investigated the microbial contamination of mobile phones in Alexandria University Students' Hospital, Egypt. Swab samples were collected from 40 mobile phones of patients and healthcare workers and all of the samples were found to be contaminated with either single or mixed bacterial agents. The work revealed that the majority of isolated bacterial contaminants were mixed with more than one organism. It was found that all mobile phones tested from the laboratory (100%) yielded mixed organisms, followed by 90% from dialysis unit and 70% from triage area. On the other hand, 60 % of the tested mobile phones from ICU revealed only one (single) isolate. Of the 4 doctors tested mobile phones, 3 (75%) revealed more than one organism. The corresponding figures for nurses, lab technicians, workers and patients were as follows, 11/16 (69%), 5/5 (100%), 6/7 (86%), 5/8 (63%), respectively. In addition, of the 29 cell phones which were recorded to be cleaned by their owners, 21 (72%) yielded more than one organism. It has been also noted that the majority of individuals enrolled in the present study reported that they perform hand hygiene (HH) practices (37/40), of these 28 (76%) grew more than one organism from their cell phones. As regards isolated organisms in this study, methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 53% of the samples, followed by CoNS (50%), Bacillus (43%), Diphtheroids (30%), methicillin-susceptible *Staphylococcus aureus* (MSSA) (18%), *E. coli* and Viridans streptococci (13% each), Micrococci (10%), *Klebsiella pneumoniae* and ESBL *Klebsiella pneumoniae* (8% each). The least encountered isolates were *Acinetobacter baumanii* and Candida (3% each).

Morubagal *et al*., (2017) conducted a study to isolate and identify different types of bacteria from mobile phones of healthcare workers and non-healthcare workers from various areas in Mysore, Karnataka, India. A total of 175 samples were examined, out of which 125 samples were from

HCWs and 50 samples were from non-HCWs. From 125 HCW's mobile phones, 203 bacteria were isolated. Out of which, 90 (43.68%) were *Staphylococcus* species, [i.e., MSSA 34 (16.64%), MRSA 31 (15.27%), MSCoNS 09 (4.43%), MRCoNS 12 (5.91%), *S. citreus* 04 (1.97%)] as the predominant pathogen, followed by 43 (21.18%) *Acinetobacter baumanii*. Among the mobile phones of HCW's from ICUs, *A. baumannii* (36.84%) was the predominant organism isolated, followed by MRSA (21.05%). Predominant organism isolated from HCW's in Operation Theater was MRSA (46.66%). Among 86 (100%) samples positive for staphylococci, excluding *S. citreus*, 34 (39.53%) were predominantly MSSA. Most of the samples positive for non-fermenters (*A. baumannii* and *P. aeruginosa*) were from HCWs working in ICUs and general wards. Among 25 mobile samples from the Doctors, MRSA (21.95%) was the predominant organism isolated followed by *A. baumannii* (17.07%). Out of 50 non-HCWs mobile phones, 23 (46.00%) samples yielded growth of six different types of bacteria. Out of which, Gram-positive spore bearer 16 (57.14%) was the predominant organism followed by *Acinetobacter baumanni* (14.28%).

Al-Abdalall *et al*., (2010) ran an investigation to determine microbial contamination of mobile phones in the city of Dammam, Saudi Arabia, and identify the most important microbial species associated with these phones. The analysis of a total of 202 samples was done to identify fungal and pathogenic bacteria isolates. There were 737 isolated of the following bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Neisseria sicca, Micrococcus luteus, Proteus mirabilis, Bacillus subtilis*, and *Enterobacter aerogenes* at the rate of 56.58, 13.57, 8.01, 7.73, 6.51, 3.66, 2.85 and 1.09% respectively. There were fungal isolates as follows: *Alternaria alternata, Aspergillus niger, Cladosporium sp., Penicillium spp., Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer, Aspergillus ochraceus* at the rate of 29.07, 26.74, 20.93, 10.47, 6.98, 2.33, 2.33, 1.16%, respectively. The study showed that all mobile phones under consideration were infected by several microbes, most of which belonged to the natural flora of the human body as well as airborne fungi and soil.

Parhizgari *et al.*, (2012) evaluated the existing levels of bacteria on mobile phones of three Jundishapur University medical and teaching hospitals administrative and medical staff in Ahvaz, Iran and also their susceptibility on certain antibiotics. Samples were collected from 170 Health Care Workers' mobile phones in Golestan, Emam Khomeini and Taleghani teaching hospitals. The samples consisted of two groups: group 1) Clinical personnel and group 2) Administrative personnel. In each group, 85 mobile phone were investigated and bacterial isolates were identified. Bacteria were isolated from 154 (90%) of the examined mobile phones: coagulase-negative

Staphylococci (69%), *Bacilli* (20.6%), *Acinetobacter spp*. (6%), *Klebsiella pneumoniae* (1.8%), *Pseudomonas aeruginosa* (1.2%), *Staphylococcus aureus* (1.2%) and *Ecsherishia coli* (0.6%). From 18 isolated pathogenic bacteria, 13 bacteria isolated from group one and five were related to bacteria isolated from group two.

Verma *et al*., (2015) aimed at the isolation and characterization of bacteria from mobile phones of students and employees of the University of Gondar located in Gondar town in Amhara Regional State, Ethiopia to show that mobile phones are a potential reservoir for a number of bacteria. Total 59 mobile samples included in this study for isolation of bacteria and 17 selected colonies of bacteria isolated from mobile phones were further processed. Out of these colonies, they found E. coli, E. aerogenes, Streptococcus spp. and S. aureus in the percentage of 23.53%, 23.53%, 17.65% and 35.30% respectively.

Dave and Shende (2015) conducted a study to find out the common microbial population inhabiting mobile phones in several regions including rural and urban areas of Durg District, in Chhattisgarh, India. The sample size consisted of 194 mobile phones from users in different areas of Durg District. The research findings indicate that *Staphylococcus aureus* (52.7%), *Staphylococcus epidermidis* (17.06%), *Pseudomonas aeruginosa* (12.2), *Micrococcus luteus* (9.1), *Enterobacter aerogenes* (1.8%) and *Bacillus subtilis* (7.07%) are the main bacterial isolates frequently associated with mobile phones. Fungal sps such as *Alternaria sps* (28.0%), *Aspergillus Niger* (32.0%), *Cladosporium sp* (18.7%), *Penicillium spp.* (14.7 %), *Aspergillus flavus* (5.34%), and *Aspergillus fumigates* (1.33%) were isolated as well.

Kotris *et al*., (2016) searched the prevalence of bacteria on mobile phones of Health Care Workers who work in the Intensive Care Unit (ICU) and medical students in Osijek, Croatia. 50 swabs were collected from HCWs who work in the ICU (University Hospital Centre Osijek) and 60 swabs from medical students (School of Medicine, University of Osijek). Out of these 110 processed mobile phones, microorganisms were not detected on 25 (22.7%); 15 (25%) students' and 10 (20%) HCW's mobile phones. The most common isolated microorganisms in both groups were coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus.* From 50 HCW samples, 34 (68%) contained coagulase-negative staphylococci (CoNS), 13 (26%) contained *Staphylococcus aureus*, 4 (8%) contained *Sarcina spp*., 2 (4%) contained *Bacillus spp.*, and 1 (2%) contained *Corynebacterium spp.* However, from the 60 medical students samples, 43 (71.67%) contained coagulase-negative staphylococci (CoNS), 9 (15%) contained *Staphylococcus aureus*, 2 (3.33%) contained *Sarcina spp.*, 2 (4%) contained *Corynebacterium spp.*, and none of the samples

contained *Bacillus spp.*

Tagoe *et al*., (2011) published an article on bacterial contamination of mobile phones after isolating and identifying the bacteria present on 100 mobile phone samples from the students of University of Cape Coast located in Cape Coast, Ghana. All 100 mobile phones sampled were contaminated with varied numbers of bacteria. Nine (9%) had a single bacterial contamination whilst 65% had above 3 bacterial contamination. Bacteria isolates include *Klebsiella pneumonia* (10%), *Citrobacter spp.* (2%), *Staphylococcus aureus* (4%), Coagulase Negative Staphylococci (CONS) (15%), *Pseudomonas aeruginosa* (4%), *Salmonella spp.* (3%), *Shigella spp.* (2%), *Proteus mirabilis* (19%), *Escherichia coli* (8%), *Bacillus cereus* (23%), *Streptococcus pneumoniae* (10%), *Salmonella spp.* (3%) and *Shigella spp.* (2%) with *Bacillus cereus* being the highest (23%) followed by *Proteus mirabilis* (19%), Coagulase Negative Staphylococci (15%). The least organisms sampled were *Citrobacter spp.* and *Shigella spp.* (2%).

Roy *et al*., (2013) conducted a research to isolate and identify the bacteria of public health importance from mobile phones of fish and animal handlers of Kashmir, India. 150 swab samples were collected from mobile phones of veterinarians, students (veterinary sciences), laboratory attendants, shepherds and meat and fish handlers of Kashmir valley for isolation, identification of public health significant bacteria. Out of 150 swab sample examined, 96.66% mobile phones were found to be contaminated with pathogenic bacteria. The mobile phone of animal handlers and veterinary surgeons showed highest and lowest total viable count, respectively. The research findings indicated that *Streptococcus* spp., *Staphylococcus* spp., *Bacillus cereus* and Enterobacteriaceae group of bacteria particularly *Klebsiella* spp., *Proteus* spp. and *E. coli* were the main isolates frequently associated with the mobile phones of laboratory attendants, animal handlers, meat handlers, fish handlers, veterinary surgeons and students of veterinary science. The highest prevalence of *Streptococcus* spp. was observed in laboratory attendants (64%) and lowest in animal handlers (40%) mobile phones. *Staphylococcus* spp. Was frequently isolated from all groups of mobile phone handlers and the highest rate of contamination was recorded in animal and fish handlers (84%), followed by meat handlers (76%), laboratory attendants (72%), veterinary surgeons (68%) and students (60%) mobile phones. Among all the mobile phone users, highest contamination of *E. coli* was isolated from fish handlers (72%) and lowest from veterinary surgeons (4%) mobile phone. Second highest contamination of *E. coli* was found in the mobile phone of animal handlers (64%). *Klebsiella* spp. was isolated from all groups of mobile phone handlers with the highest prevalence in fish handlers (60%) and lowest in veterinary surgeons

(24%) mobile phone. 44% animal handlers, 40% laboratory attendants and 12% veterinary Surgeons' phones were carrying *Proteus* spp. *Bacillus cereus* was predominantly present in the mobile phone of meat handlers (84%) followed by animal handlers (80%), fish handlers (60%), laboratory attendants (48%), veterinary surgeons (20%) and students (12%) mobile phones.

1.10 Aims and objectives:

The aims of this research work carried out at BRAC University were to isolate, identify and evaluate the prevalence of bacterial contaminants from the Cellphones of the demography of Dhaka North and their harmful implication to public health. This study aims to find the correlation between the availability of organisms and if any behavioral traits are responsible for the said organisms to be found in the phones. A Survey is also conducted to gather data on the usage and the behavioral traits of the person the samples are collected from. How much effective the commercially available cleansing solvents which are available in the market against these microorganisms will also be checked and cataloged. Besides the purpose of this study was also to raise awareness about cell phone hygiene and importance of cleaning cell phones among the People of Dhaka North.

On the basis of above context, the objectives of the present study are:

- Isolating the bacterial contaminants present in Random samples collected from the 10 thanas of Dhaka City North.
- Complete a survey form focused on the type of usage and state of the cellphone from which the samples are being collected while taking consent from the owner.
- Identifying and characterizing the bacterial contaminants.
- Determining the prevalence of the isolated organisms.
- Investigating the relationship between the bacterial contaminants present and the behavioral traits of the owner of the cellphone.
- Determining the potency and the combat effectiveness of the commercially available cleaning agents against the bacterial contaminants found in the cell phone.

Chapter 2

Materials and Methods

2.1 Study area:

The study was conducted at the BRAC University in Dhaka, Bangladesh. The laboratory processing, analysis of data and the overall experimental work were done in Biotechnology Research Laboratory of the Department of Mathematics and Natural Sciences of BRAC University.

2.2 Study duration:

The study was conducted during the period September 2017 to March 2018

2.3 Sample size:

A total of about 100 Sample were collected from the 100 mobile samples from the area of the 10 thanas of Dhaka City North is included in this study. The samples were taken from people of all walks of life while taking consideration of their lifestyle, locality, and personal preferences. Cellphone models are also considered as a category. Samples are collected from throughout Dhaka city North, in the number of 10 from each Thana. The number of samples was constant in the area and population of the designated zone.

2.4 Materials:

2.4.1 Equipment:

Equipment that was used in this study include:

- Laminar airflow cabinet (Model-SLF-V, vertical, SAARC group Bangladesh)
- Incubator (Model-0SI-500D, Digi system Laboratory Instruments Inc. Taiwan)
- Vortex machine (Digi system Taiwan, VM-2000)
- Autoclave machine (Model: WIS 20R Daihan Scientific Co. ltd, Korea)
- Centrifuge machine

- Gel apparatus
- Glasswares, Laboratory distillation apparatus- fractional distillatory set up, Microscope, Petri-dishes, Test-tubes, Micro-pipettes, Bunsen burner, Electric balance, etc.

2.4.2 Culture Media:

Culture media used for bacterial isolation and identification include**:**

2.4.2.1 MacConkey Agar:

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particular members of the family Enterobacteriaceae. It also can distinguish between lactose fermenting from non-fermenting bacteria. After 24-48 hours at 37ºC of incubation period, *E.coli* and *Klebsiella* will produce pink colonies. Bacteria which can't ferment lactose like *Pseudomonas aeruginosa*, *Salmonella* species, and *Proteus* species will appear colorless on the medium and the agar surrounding the bacteria remains relatively transparent.

2.4.2.2 Mannitol Salt Agar (MSA):

Mannitol Salt Agar is used as a selective media for the isolation of pathogenic *Staphylococci*. *S.aureus* ferment mannitol and produce yellow-colored colonies surrounded by yellow zones. Nonmannitol fermenters such as *S.epidermidis* will give colorless colonies and the media will remain red. MSA is also used to differentiate between *S.aureus* and *S.epidermidis*.

2.4.2.3 Membrane fecal coliform agar (MFC):

M-FC Agar Base is used for the detection and enumeration of fecal coliforms at a higher temperature (44.5°C). After 24-48 hours incubation Fecal Coliforms will form blue colored colonies whereas non-fecal-coliforms will form gray colored colonies on M-FC Agar Base.

2.4.2.4 Nutrient Agar (NA):

Nutrient Agar is used for the cultivation of microbes supporting the growth of a wide range of non- fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

2.4.2.5 Eosine Methylene Blue Agar (EMB):

This media can differentiate among lactose fermenters and lactose non-fermenters bacteria. In case of lactose fermenters such as *E. coli*, the colonies will be blue/black in color with a metallic green sheen and for lactose non-fermenters colorless, transparent colonies will be obtained. Other coliform such as *Enterobacter aerogenes* can also ferment lactose and grow on EMB media. They will give thick mucoid pink colored colonies.

2.4.2.6 Hi-Crome agar:

This agar media is selective for urine infection-causing microorganisms such as *Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus, Proteus mirabilis, E. coli, Pseudomonas aeruginosa* and they produce distinctive different colors on media. *E. coli* gives pink-purple colonies, *Staphylococcus aureus* gives golden-yellow colonies, *Proteus, Morganella and Providencia give* brown colonies, *Enterococcus faecalis* produce blue colonies, *Klebsiella pneumoniae* produce blue, mucoid colonies and *Pseudomonas* give colorless colonies on Hi-Crome agar after 24-48 hours of incubation.

2.4.2.7 Bacillus cereus Agar (BC Agar):

Bacillus Cereus Agar Base with added supplements is used as a selective medium for the isolation and enumeration of *Bacillus cereus*. Colonies of *Bacillus cereus* give turquoise to peacock blue color surrounded by a good egg yolk precipitate of the same color. Other species of *Bacillus* are also able to grow in BC agar but they will produce green colonies.

2.4.2.8 Blood agar (BA):

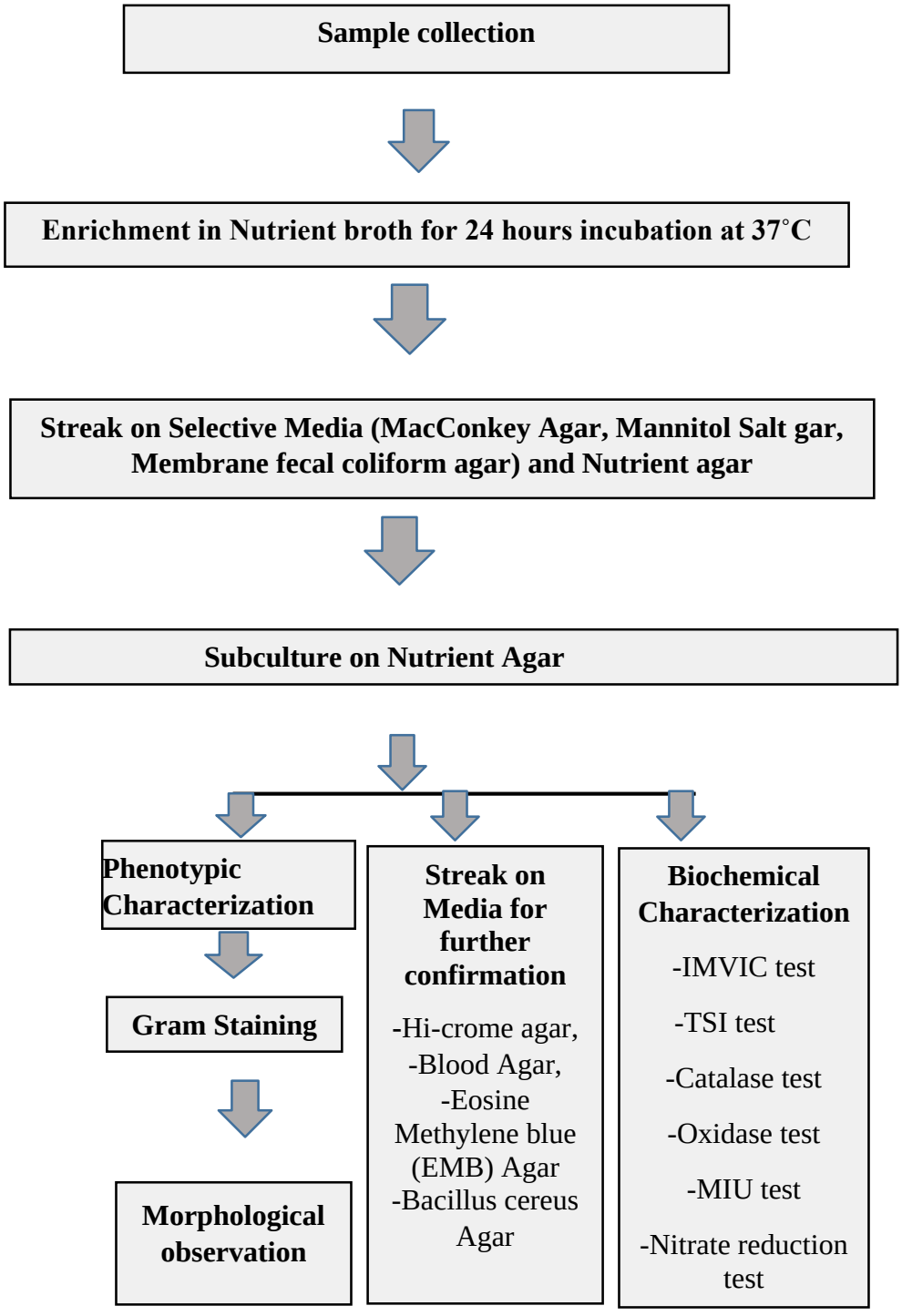
Blood Agar (BA) is an enriched medium used to culture those bacteria or microbes that do not grow easily. Such bacteria are called fastidious as they demand a special, enriched nutritional environment compared to the routine bacteria. Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow such as *Haemophilus influenzae, Streptococcus pneumoniae* and *Neisseria* species. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Aerococcus*. It is used to see the lysis of red blood cells by the organisms. Usually, three types of hemolysis are found including alpha hemolysis, beta hemolysis, and gamma hemolysis. Hemolysis is determined by observing the clear zones around the bacterial growth.

2.4.3 Biochemical test media:

Table: Media used for biochemical tests

Media used for biochemical tests
✓ **Indole broth**
✓ **Methyl Red (MR) broth**
✓ **Voges-Proskauer (VP) broth**
✓ **Simmons citrate agar**
✓ **Triple Sugar Iron (TSI) agar**
✓ **Motility Indole Urease (MIU) agar**
✓ **Nitrate reduction broth**

2.5 flow chart of the overall study design



2.6 Methods:

2.6.1 Sample collection:

Mobile phones of people are randomly sampled by taking written and oral consents from all the participants included in this study. The samples were collected aseptically using sterile cotton-tipped applicators which were immersed in 0.85% sterilized normal saline solution (NSS). All the collected samples are being analyzed and screened in accordance with the previously reported method (Sepehri et al.,2009). The mobile phone is first held with the aid of sterile gloves. Sterile cotton swab moistened with the sterile (0.85%) normal saline solution is rotated over the surface of both sides of the mobile phone.

The cotton swabs are transferred immediately to the laboratory with one hour of collection to prevent dryness. Sampled mobile phone swab was streaked onto nutrient agar. The inoculated plates are then incubated aerobically in an inverted position at 37 °C for 48 hours. The plates are then observed for the presence of isolated colonies and selected colonies were again sub-cultured on nutrient agar in petri-plates to isolate pure culture. After isolating pure cultures, bacterial isolates are further identified and characterized by Gram staining, PEA Agar, Mac-Conkey agar and biochemical tests (Ekrakene and Igeleke, 2007). Biochemical tests are performed on pure culture for final identification of the isolates on the basis of their biochemical reaction.

2.6.2 Sample Analysis:

The collected samples were processed to identify the bacteria in the sample. The following processing techniques were applied:

1. Culture
2. Gram staining
3. Biochemical tests

2.6.2.1 Culture technique:

After 24 hours, each sample was streaked onto Nutrient agar, MacConkey agar, Mannitol salt agar and Membrane fecal coliform agar plates. Here, using the swab stick, a primary streak was made while secondary and tertiary streaks were made from the primary streak in parallel pattern with the aid of a sterilized wire loop to make a four-quadrant streak plate technique. All the plates were incubated for 24 hours at 37ºC. After the overnight incubation, the plates were removed from the incubator and presumptively observed for colony characteristics. Isolated colonies were then subcultured onto fresh nutrient agar. Single isolated colonies from nutrient agar plates were subjected to Gram staining, Spore staining, and Standard Biochemical tests to identify the organism.

2.6.2.1.1 Streak plate method:

Streak plate technique is used for the isolation of pure culture of the organisms from a mixed population. It is necessary to study the colony morphology of an organism to perform the biochemical tests needed to identify the organism.

Materials needed for streak plate method:

- A source of bacteria (stock culture, previously streaked agar plate or any other inoculum)
- Inoculating loop
- Bunsen burner
- Agar plate (Nutrient agar or any other agar medium)

Procedure:

Four quadrant streaking:

1. The inoculating loop is sterilized in the Bunsen burner by putting the loop into the flame until it is red hot. Then the loop is allowed to cool.
2. The inoculating loop is inserted into the test-tube containing bacterial culture and some of the inoculums are taken with the help of the loop.
3. The inoculating loop is streaked immediately very gently over a quarter of the plate using a back and forth motion.
4. The loop is flamed again and is allowed to cool
5. By going back to the edge of the area one which has been just streaked, the streaks are extended into the second quarter of the plate
6. The loop is flamed again and is allowed to cool
7. Going back to the edge of area two which has been just streaked, the streaks are extended into the third quarter of the plate
8. The loop is not burned after streaking the third quadrant of the plate
9. The loop is touched over the surface of the third quadrant and zigzag line is drawn from the third quadrant
10. The loop is flamed and cooled.

Figure 2.1: Four quadrant streaking on EMB agar plate

2.6.2.2 Gram staining:

Gram staining was done for differentiating between two principal groups of bacteria: Gram positive and Gram negative.

- A sterile microscopic glass slide was taken.
- A drop of saline was taken by the loop and added to the slide.
- A colony from fresh culture of the experimented bacteria was taken and was smeared on the glass slide with the saline. Then the smear was heat fixed and was allowed to dry for few minutes.
- One drop of crystal violet was added to the smear and after one minute, the crystal violet was gently washed off the glass slide with the tap water.
- Then one drop of Grams iodine was added and then after one minute the Grams iodine was gently washed off the slide with the tap water.
- Few drops of 70% ethanol were added and were washed immediately.
- One drop of Safranin was added and after 45 seconds it was washed off the glass slide.

- The slide was allowed to dry off completely, after which it was observed under the microscope.

2.6.2.3 Spore staining:

Spore staining was done to determine whether the bacteria was endospore-forming or not.

- A sterile microscopic glass slide was taken.
- A drop of saline was taken by the loop and added to the slide.
- A colony from fresh culture of the experimented bacteria was taken and was smeared on the glass slide with the saline. Then the smear was heat fixed and was allowed to dry for few minutes.
- The slide was placed over a water bath and malachite green was added continuously so that the dye did not dry out.
- The slide was heated for 2 to 3 minutes.
- After heating, the slide was cooled and rinsed thoroughly with tap water.
- Then the smear was stained with safranin for 30 seconds, washed with tap water and blot dried with bibulous paper.
- Finally, the bacterial observation was made under the oil immersion lens (1000X) for the presence of endospores.

2.6.2.4 Biochemical tests:

2.6.2.4.1 Indole test:

Indole production test was done to determine the ability of microorganisms to degrade the amino acid tryptophan by the enzyme tryptophanase.

- For indole test, each indole broth containing 6ml of peptone, sodium chloride was taken.
- Using sterile technique, a small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of loop inoculation method with an inoculating loop
- The tubes were then incubated for 24 hours at 37ºC.
- In order to detect the indole production, 10 drops of Kovacs reagent were added to all the tubes.
- If red reagent layer develops then it indicates indole positive and absence of red color indicates that the substrate tryptophan was not hydrolyzed and it indicates indole negative reaction. (Cappuccino & Sherman, 2005)

2.6.2.4.2 Methyl red (MR) test:

Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of high concentration of acid end products.

- For methyl red test each MR broth containing 5 ml of dipeptone, dextrose and potassium phosphate was taken.
- Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method.
- The tubes were then incubated for 48 hours at 37ºC.
- After 48 hours, 5 drops of methyl red indicator were added to each tube and the color of the tubes was observed.
- If red color develops then it indicates that the organism was capable of fermenting glucose with the production of high concentration of acid.
- If orange or yellow color develops then it indicates methyl red negative result

(Cappuccino & Sherman, 2005).

2.6.2.4.3 Voges-Proskauer (VP) test:

The Voges-Proskauer (VP) test was done to determine if an organism produces acetylmethyl carbinol from glucose fermentation.

- For Voges-Proskauer test each VP broth containing dipeptone, dextrose and potassium phosphate was taken.
- Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method.
- The tubes were then incubated for 48 hours at 37ºC.
- After 48 hours, 10 drops of Barritt's reagent A was added to each tube and the tubes were shaken. Then immediately 10 drops of Barritt's reagent B was added and the tubes were shaken.
- The colour was observed after 15-30 minutes of the reagent addition.
- If red colour developed then it indicates that the organism was capable of fermenting glucose with ultimate production of acetyl methyl carbinol and it indicates positive result.
- If no colour developed then it indicates voges- proskauer negative result. (Cappuccino & Sherman, 2005)

2.6.2.4.4 Citrate utilization test:

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrase.

- For citrate utilization test each vial containing 2.5 ml of simmons citrate agar was taken.
- Using sterile technique, small amount of the experimental bacteria from 24-hours fresh culture was inoculated into the vials by means of a streak inoculation method with an inoculating loop.
- The vials were then incubated at 37ºC for 24-48 hours.
- After 48 hours incubation, if the Prussian blue colour developed then it indicates the citrate positive result which means the organism was capable of fermenting citrate as a sole source of carbon.
- If there was no colour change then it indicates citrate negative result. (Cappuccino & Sherman, 2005)

2.6.2.4.5 Triple sugar-iron (TSI) agar test:

Triple sugar iron agar test was done to differentiate between Gram-negative enteric bacilli based on their ability to ferment carbohydrate and reduce hydrogen sulfide.

- For TSI test each tube containing TSI agar was taken.
- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of stab inoculation method with an inoculating needle.
- The tubes were then incubated at 37˚C for 24-48 hours.
- After 24-48 hours the color of both the butt and slant of agar slant cultures were observed.
- The results were recorded based on the following observation (Cappuccino & Sherman, 2005).

Table 2.1: Interpretation of Triple sugar iron (TSI) test result

Result	Interpretation	Symbol
Yellow slant/yellow butt	Glucose and lactose and/or sucrose fermentation with acid accumulation in slant and butt.	A/A
Red slant/yellow butt	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products (reversion).	K/A
Red slant/red butt	No fermentation. Peptone catabolized aerobically and anaerobically with alkaline products. Not from *Enterobacteriaceae*.	K/K
Red slant/no change in butt	No fermentation. Peptone catabolized aerobically with alkaline products. Not from *Enterobacteriaceae*.	K/NC
No change in slant / no change in butt	Organism is growing slowly or not at all. Not from *Enterobacteriaceae*.	NC/NC
Black precipitate in the agar	Sulfur reduction. (An acid condition, from fermentation of glucose or lactose and/or sucrose, exists in the butt even if the yellow color is obscured by the black precipitate.)	H_2S
Cracks in or lifting of agar	Gas production.	G

2.6.2.4.6 Catalase test:

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase.

- For catalase test a sterile microscopic slide was taken.
- A drop of the catalase reagent 3% Hydrogen peroxide was placed on the glass slide
- Using a sterile inoculating loop, a small number of bacteria from 24-hour pure culture was placed onto the reagent drops of the microscopic slide
- An immediate bubble formation indicated a positive result and no bubble formation indicated catalase negative result (Reiner, 2010).

2.6.2.4.7 Oxidase test:

Oxidase test was done to determine the presence of the enzyme cytochrome oxidase in the bacteria.

- Filter papers were taken, and two drops of oxidase reagent (p-Amino dimethyl aniline oxalate) were added to the filter papers (Whatman, 1MM).
- The filter papers were labeled according to the sample being tested.
- Using an inoculating loop, a well-isolated colony from pure 24-hour culture was

picked and rubbed onto filter paper (Whatman, 1MM) and observed for color change.

- A positive reaction would turn the paper from violet to purple within 1 to 30 seconds.
- Delayed reactions should be ignored as that might give false positive result (Shields & Cathcart, 2010).

2.6.2.4.8 MIU (Motility-indole-urease) test:

MIU test was done for determining the motility of bacteria, indole production and urea degradation by means of the enzyme urease.

- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of stab inoculation method with an inoculating needle
- The tubes were then incubated for 24 hours at 37ºC.
- The growth of the organism would spread throughout the test tube from downward to the upward of the test tube if the organism is motile.
- The colour of the media will turn to deep pink if the organism is positive for urease test. If yellow colour develops then it indicates urease negative result.
- To confirm the indole test, five drops of Kovac's reagent was added following overnight incubation. Then the colour of the media was examined and the results were recorded. Formation of a rose red ring at the top indicates a positive result. A negative result can have a yellow or brown layer (Cappuccino & Sherman, 2005).

2.6.2.4.9 Nitrate reduction test:

Nitrate reduction test was done to determine the ability or inability of the bacteria to reduce nitrate to nitrite or beyond the nitrite stage using anaerobic respiration by the enzyme nitrate reductase.

- 5 ml of nitrate broth containing peptone, beef extract, potassium nitrate was prepared.
- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of loop inoculation method with an inoculating loop.
- The tubes were then incubated at 37ºC for 24-48 hours.
- After 48 hours, five drops of nitrate reagent A and five drops of nitrate reagent B were

added to all nitrate broth cultures.

- If red color develops then it indicates nitrate positive result which means nitrate has been reduced to nitrite.
- If there was no red color development, a small amount of zinc was added to each broth. If red color develops after addition of zinc powder then it indicates nitrate negative result. (Cappuccino & Sherman, 2005)

2.7 Questionnaire

The data was collected using a structured questionnaire which was presented to the participants to complete. A consent form was also signed to let them know how their information would be used. All possible precautions were taken to maintain the reliability of the responses. The entire process of data collection was completed during October 2017 to April 2018.

2.8 Statistical Analysis

The current study has 2 categories of variables, viz. Dependent and independent variable. The dependent variable was the behaviors and characteristically traits towards using and sharing of cellphone, while the independent variables were age, occupation, cellphone brand, type etc. The occupation and area of the collected sample were nominal measurements and the other variables were scale. Occupation had 10 string and area had 12 strings. Variables were total 14 in number.

All the data were entered under the different variables and then it was analyzed. According to descriptive statistic, the frequency was calculated. For each variable, maximum value, minimum value, percentage were calculated alongside frequency. The percentages, to show more clearly, was graphically represented using a pie chart.

For each question present, four variables were used on which one is nominal while the other 3 is scale. The nominal value is represented on the X-axis which was the presence of Microorganism. Y-axis showed the percentage of the presence of the microorganism as three scale value.

Collected Data were coded for processing and analysis. The SPSS computer software program version 25 version was used to perform the data analysis. Qualitative Data were converted into quantitative ones by means of suitable scoring, whenever needed. For describing the particular dependent and independent variables, the respondents were classified into several categories in respect of each variable. Frequency counts and percentage as well as means, standard deviations, rank order, cross- tabulation were used for descriptive data.

Chapter 3 Results

3.1 Analysis of the Survey according to Questionnaire

3.1.1 age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	16-20	32	32.0	32.0	32.0
	21-25	40	40.0	40.0	72.0
	26-30	14	14.0	14.0	86.0
	above 30	14	14.0	14.0	100.0
	Total	100	100.0	100.0	

The age group of 21-25 is dominant in the age group.

3.1.2 occupation

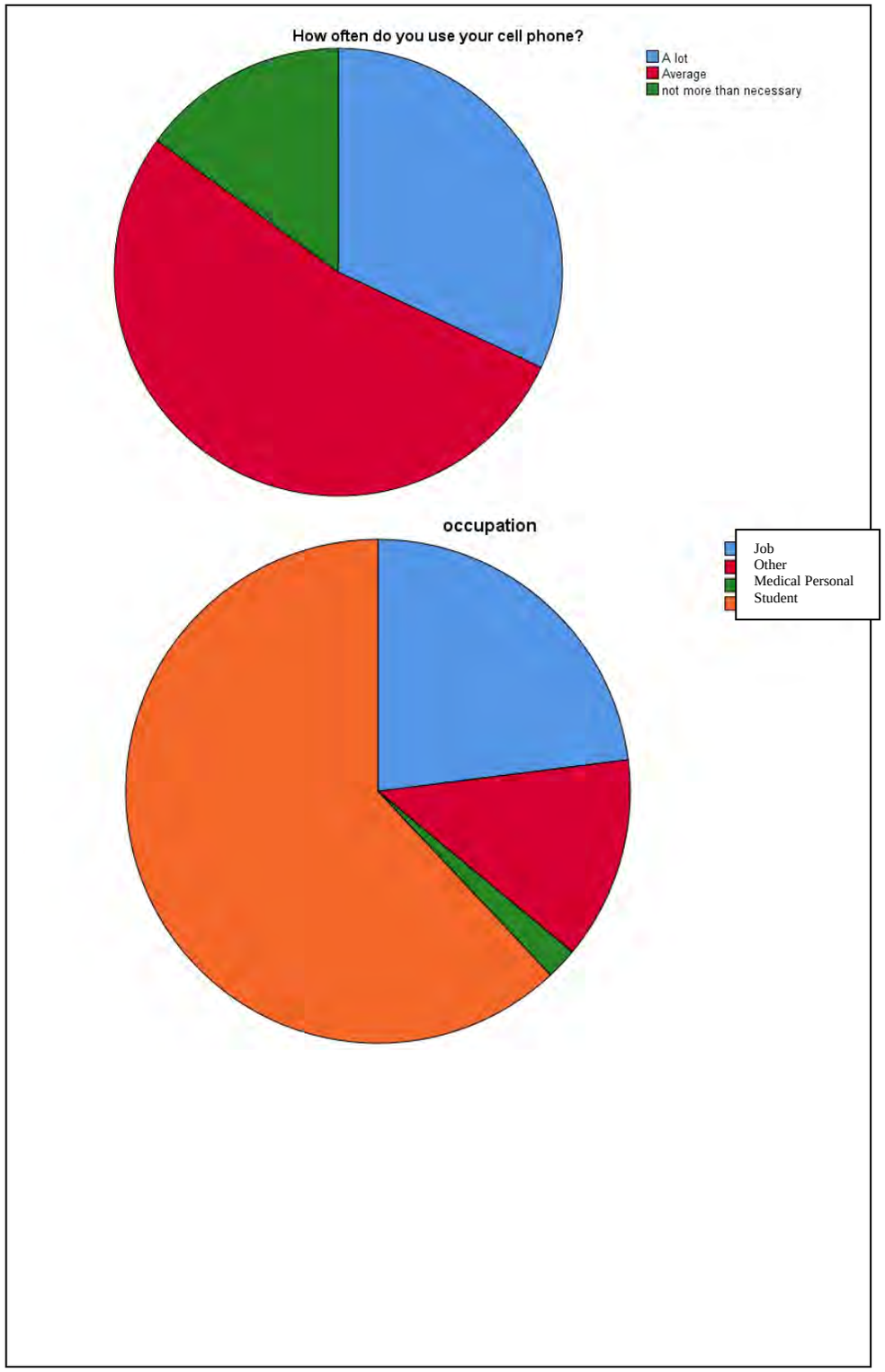
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	JOB	23	23.0	23.0	23.0
	OTHER	13	13.0	13.0	36.0
	MEDICAL PERSONAL	2	2.0	2.0	38.0
	STUDENT	62	62.0	62.0	100.0
	Total	100	100.0	100.0	

Students are the dominant group sitting at 62%.

3.1.3 How often do you use your cell phone?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A lot	32	32.0	32.0	32.0
	Average	53	53.0	53.0	85.0
	not more than necessary	15	15.0	15.0	100.0
	Total	100	100.0	100.0	

Average usage of cellphone is observed in more than half the participants throughout the survey.



3.1.4 Do you use your cell phone while eating?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Always	20	20.0	20.0	20.0
	Sometimes	49	49.0	49.0	69.0
	Not at all	31	31.0	31.0	100.0
	Total	100	100.0	100.0	

49% of the participants use their cellphone while eating.

3.1.5 Do you use your cell phone inside washroom?

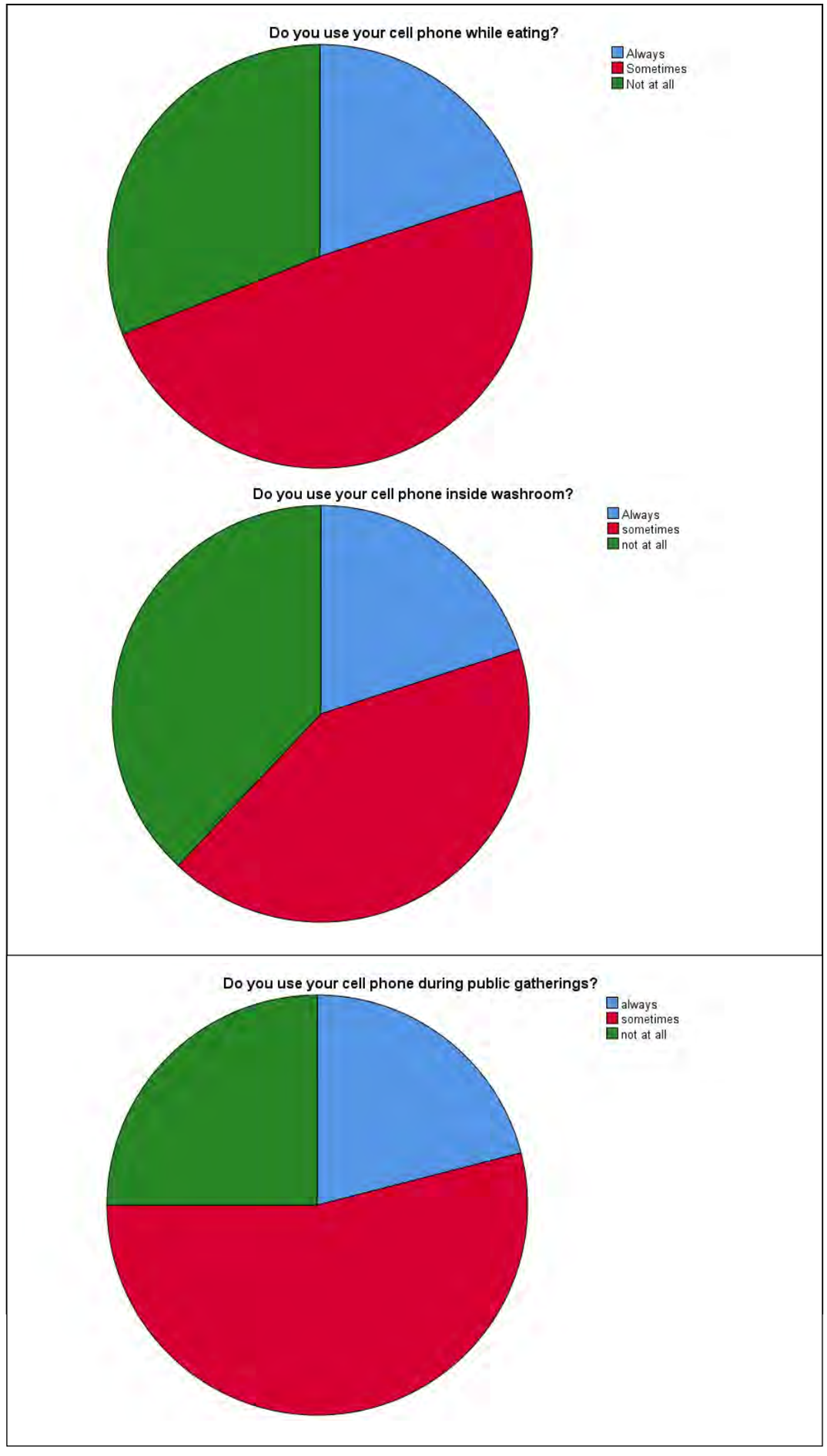
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Always	20	20.0	20.0	20.0
	sometimes	42	42.0	42.0	62.0
	not at all	38	38.0	38.0	100.0
	Total	100	100.0	100.0	

Cellphone usage in the washroom is mostly sometimes or not at all, sitting at 42 and 38 percent respectively.

3.1.6 Do you use your cell phone during public gatherings?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	always	21	21.0	21.0	21.0
	sometimes	54	54.0	54.0	75.0
	not at all	25	25.0	25.0	100.0
	Total	100	100.0	100.0	

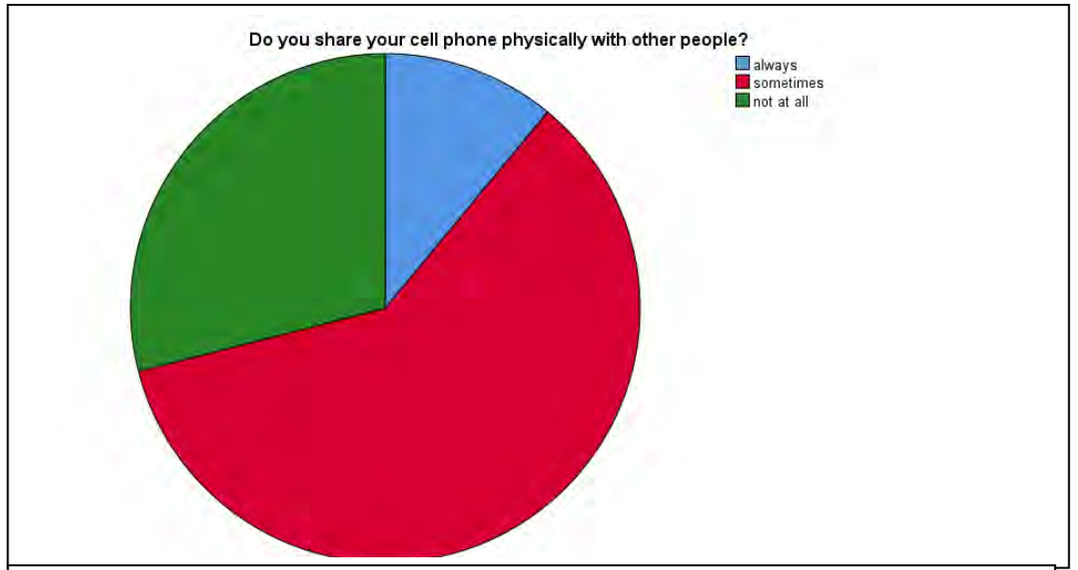
Cellphone usage in public gatherings like meetings or party is mostly on the need to use basis as seen from the information of participants.



3.1.7 Do you share your cell phone physically with other people?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	always	11	11.0	11.0	11.0
	sometimes	60	60.0	60.0	71.0
	not at all	29	29.0	29.0	100.0
	Total	100	100.0	100.0	

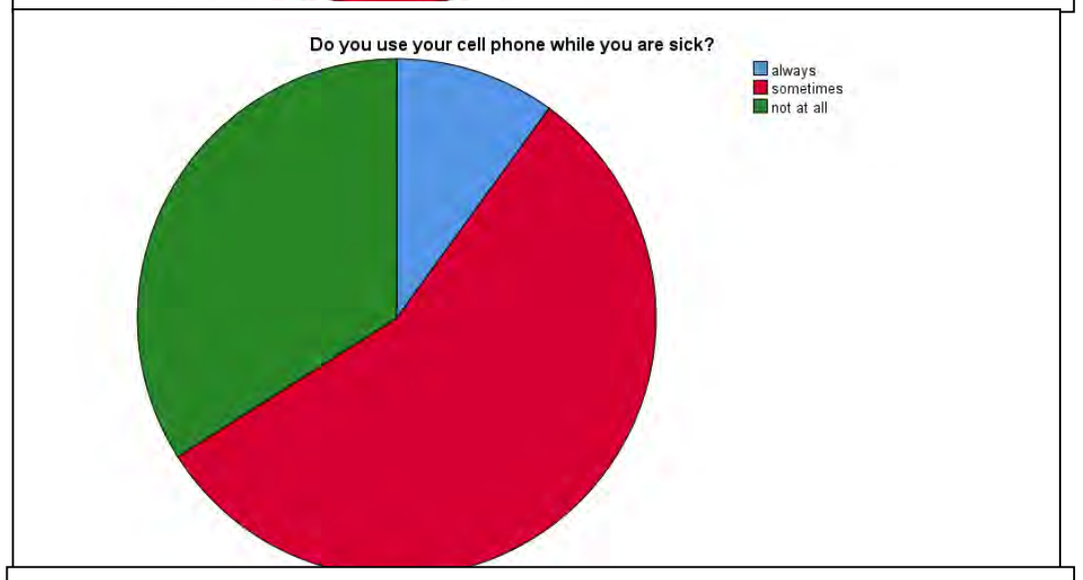
Sharing a cellphone physically is mostly reserved for necessary use, as seen by the high percentage of “sometimes” at 60%



3.1.8 Do you use your cell phone while you are sick?

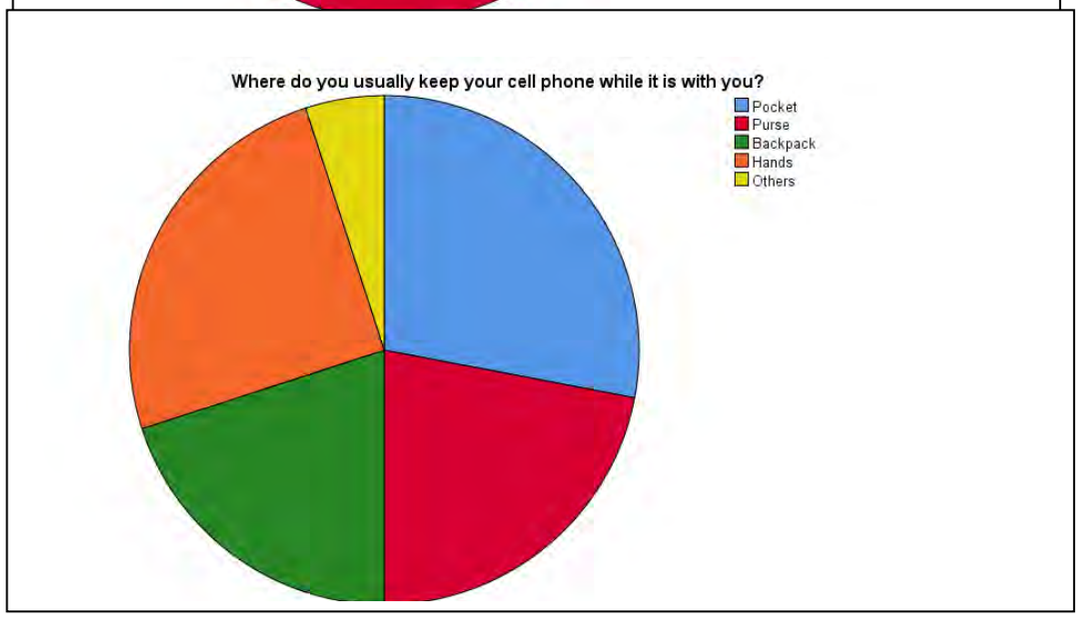
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	always	10	10.0	10.0	10.0
	sometimes	56	56.0	56.0	66.0
	not at all	34	34.0	34.0	100.0
	Total	100	100.0	100.0	

Cellphone usage while sick is mostly sometimes, sitting at 56%.



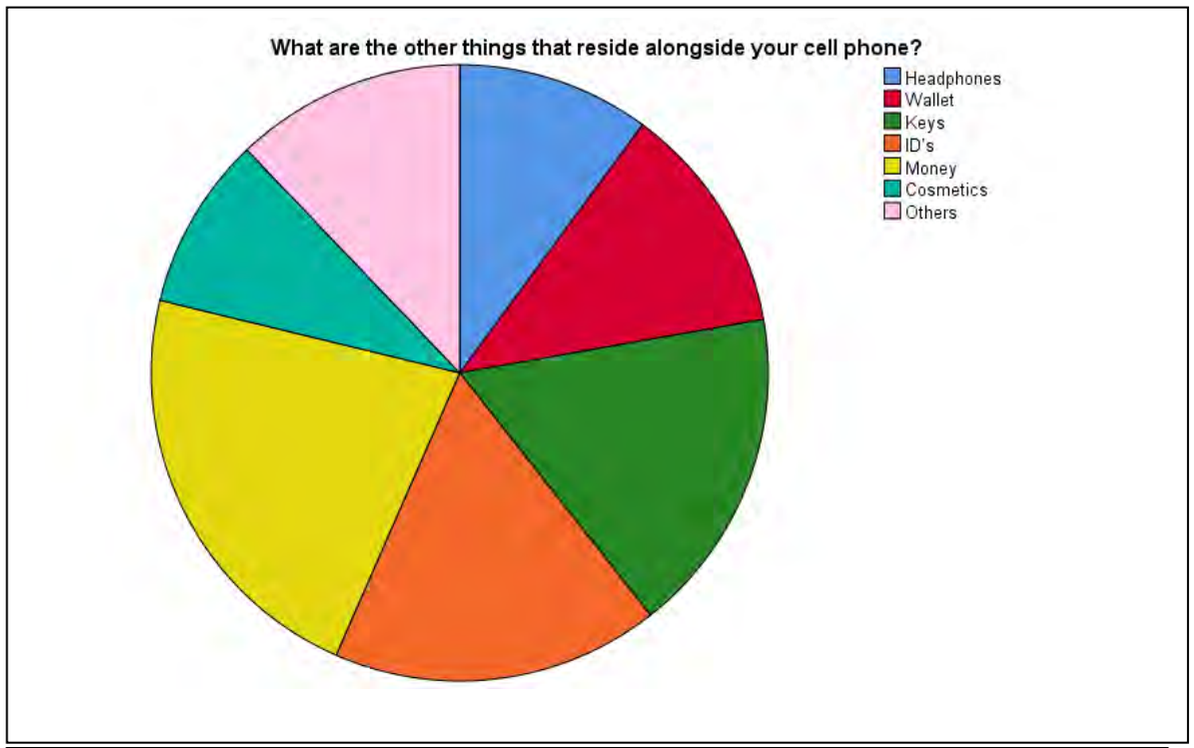
3.1.9 Where do you usually keep your cell phone while it is with you?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Pocket	28	28.0	28.0	28.0
	Purse	22	22.0	22.0	50.0
	Backpack	20	20.0	20.0	70.0
	Hands	25	25.0	25.0	95.0
	Others	5	5.0	5.0	100.0
	Total	100	100.0	100.0	



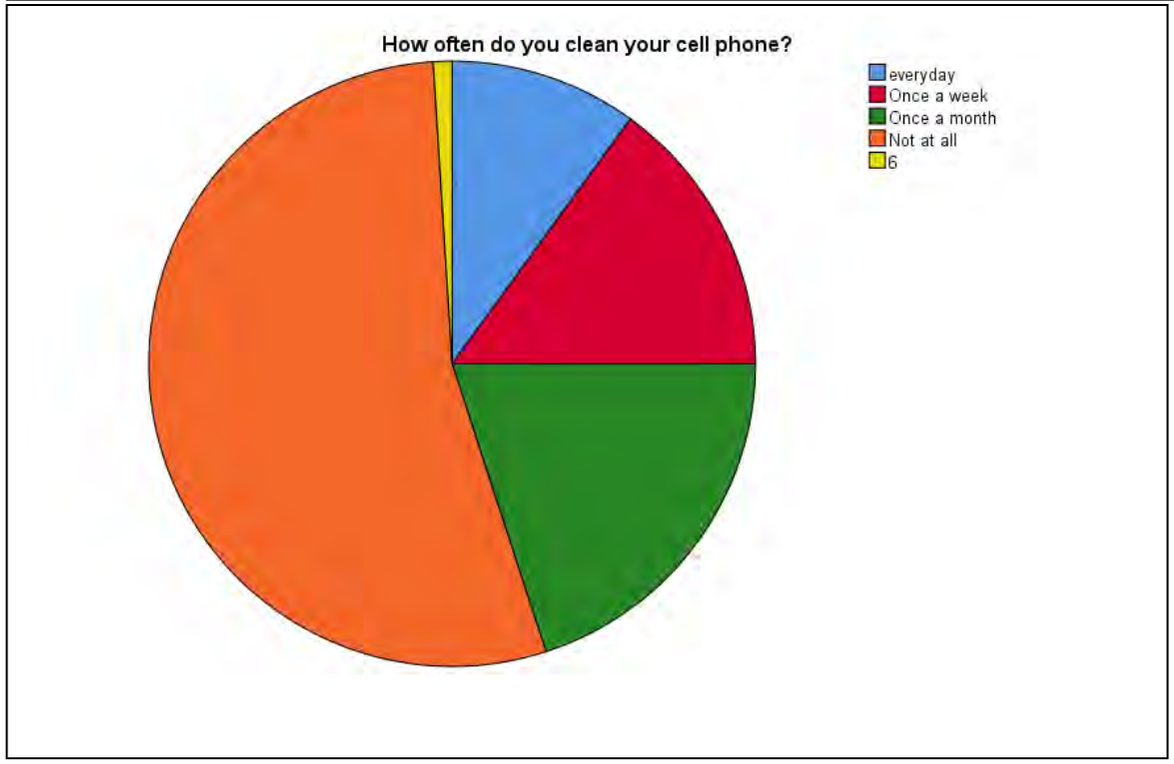
3.1.10 What are the other things that reside alongside your cell phone?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Headphones	10	10.0	10.1	10.1
	Wallet	12	12.0	12.1	22.2
	Keys	17	17.0	17.2	39.4
	ID's	17	17.0	17.2	56.6
	Money	22	22.0	22.2	78.8
	Cosmetics	9	9.0	9.1	87.9
	Others	12	12.0	12.1	100.0
	Total	99	99.0	100.0	
Missing	System	1	1.0		
Total		100	100.0		



3.1.11 How often do you clean your cell phone?

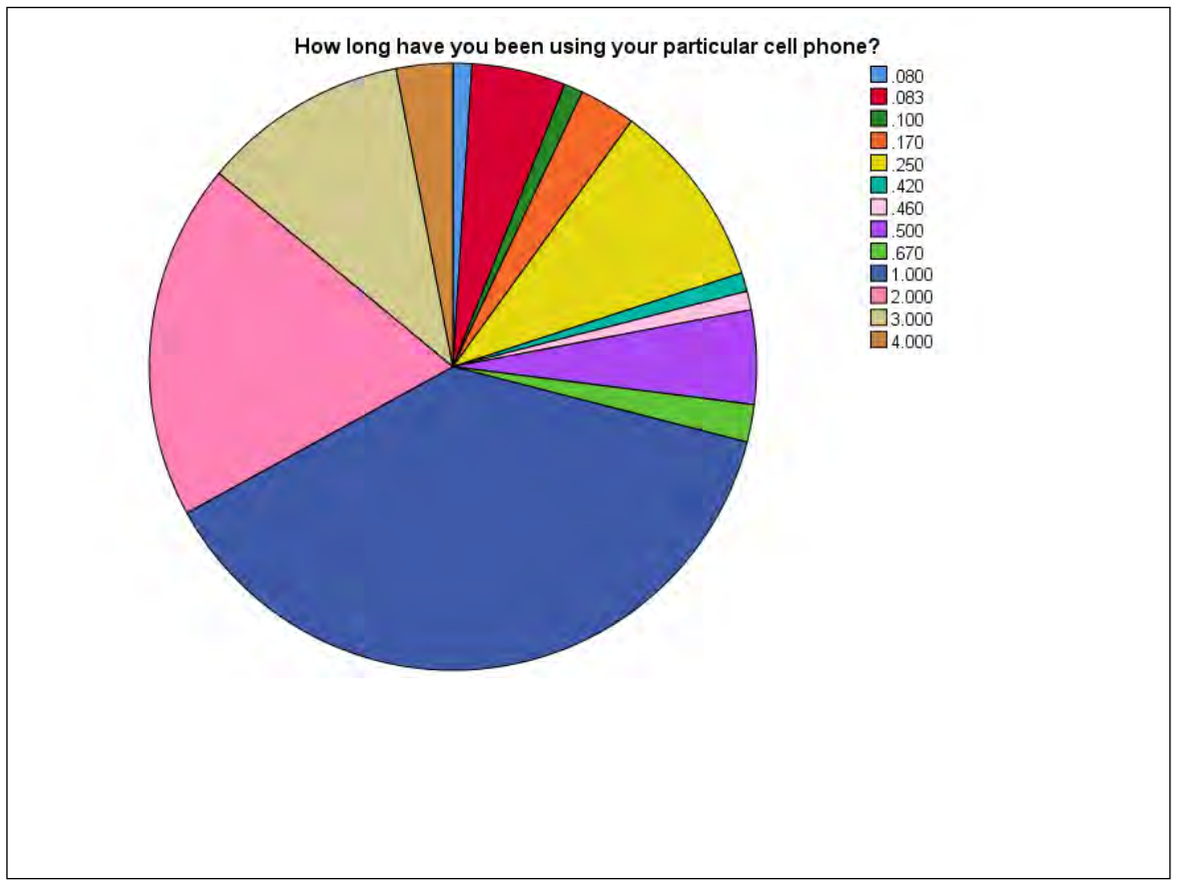
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	everyday	10	10.0	10.0	10.0
	Once a week	15	15.0	15.0	25.0
	Once a month	20	20.0	20.0	45.0
	Not at all	54	54.0	54.0	99.0
	6	1	1.0	1.0	100.0
	Total	100	100.0	100.0	



Cellphone cleaning hygiene is at an alarmingly low level, showing only 10% cleaning every day against the massive 54% number of people not cleaning their cellphone at all.

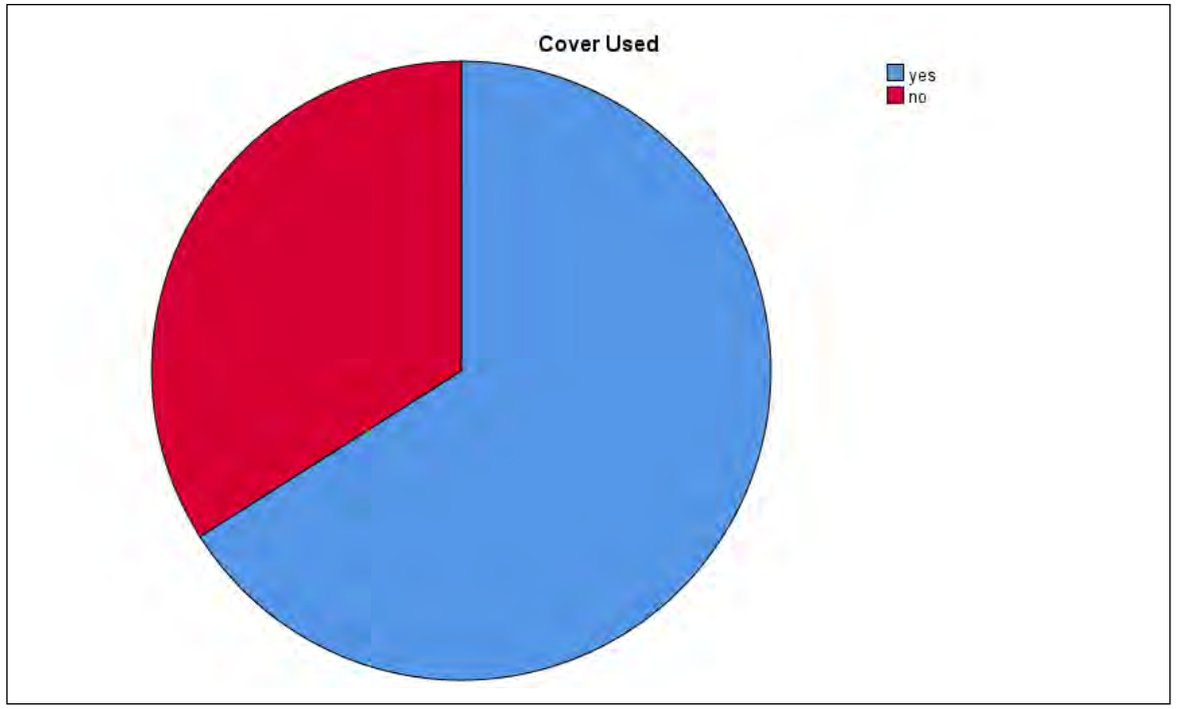
3.1.12 How long have you been using your particular cell phone?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.080	1	1.0	1.0	1.0
	.083	5	5.0	5.0	6.0
	.100	1	1.0	1.0	7.0
	.170	3	3.0	3.0	10.0
	.250	10	10.0	10.0	20.0
	.420	1	1.0	1.0	21.0
	.460	1	1.0	1.0	22.0
	.500	5	5.0	5.0	27.0
	.670	2	2.0	2.0	29.0
	1.000	38	38.0	38.0	67.0
	2.000	19	19.0	19.0	86.0
	3.000	11	11.0	11.0	97.0
	4.000	3	3.0	3.0	100.0
	Total	100	100.0	100.0	



3.1.13 Cover Used

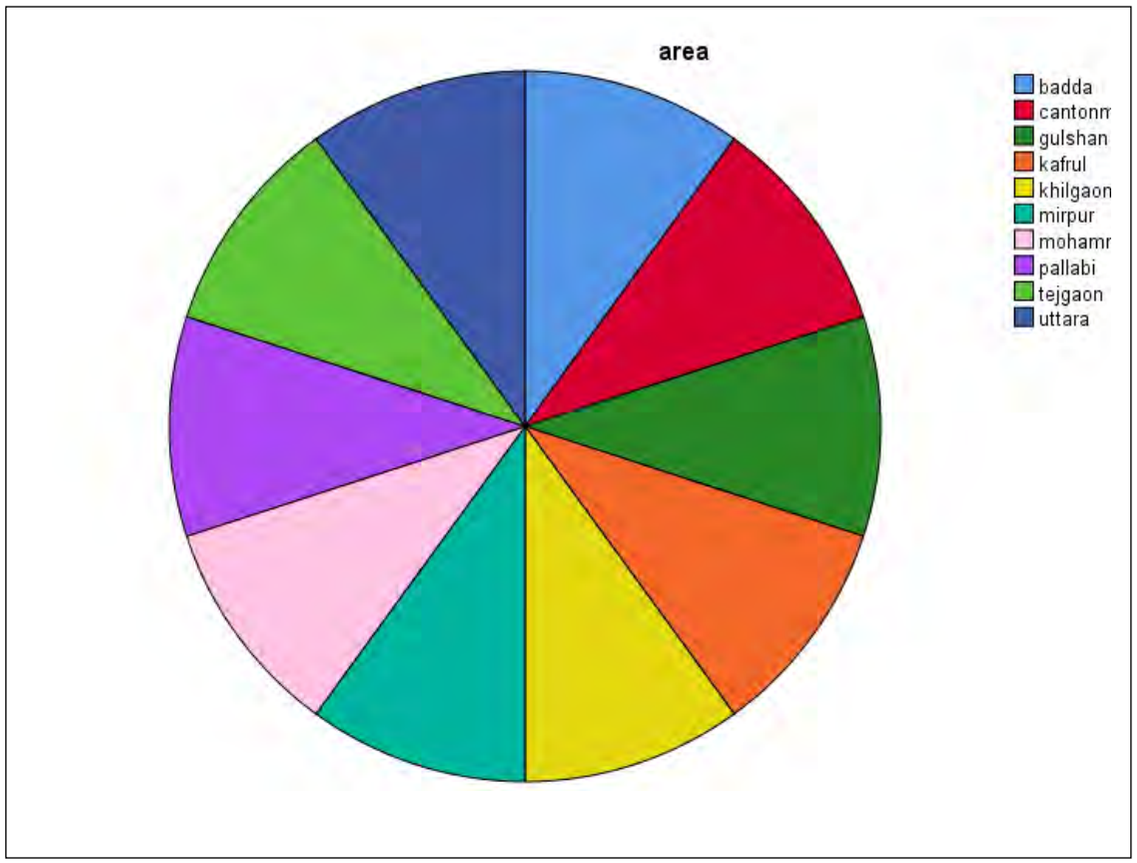
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	66	66.0	66.0	66.0
	no	34	34.0	34.0	100.0
	Total	100	100.0	100.0	



Covers are seemed to be popular with the percentage of 66 among the sample population.

3.1.14 area

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	badda	10	10.0	10.0	10.0
	cantonment	10	10.0	10.0	20.0
	gulshan	10	10.0	10.0	30.0
	kafrul	10	10.0	10.0	40.0
	khilgaon	10	10.0	10.0	50.0
	mirpur	10	10.0	10.0	60.0
	mohammadpur	10	10.0	10.0	70.0
	pallabi	10	10.0	10.0	80.0
	tejgaon	10	10.0	10.0	90.0
	uttara	10	10.0	10.0	100.0
	Total	100	100.0	100.0	



The areas of Dhaka City North is selected as per its Thana basis. A sample size of 10 was selected. From each area, focusing on diverse backgrounds to get a more accurate representation. The total Sample size was 100, with male and female almost evenly matched.

3.2 Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media

The cultural and morphological characteristics of the Bacterial isolates from cellphone, which were collected from the 10 Thana of Dhaka City North, were seen on Macconky Agar, Mannitol Salt Agar (MSA), Membrane Fecal Coliform Agar (MFC), Eosine Methylene Blue Agar (EMB), Bacillus Cereus Agar (BC), Hi Chrome Agar and on Blood agar containing Human blood. The morphological characteristics of the colonies were observed on Nutrient Agar, concentrating on Size, Color, Form, Margin and Elevation. These observations are noted and their characteristics are used to determine the probable species of the isolated colony.

Out of 187 isolates, *Staphylococci spp* were found in 97 isolates which indicates 50.7% of the total colony. Moreover, *E. coli* were seen in 26 cases and *Bacillus spp* were seen in 25 cases. 21 and 25 colonies were identified as *Micrococcus sp*p and Fecal Coliform respectively. 8 samples overall showed no colonies.

Pallabi Thana (table 3.2.1) is dominated by *Staphylococcus spp* at 10 colony and the others being *Micrococcus spp* and *Bacillus* Spp numbering at 2 each. Uttara Thana (Table 3.2.2) had 8 *Staphylococcus Spp* and 1 of Fecal coliform and *Bacillus sp*. Mohammadpur Thana (Table 3.2.3) had 3 colonies of *E.coli* and 2 of Fecal Coliform and *Bacillus spp*. Badda (Table 3.2.4) Had *E.coli, Micrococcus spp* and *Bacillus spp* in colony numbers 1,2 and 3. Gulshan (Table 3.2.5) had a larger variety, with *Staphylococcus Spp, E.coli, Micrococcus Spp,* Fecal Coliform and *Bacillus Spp* present in Colony Number 10,6,2,4 and 2 respectively. Mirpur (Table 3.2.6) Showed similar result in organisms at 10,6,4,2 and 3 number. Khilgaon (Table 3.2.7) had the highest number of Fecal Coliform presence in the entire study, at 7 colonies along with highest *Micrococcus spp* present numbering in 5 colonies. Cantonment (Table 3.2.8) showed *E. coli* and *Bacillus* number both in 3 colonies each. Kafrul Thana (Table 3.2.9) had *E. coli, Micrococcus spp,* Fecal Coliform and *Bacillus spp* presence numbering in 2,2,2 and 1. Lastly, Tejgaon thana (Table 3.2.10) showed a similar number of organisms in the previous format at 1,3,1 and 4 colony.

Table 3.2.1: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Pallabi.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.Pl 1	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.Pl 2	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
3.PL 3	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
4.PL 4(a)	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
5.PL 4(b)		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
6.PL 5 (a)	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.1: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Pallabi.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.PL 5(b)		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
8.PL 6(a)					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
9.PL 6(b)	Small, Pink Colour colonies			Metallic Green Sheen colonies		Purple Colour colonies	Gamma Hemo-Lysis	Small	Creamy	Circular	Entire	Raised	***E. coli***
10.PL 7	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
11.PL 7(b)					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
12.PL 8	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.1: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Pallabi.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.Pl 9	-	Pinpoint, Yellow Colonies	-	-		Golden Yellow Colour colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Raised	***Staphylococcus* spp.**
14.PL 10		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.2: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Uttara.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1. UT1													**None**
2. UT2													**None**
3. UT3		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
4. UT4		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
5. UT5		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
6. UT6(a)		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.2 : Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Uttara.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7. UT6b					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
8. UT7		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
9. UT8a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
10. UT8b			Blue Colour Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Convex	**Fecal coliform**
11. UT9		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
12. UT10		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.3 : Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mohammadpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1. MM1		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
2. MM2		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
3. MM 3a		Pinpoint, White Colonies				Cream Colour colonies	Alpha Hemo-Lysis	Pinpoint	White	Circular	Entire	Convex	***Staphylococcus* spp.**
4. MM3 b					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
5. MM4 a		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
6. MM4 b	Medium Pink coloured colonies			Metallic Green sheen colonies	-	Purple Colour colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***

Table 3.2.3 : Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mohammadpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7. MM5a		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
8. MM5b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
9. MM5c	Small, pink colonies			Metallic GreeShe en colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
10. MM6a		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
11. MM6b					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
12. MM7	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.3 : Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mohammadpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13. MM8													***None***
14. MM9a		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**
15. MM9b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
16. MM9c	Medium Pink coloured colonies			Metallic Green sheen colonies	-	Purple Colour colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
17. MM 10		Small, Yellow colonies				Off-White colonies	Beta Hemo-Lysis	Small	Off White	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.4: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Badda.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.BD 01a		Small, yellow coloured colonies				Golden Yellow Colour colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.BD 01b	-		-	-	Blue Colour Colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
3.BD 01c		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
4.BD 02		Small, yellow coloured colonies				Golden Yellow Colour colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
5.BD 03a		Small, yellow coloured colonies				Golden Yellow Colour colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
6.BD 03b					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

Table 3.2.4: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Badda.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.BD 04a	-	Pinpoint, Yellow Colonies	-	-		Golden Yellow Colour colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Raised	***Staphylococcus* spp.**
8.BD 04b	-		-	-	Blue Colour Colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
9.BD 04c		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
10.BD05													None
11.BD06		Small, yellow coloured colonies				Golden Yellow Colour colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
12.BD07a		Small, yellow coloured colonies				Golden Yellow Colour colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.4: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Badda.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.BD 07b	-		-	-	Blue Colour Colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
14.BD 08	-	Pinpoint, Yellow Colonies	-	-		Golden Yellow Colour colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Raised	***Staphylococcus* spp.**
15.BD 09		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
16.BD 10													**None**

Table 3.2.5: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Gulshan.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.GL 01a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
2.GL 01b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
3.GL 02a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
4.GL 02b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
5.GL 02c					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
6.GL 03a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.5: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Gulshan.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected Organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.GL 03b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
8.GL 03c	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
9.GL 03d					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
10.GL04a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
11.GL04b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
12.GL05a		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.5: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Gulshan.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.GL 05b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
14.GL 05c						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
15.GL 06a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Cream y	Circular	Entire	Convex	***Staphylococcus* spp.**
16.GL 06b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
17.GL 07a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Cream y	Circular	Entire	Convex	***Staphylococcus* spp.**
18.GL 07b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
19.GL 07c	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***

Table 3.2.5: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Gulshan.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
20.GL 08a		Small, yellow coloured colonies				Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	circular	Entire	Convex	***Staphylococcus* spp.**
21.GL 08b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
22.GL 09		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Cream y	Circular	Entire	Convex	***Staphylococcus* spp.**
23.GL 10a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Cream y	Circular	Entire	Convex	***Staphylococcus* spp.**
24.GL 10b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**

Table 3.2.6: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mirpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.MR 01a		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**
2.MR 01b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
3.MR 02a		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**
4.MR 02b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
5.MR 02c	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
6.MR 03a		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.6: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mirpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.MR 03b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
8.MR 03c	Small, Pink Colour colonies			Metallic Green Sheen colonies		Purple Colour colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
9.MR 03d					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
10.MR04		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**
11.MR05a		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
12.MR05b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**

Table 3.2.6: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mirpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected Organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.MR05c					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
14.MR06a		Medium, Light Yellow colonies				Cream Colour colonies	Beta Hemo-Lysis	Medium	Cream y	Circular	Entire	Convex	***Staphylococcus* spp.**
15.MR06b					Blue Colour colonies	Green Colour colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
16.MR07a		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
17.MR07b			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
18.MR08a		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
19.MR08b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***

Table 3.2.6: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Mirpur.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
20.MR08c						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
21.MR08d			Blue colour colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
22.MR09a		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
23.MR09b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
24.MR10a		Medium, Yellow colonies			Beta Hemo Lysis	Golden Yellow	Beta Hemo-Lysis	Medium	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
25.MR10b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***

Table 3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Khilgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.KG 1a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.KG 1b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
3.KG 1c					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
4.KG 2a	-	Small, Yellow coloured colonies	-	-	-	Golden Yellow colour colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
5.KG 2b		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
6.KG 3a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Khilgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.KG 3b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
8.KG 3c		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
9.KG 3d	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
10.KG4a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
11.KG4b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
12.KG4c					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

Table 3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Khilgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.KG5a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
14.KG5b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
15.KG5c					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
16.KG6a	-	Pinpoint, Yellow Colonies	-	-		Golden Yellow Colour colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Raised	***Staphylococcus* spp.**
17.KG6b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
18.KG6c			Blue Colour Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
19.KG7a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Khilgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
20.KG7b		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
21.KG8a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
22.KG8b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
23.KG8c					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
24.KG9a		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
25.KG9b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
26.KG9c	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**

Table 3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Khilgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
27.KG9d		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
28.KG10a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
29.KG10b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
30.KG10c	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**

Table 3.2.8: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Dhaka Cantonment.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.CT 01a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.CT 01b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
3.CT 02a		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
4.CT 02b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
5.CT 02c	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
6.CT 03		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.8: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Dhaka Cantonment.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.CT04		Small, White colonies				Cream Colour colonies	Alpha Hemo-Lysis	Small	White	Circular	Entire	Convex	***Staphylococcus* spp.**
8.CT05													***None***
9.CT06a		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
10.CT-06b			Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
11.CT06c	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
12.CT06d					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
13.CT07		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.8: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Dhaka Cantonment.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
14.CT 08a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
15.CT 08b		Small, White Colonies				Yellow Colour colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
16.CT 08c					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
17.CT 09	-	Pinpoint, Yellow Colonies	-	-		Golden Yellow Colour colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Raised	***Staphylococcus* spp.**
18.CT 10a		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
19.CT 10b					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

Table 3.2.9: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Kafrul.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.KF 01a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.KF 01b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
3.KF 02a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
4.KF 02b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
5.KF 03		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
6.KF 04a		Pinpoint, Yellow colonies				Golden Yellow colonies	Beta Hemo-Lysis	Pinpoint	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.9: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Kafrul.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected Organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
7.KF 04b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
8.KF 04c	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
9.KF 05a		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
10.KF05b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
11.KF06		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
12.KF07		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.9: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Kafrul.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13.KF08		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
14.KF09a		Pinpoint, White Colonies				Cream Colour colonies	Alpha Hemo-Lysis	Pinpoint	White	Circular	Entire	Convex	***Staphylococcus* spp.**
15.KF09b	-		Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
16.KF9c			Blue Colour Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
17.KF10		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.2.10: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Tejgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
1.TG01a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
2.TG01b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
3.TG01c					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
4.TG0-1d			Blue coloured Colonies				Gamma Hemo-Lysis	Medium	White	Circular	Entire	Raised	**Fecal coliform**
5.TG02		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
6.TG03a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
7.TG03b					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

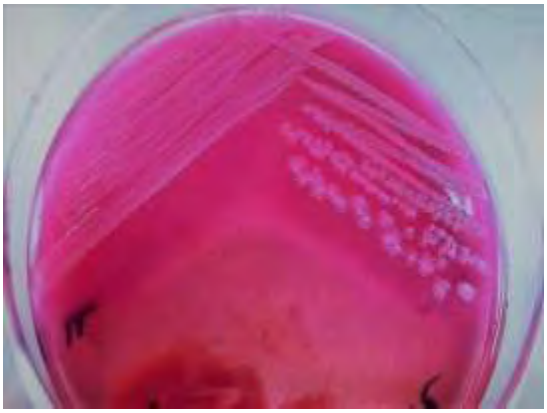
Table 3.2.10: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Tejgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
8.TG 04		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
9.TG 05		Small, Yellow Colonies				Golden Yellow colonies	Beta Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
10.T G06													**None**
11.T G07a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
12.T G07b	Small, pink colonies			Metallic Green Sheen colonies		Purple Colour Colonies	Gamma Hemo-Lysis	Small	White	Circular	Entire	Raised	***E. coli***
13.T G07c						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**

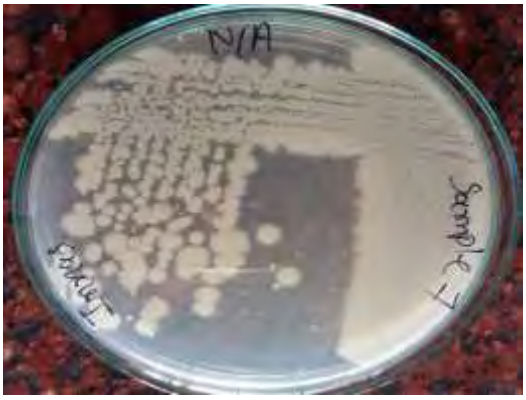
Table 3.2.10: Cultural and Morphological Characteristics of the Bacterial Isolates from Cellphone on various Selective, Differential, Enriched and Nutrient Media on Tejgaon.

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Membrane Fecal Coliform Agar (MFC)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
14.TG08a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
15.TG08b						Yellow Colour colonies	Gamma Hemo-lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus* spp.**
16.TG09a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
17.TG09b					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
18.TG10a		Small, Yellow colonies				Cream Colour Colonies	Alpha Hemo-Lysis	Small	Creamy	Circular	Entire	Convex	***Staphylococcus* spp.**
19.TG10b					Blue Colour colonies	Green Colour colonies	Alpha Hemo-Lysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

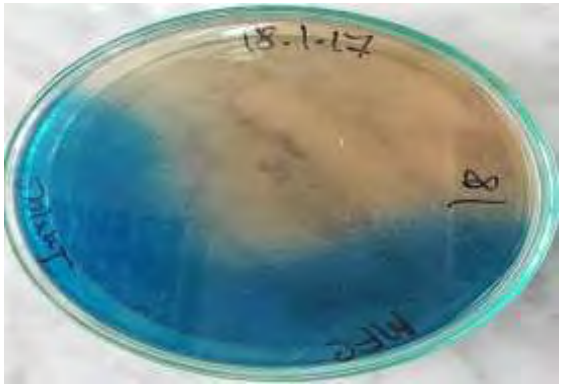
Growth of *Staphylococcus* species on MSA



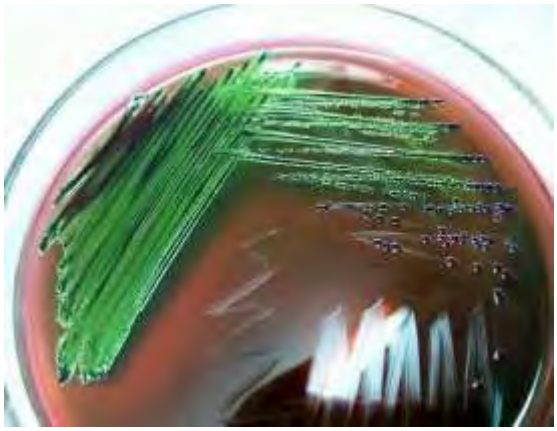
Growth of *E.coli* on MacConkey agar



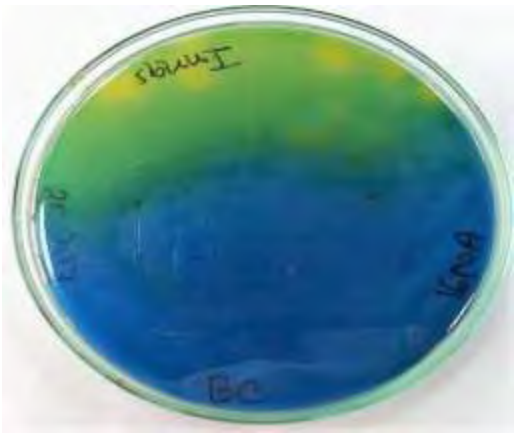
Growth of *Bacillus* species on Nutrient Agar



Growth of Fecal coliform on MFC



Growth of *E.coli* on EMB Agar



Growth of *Bacillus* on BC Agar

Figure 3.1: Bacterial growth on various selective media

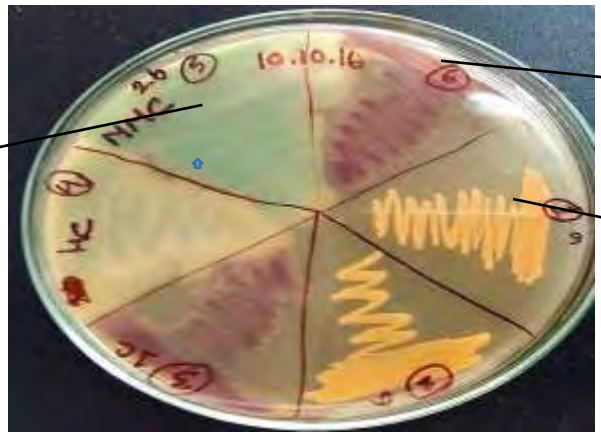
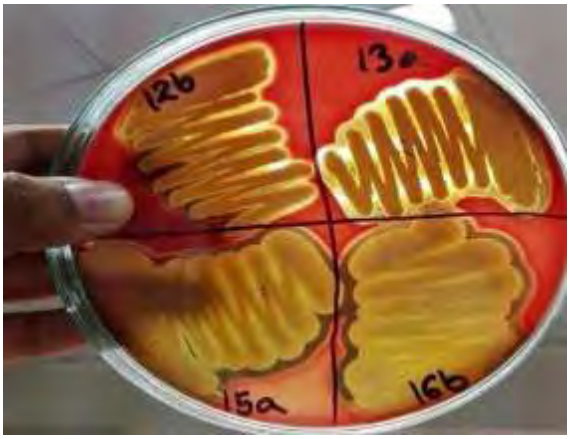
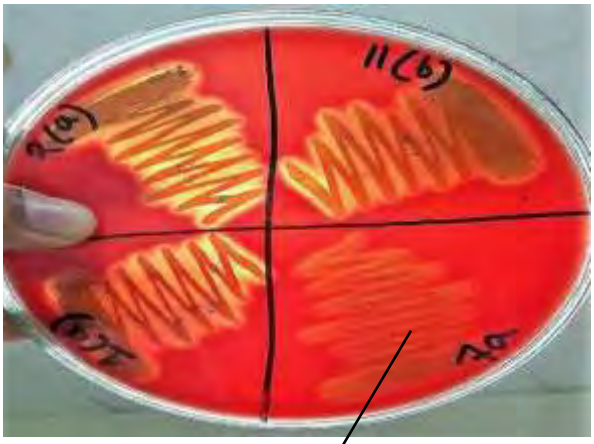


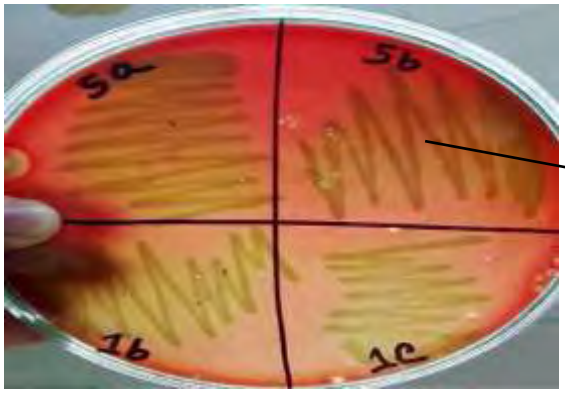
Figure 3.2: Growth of various organisms on Hi-Chrome agar



Beta hemolysis



Alpha hemolysis



Gamma Hemolysis

Figure 3.3: Bacterial growth on Blood agar

3.3 Biochemical characteristics of the bacterial isolates:

Bacterial isolates extracted from Cellphones were tested by different types of biochemical tests. Biochemical tests are important for identification and confirmation of the unknown organisms. After spreading and streaking on the agar plates, microorganisms were isolated and sub-cultured for biochemical tests. These tests were done with 24 hours fresh culture of the isolates. After subculture, some specific biochemical tests were done and recorded. Then organisms were analyzed and identified with the help of reference books including Bergey's Manual of Systematic Bacteriology and Cappuccino and Sherman. The biochemical tests that were performed are described precisely in materials and method chapter 2 and the biochemical test results of the isolates are given below in Table 3.3.

The biochemical Tests confirmed the suspected organisms which are identified during the morphological tests. All the findings of the species found in the morphological analysis were similar in nature to the biochemical tests.

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
1.	PL1	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
2.	PL2	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
3	PL3	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
4	PL4a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
5	PL4b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
6	PL5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
7	PL5b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
8	PL6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
9	PL6b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
10	PL7a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
11	PL7b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
12	PL8	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
13	PL9	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
14	PL10	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges-Proskauer (VP) T	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
17	UT 1																			none
18	UT 2																			None
19	UT 3	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
20	UT 4	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
21	UT 5	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
22	UT 6a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
23	UT 6b	+	Long rods	-	+	+	-	R/Y	+	-	-	-		-	-	+	+	+	-	*Bacillus* spp.
24	UT 7	+	Cocci in Cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
25	UT 8a	+	Cocci in Cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
26	UT 8b	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
27	UT 9	+	Cocci in Cluster	+	-	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp
28	UT 10	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
29	MM 1	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
30	MM 2	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

31	MM 3a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
32	MM 3b	+	Long rods	-	-	+	-	R/Y	+	-	-	+	-	-	-	+	+	+	-	*Bacillus* spp.
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																				

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
32	MM 3b	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
33	MM 4a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
34	MM 4b	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
35	MM 5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
36	MM 5b																			Fecal Coliform
37	MM 5c	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
38	MM 6a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
39	MM 6b	+	Long rods	-	-	+	-	R/Y	+	-	-	+	-	-	-	+	+	+	-	*Bacillus* spp.
40	MM 7	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
41	MM 8																			None
42	MM 9a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

43	MM 9b																		Fecal Coliform
44	MM 9c	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E.coli*
45	MM 10	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
46	BD1a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
47	BD1b	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges-Proskauer (VP) T	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
48	BD1c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
49	BD2	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
50	BD3a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
51	BD3b	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
52	BD4a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
53	BD4b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
54	BD4c	-	Short rods	+	+	-	+	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E.coli*
55	BD5																			None
56	BD6	+	Cocci in Cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
57	BD7a	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
58	BD7b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
59	BD8	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
60	BD9	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
61	BD10																			*None*

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
62	GL01a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
63	GL01b	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
64	GL02a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
65	GL02b	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
66	GL02c	+	Long Rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
67	GL03a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
68	GL03b																			Fecal Coliform
69	GL03c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
70	GL03d	+	Long Rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
71	GL04a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
72	GL04b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E.coli*
73	GL05a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
74	GL05b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
75	GL05c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
76	GL06a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
77	GL06b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																				

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges- Proskau (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
78	GL07a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
79	GL07b																			Fecal Coliform
80	GL07c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
81	GL08a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
82	GL08b																			Fecal Coliform
83	GL09	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
84	GL10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
85	GL10b																			Fecal Coliform
86	MR1a	+	Cocci in Cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
87	MR1b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
88	MR2a	+	Cocci in Cluster	+	-	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp
89	MR2b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
90	MR2c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
91	MR3a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
92	MR3b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
93	MR3c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
94	MR3d	+	Long Rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
95	MR4	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
96	MR5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
97	MR5b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
98	MR5c	+	Long Rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
99	MR6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
100	MR6b	+	Long Rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
101	MR7a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
102	MR7b																			Fecal Coliform
103	MR8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
104	MR8b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
105	MR8c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
106	MR8d																			Fecal Coliform
107	MR9a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
108	MR9b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
109	MR10a	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges- Proskau (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
110	MR10b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
111	KG1a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
112	KG1b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
113	KG1c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
114	KG2a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
115	KG2b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
116	KG3a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
117	KG3b																			Fecal Coliform
118	KG3c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
119	KG3d	-	Short rods	+	+	-	+	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
120	KG4a	+	Cocci in Cluster	+	-	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp
121	KG4b																			Fecal Coliform
123	KG4c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
124	KG5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
125	KG5b																			Fecal Coliform
126	KG5c	+	Long rods	-	-	+	-	R/Y	+	-	-	+	-	-	-	+	+	+	-	*Bacillus* spp.

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
127	KG6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
128	KG6b	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
129	KG6c																			Fecal Coliform
130	KG7a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
131	KG7b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
132	KG8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
133	KG8b																			Fecal Coliform
134	KG8c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
135	KG9a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
136	KG9b	-	Short rods	+	+	-	+	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
137	KG9c																			Fecal Coliform
138	KG9d	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
139	KG10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
140	KG10b	-	Short rods	+	+	-	+	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
141	KG10c																			Fecal Coliform
142	CT01a	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges- Proskau (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
143	CT01b	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
144	CT02a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
145	CT02b																			Fecal Coliform
146	CT03	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
147	CT04	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
148	CT05																			none
149	CT06a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
150	CT06b																			Fecal Coliform
151	CT06c	-	Short Rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
152	CT06d	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
153	CT07	+	Cocci in Cluster	+	-	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp
154	CT08a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
155	CT08b	+	Cocci in Cluster	+	+	-	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
156	CT08c	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
157	CT09	+	Cocci cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus*
158	CT10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
159	CT10b	+	Long rods	+	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
160	KF01a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
161	KF01b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
162	KF02a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
163	KF02b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
164	KF03	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
165	KF04a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
166	KF04b																			*Fecal*
167	KF04c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	*E. coli*
168	KF05a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
169	KF05b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
170	KF06	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
171	KF07	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
172	KF08	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
173	KF09a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
174	KF09b																			Fecal

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges- Proskau (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reductio	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
175	KF09c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
176	KF10	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
177	TG01a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
178	TG01b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
179	TG01c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
180	TG01d																			Fecal
181	TG02	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
182	TG03a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
183	TG03b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
184	TG04	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
185	TG05	+	Cocci in Cluster	+	-	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp
186	TG06																			None
187	TG07a	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
188	TG07b	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
189	TG07c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
190	TG08a	+	Cocci cluster	-	+	+	-	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus Sp*

'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)

Table 3.3: Biochemical characteristics of the bacteria isolated from Cellphones of the Demography of Dhaka North.

Isolates No	Isolates ID	Gram Staining		Indole test	Red Methyl (MR) Test	Voges- Prosk (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease				
191	TG08b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	*Micrococcus* spp.
192	TG09a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
193	TG09b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
194	TG10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus* spp.
195	TG10b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	*Bacillus* spp.
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																				

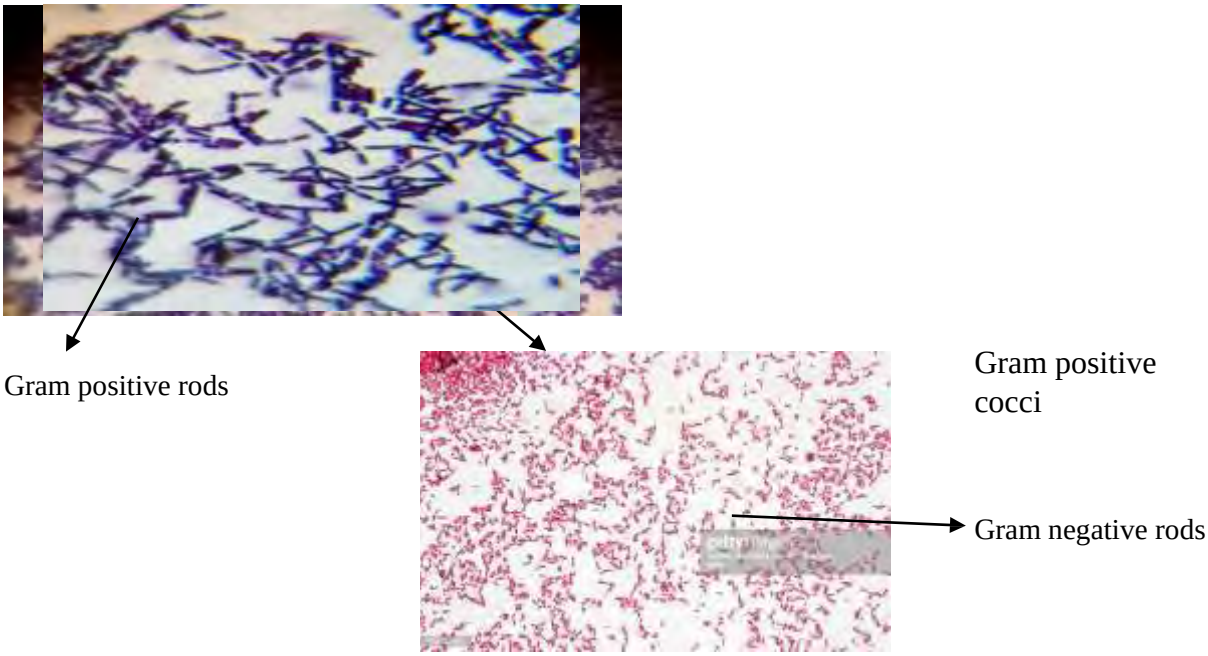


Figure: Gram staining of bacterial isolates

Indole test (positive) **Indole test (negative)**

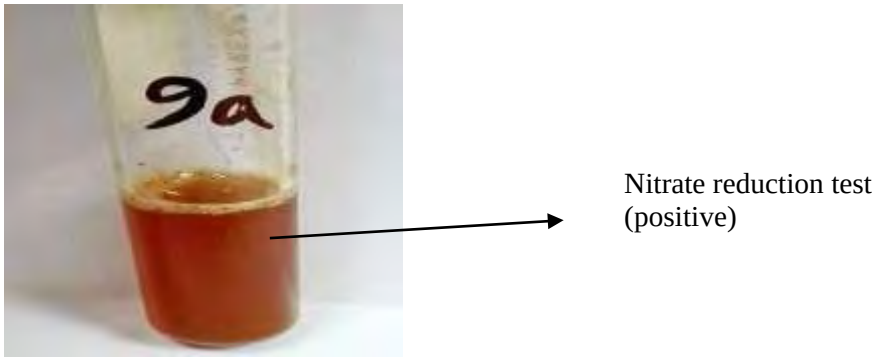
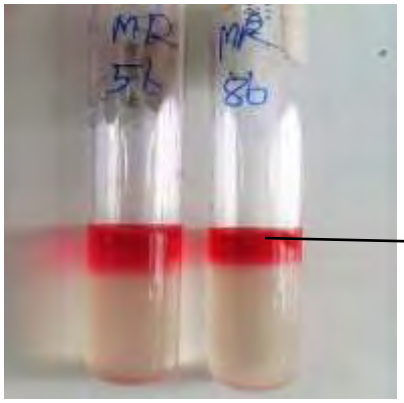


Figure : Biochemical test results of bacterial isolates

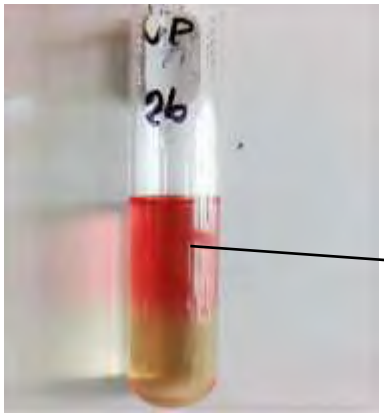


Red color

Methyl red test (positive)

Pink color

Methyl red test (negative)



Red color

Voges-Proskauer test (positive)

No color change

Voges-Proskauer test (negative)

Yellow slant, yellow butt

Red slant, yellow butt

Yellow slant, yellow butt (gas produced)

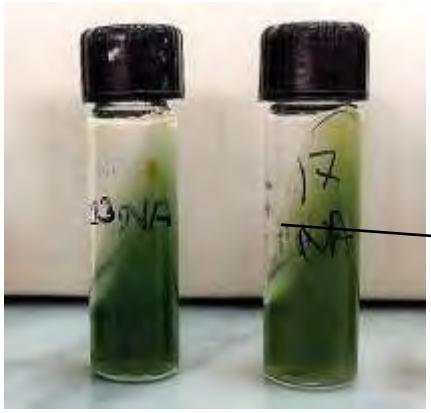
Red slant, red butt

TSI test

Figure.: Biochemical test results of bacterial isolates

Blue color

Citrate test (positive)



No color change

Citrate test (negative)

Pink colour

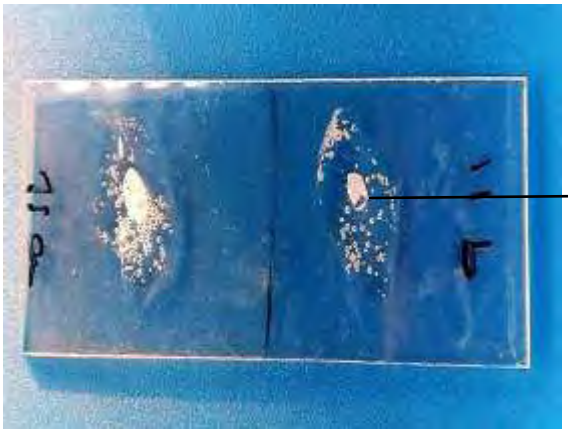
Non-motile

MIU test (Urease +ve, Non-motile)

Yellow color

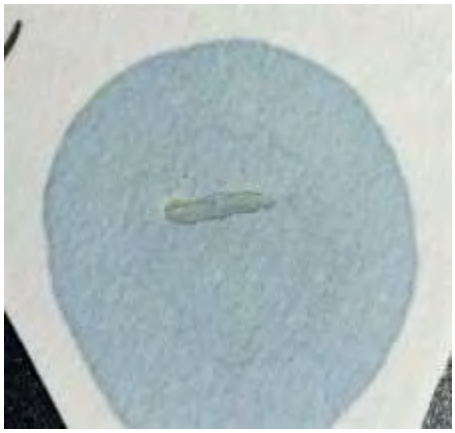
Non-motile

MIU test (Urease -ve, Non-motile)



Bubble formation

Catalase test (positive)

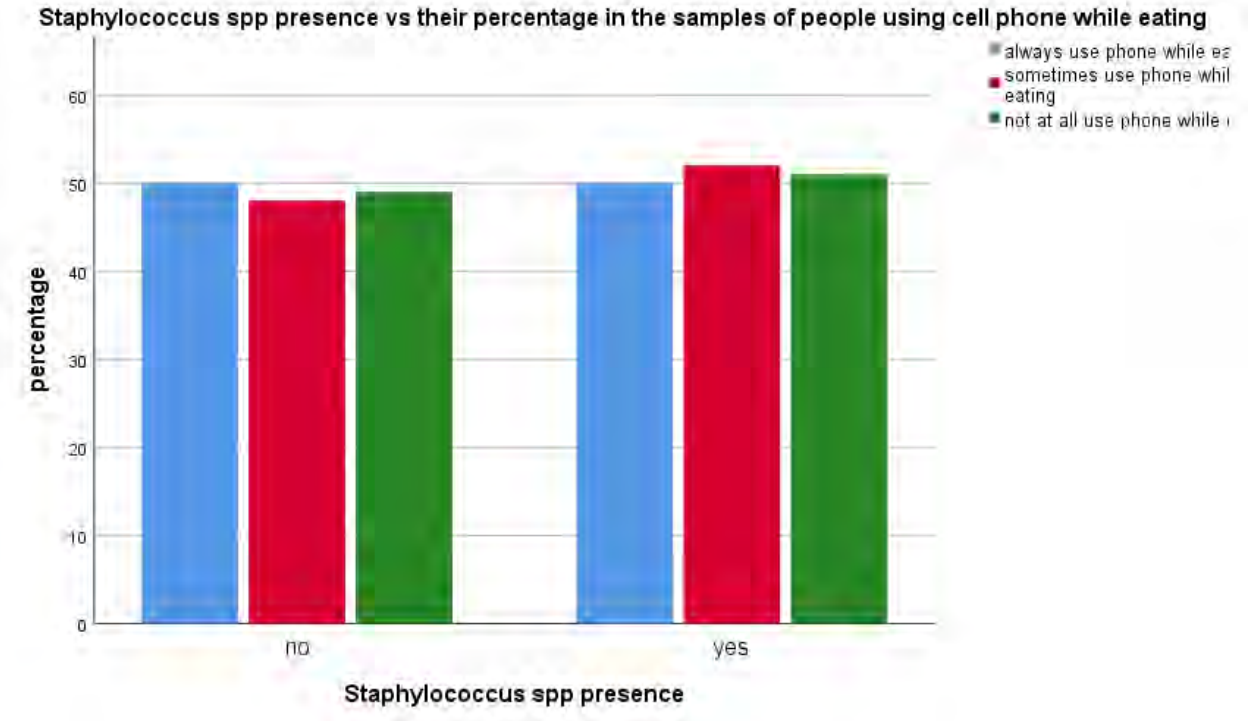


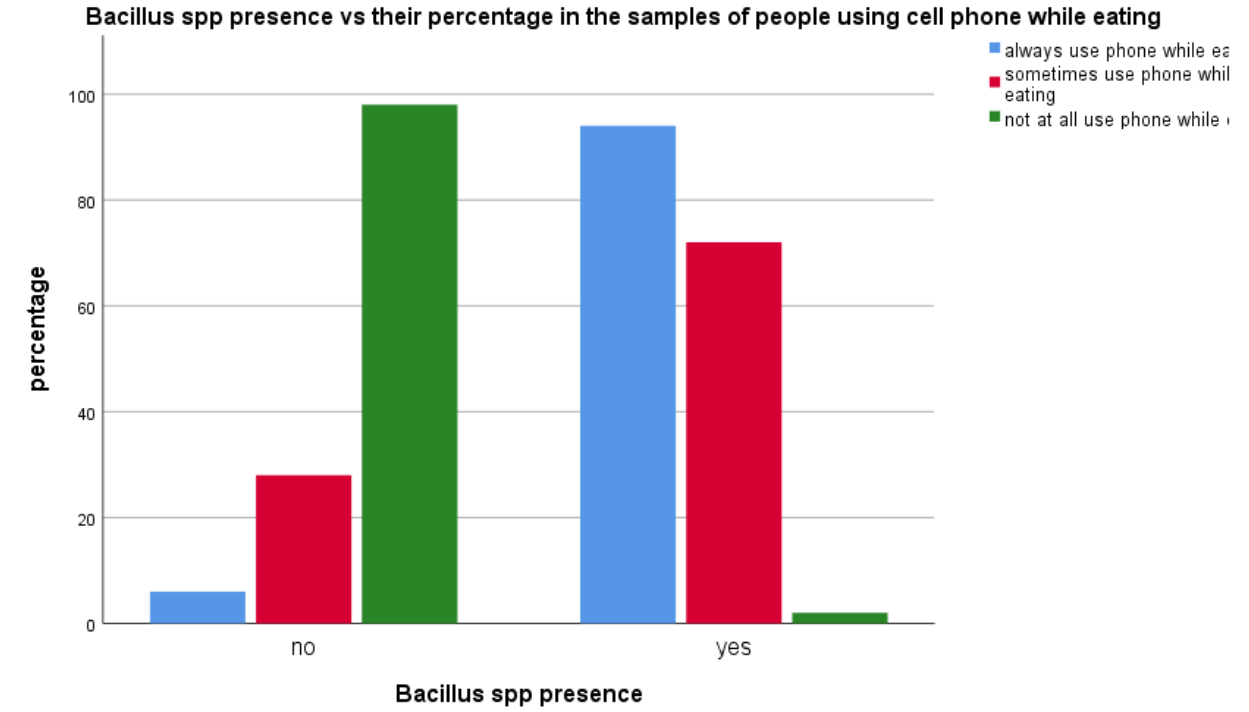
Oxidase Test (Negative)

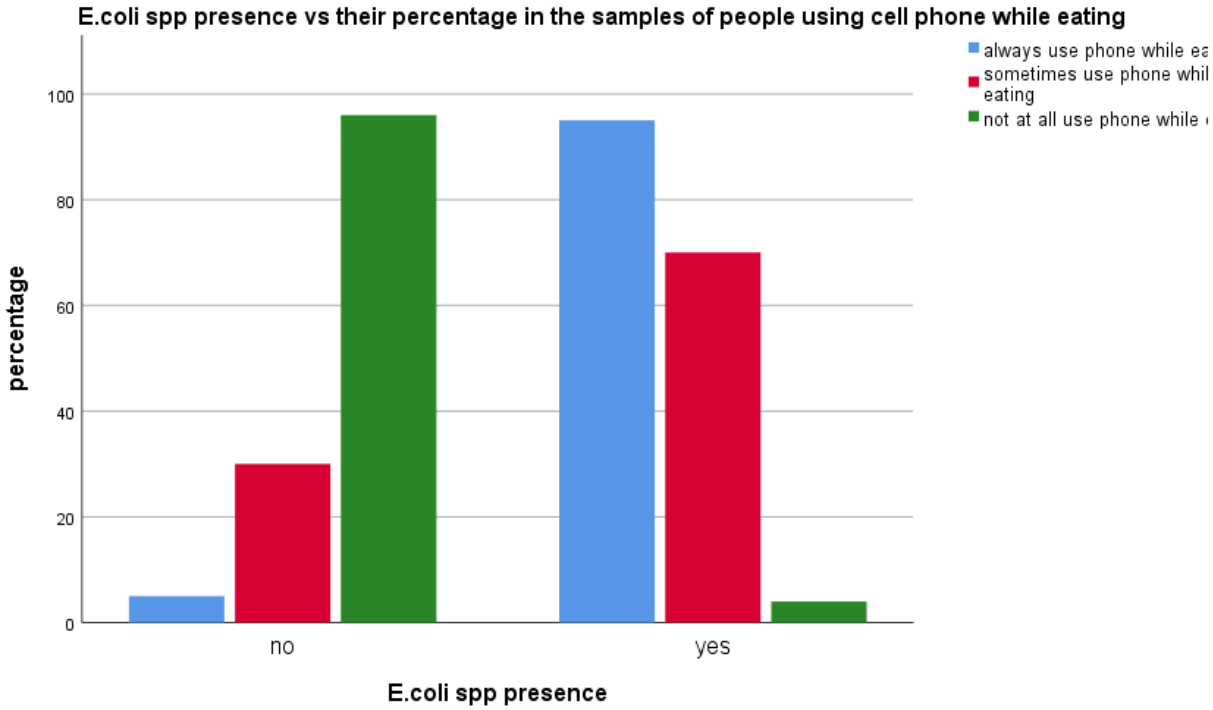
Figure 3.4.: Biochemical test results of bacterial isolates

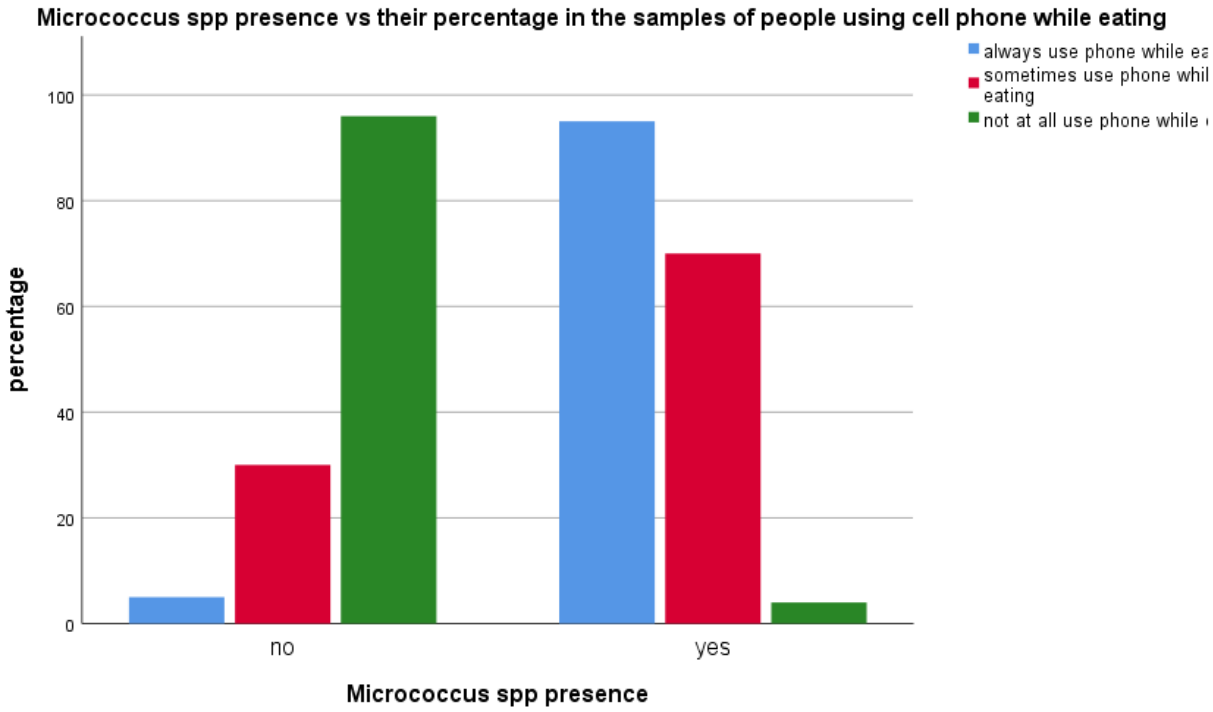
3.4 Correlation between the behaviors of cellphone users and the organisms present in the cellphone

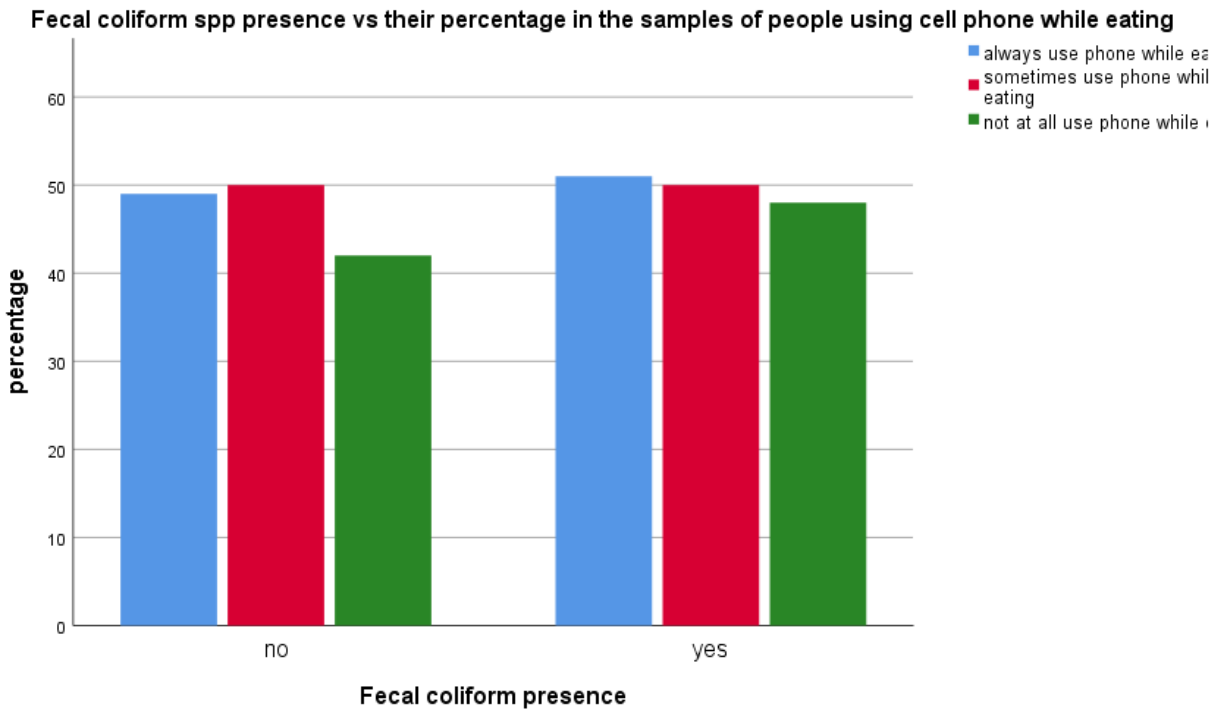
3.4.1 Presence of organism in the samples of people based on whether they use their cellphone while eating.





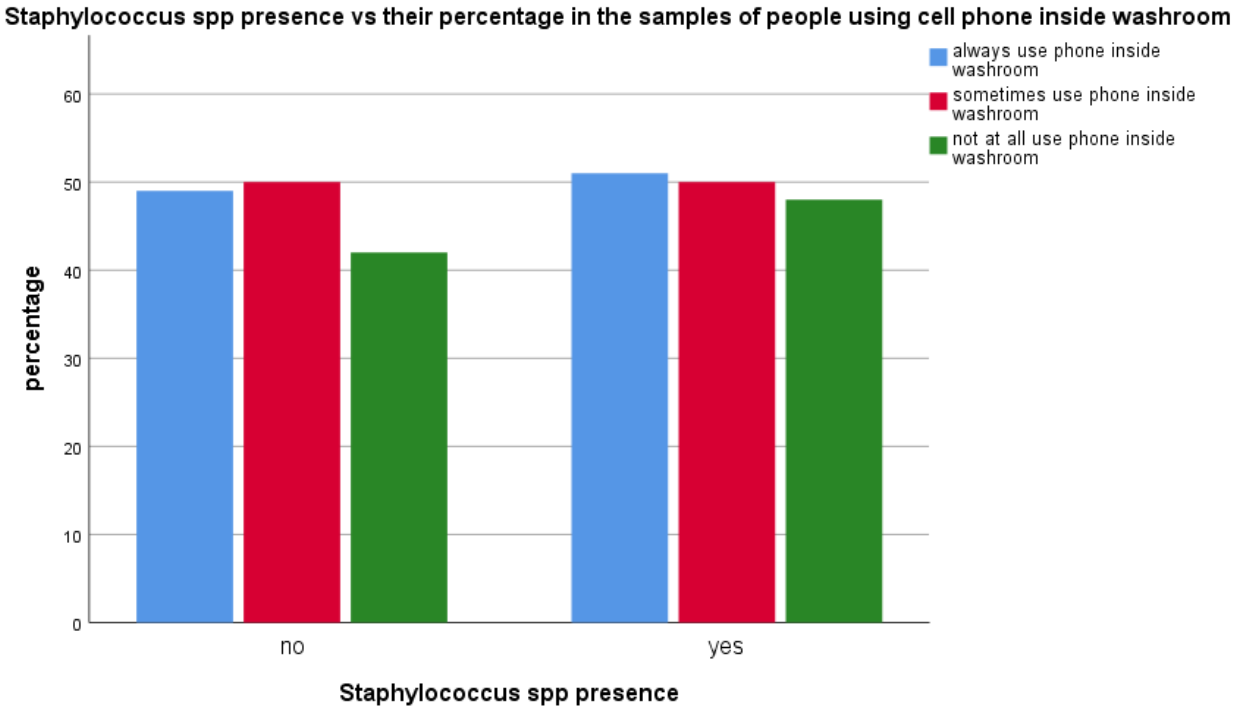

E.coli spp presence vs their percentage in the samples of people using cell phone while eating
always use phone while ea
sometimes use phone whil
eating
not at all use phone while
percentage
100
80
60
40
20
0
no
yes
E.coli spp presence

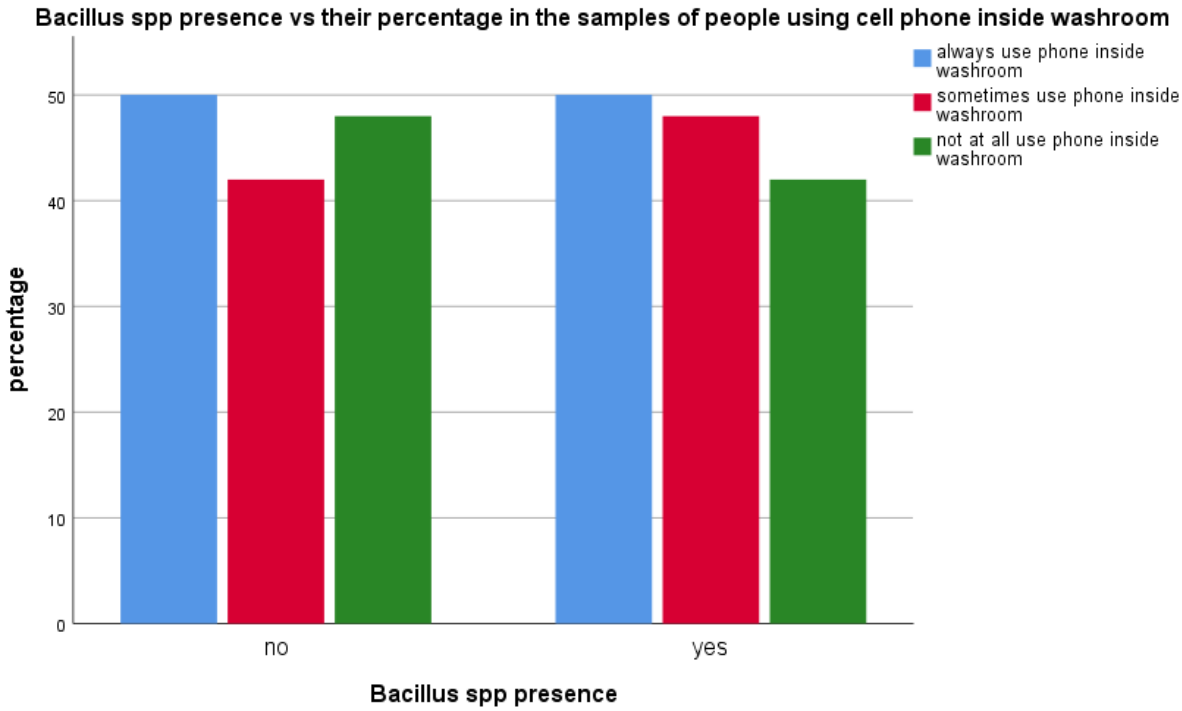

Micrococcus spp presence vs their percentage in the samples of people using cell phone while eating
always use phone while ea
sometimes use phone whil
eating
not at all use phone while
percentage
100
80
60
40
20
0
no
yes
Micrococcus spp presence

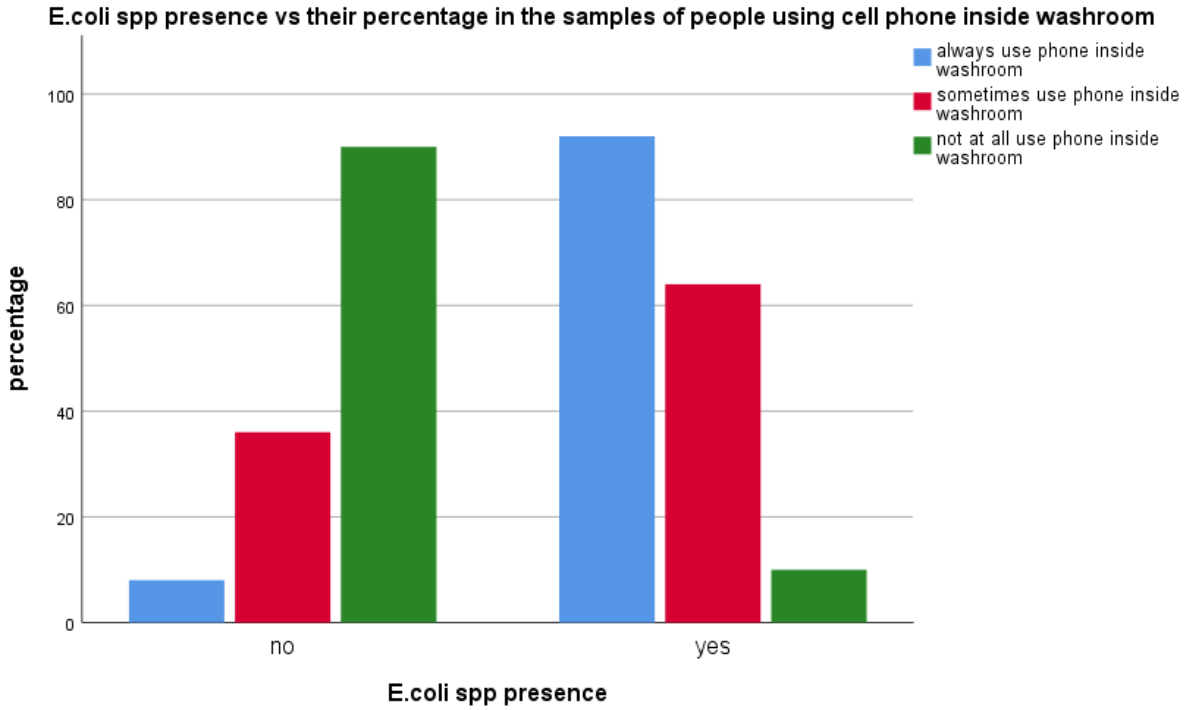


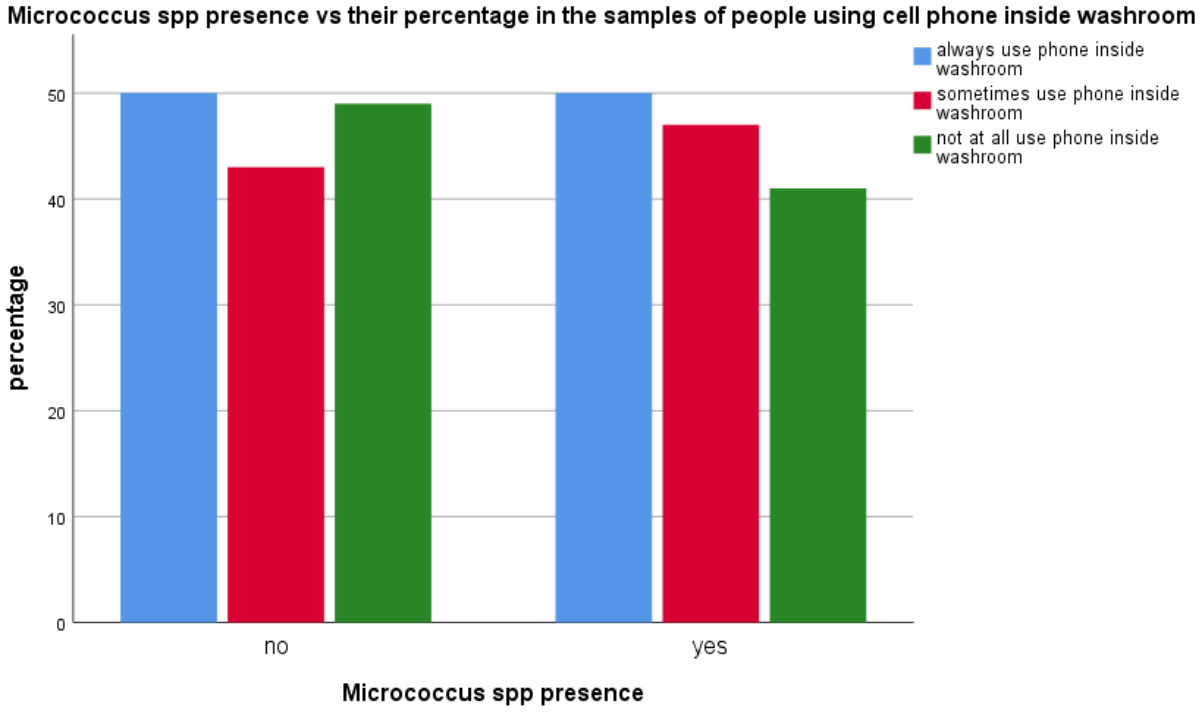
In graph 3.4.1 it is observed that those who used their cellphone while eating had the highest concentration of *Bacillus spp* present in the study, while those who did not use had a minimal *Bacillus Spp* presence of less than 1% of the total bacilli isolated. It also shows *Micrococcus Spp* and E.coli presence in the cellphones whose owners used their phones while eating, which showed a similar result with bacillus highest concentration of *Micrococcus Spp* among those who used a cell phone while eating and lowest concentration amongst those who did not. Fecal Coliform and *Staphylococcus spp* is observed to be neutral in number, averaging at 50% in both usage and non-usage cases, showing no relationship between the behavioral pattern and the presence of these two organisms.

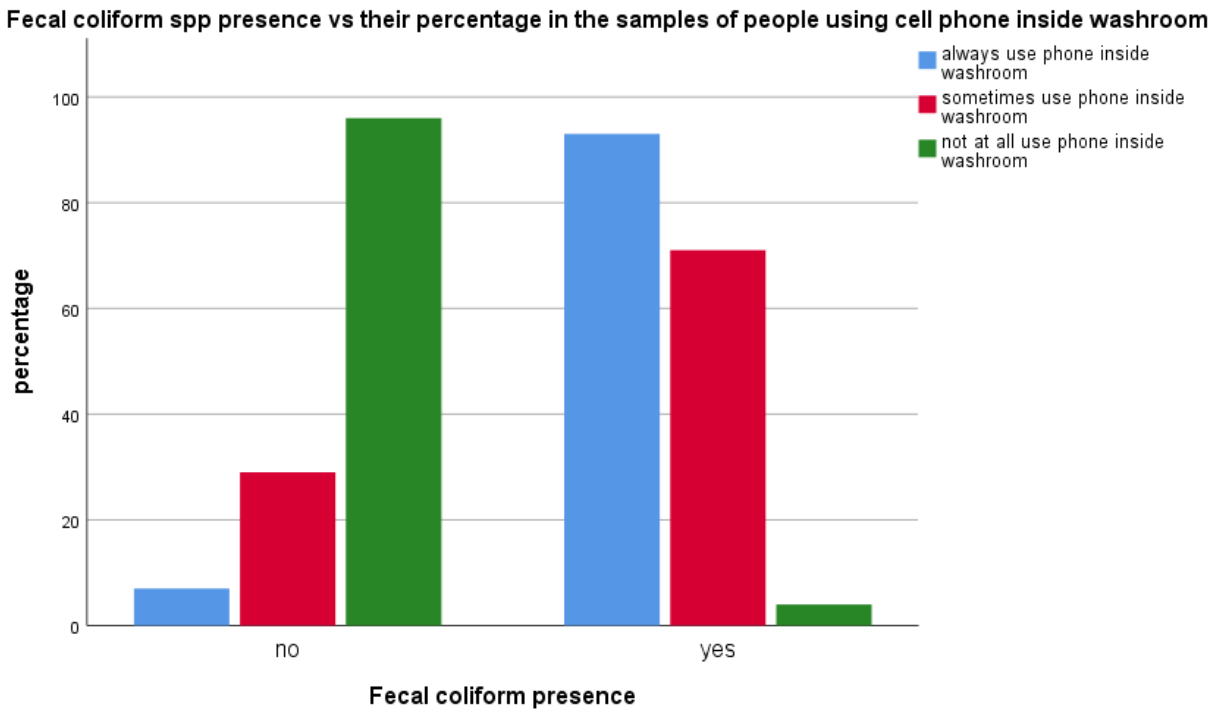
3.4.2 Presence of organism in the samples of people based on whether they use their cellphone inside the washroom





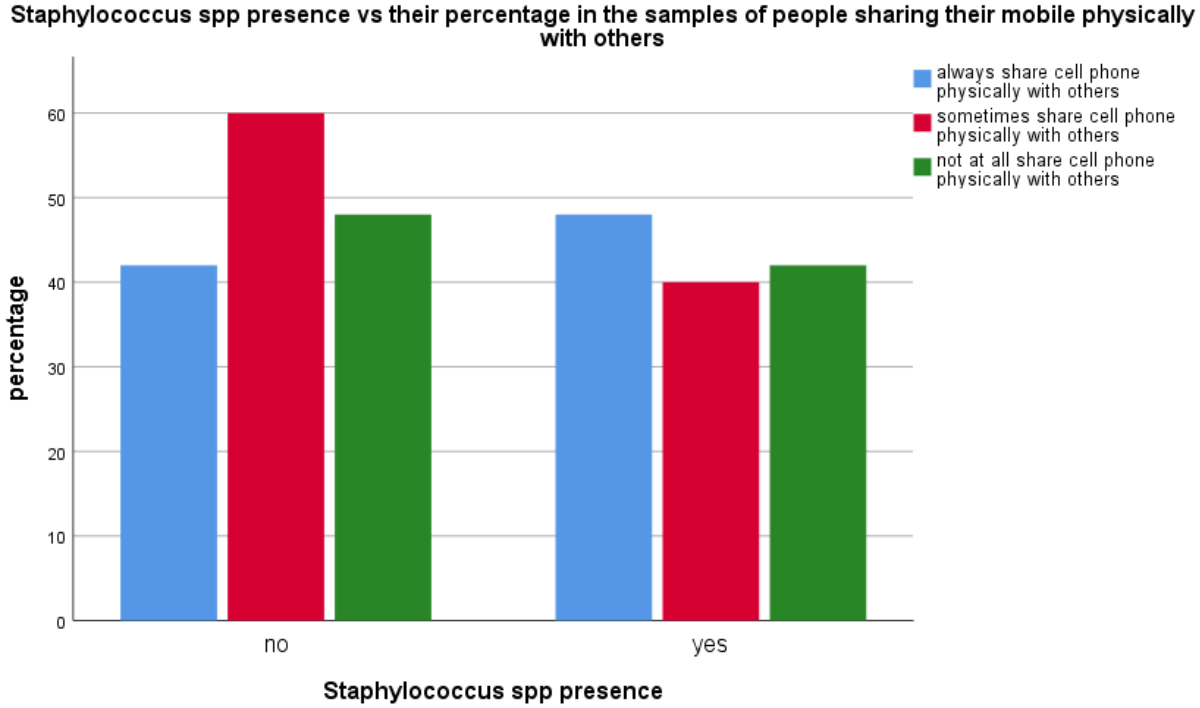

E.coli spp presence vs their percentage in the samples of people using cell phone inside washroom
always use phone inside washroom
sometimes use phone inside washroom
not at all use phone inside washroom
percentage
100
80
60
40
20
0
no
yes
E.coli spp presence

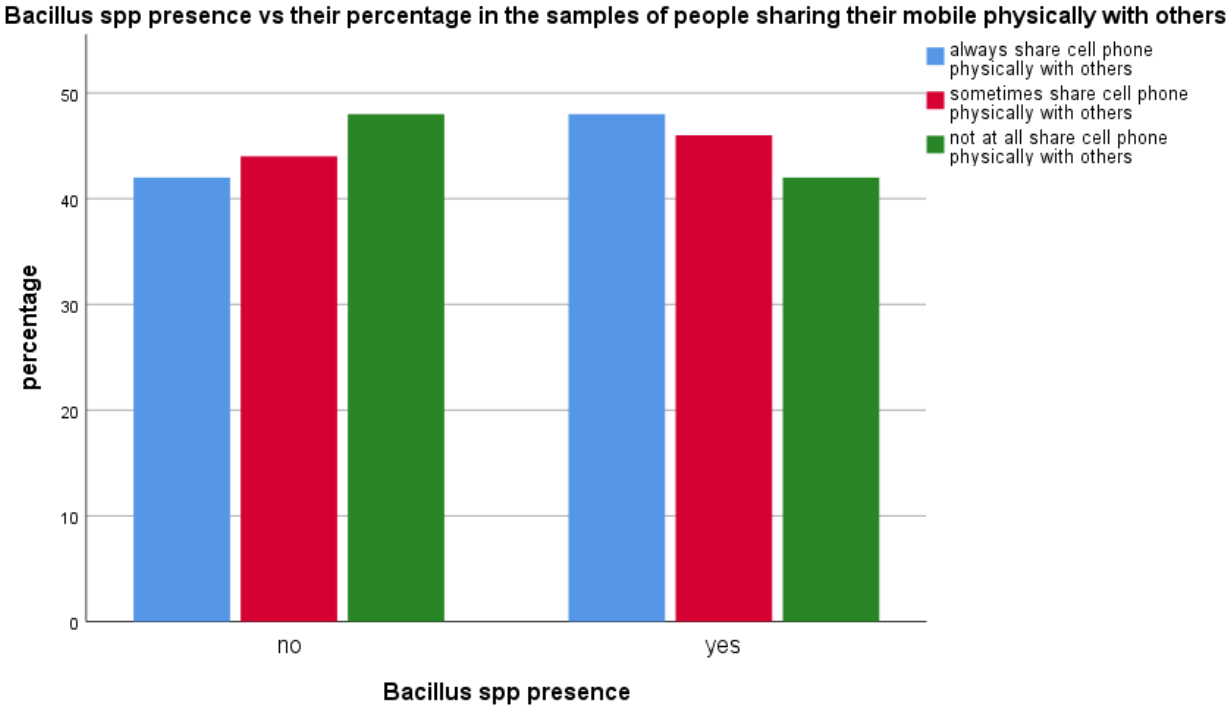

Micrococcus spp presence vs their percentage in the samples of people using cell phone inside washroom
always use phone inside washroom
sometimes use phone inside washroom
not at all use phone inside washroom
percentage
50
40
30
20
10
0
no
yes
Micrococcus spp presence

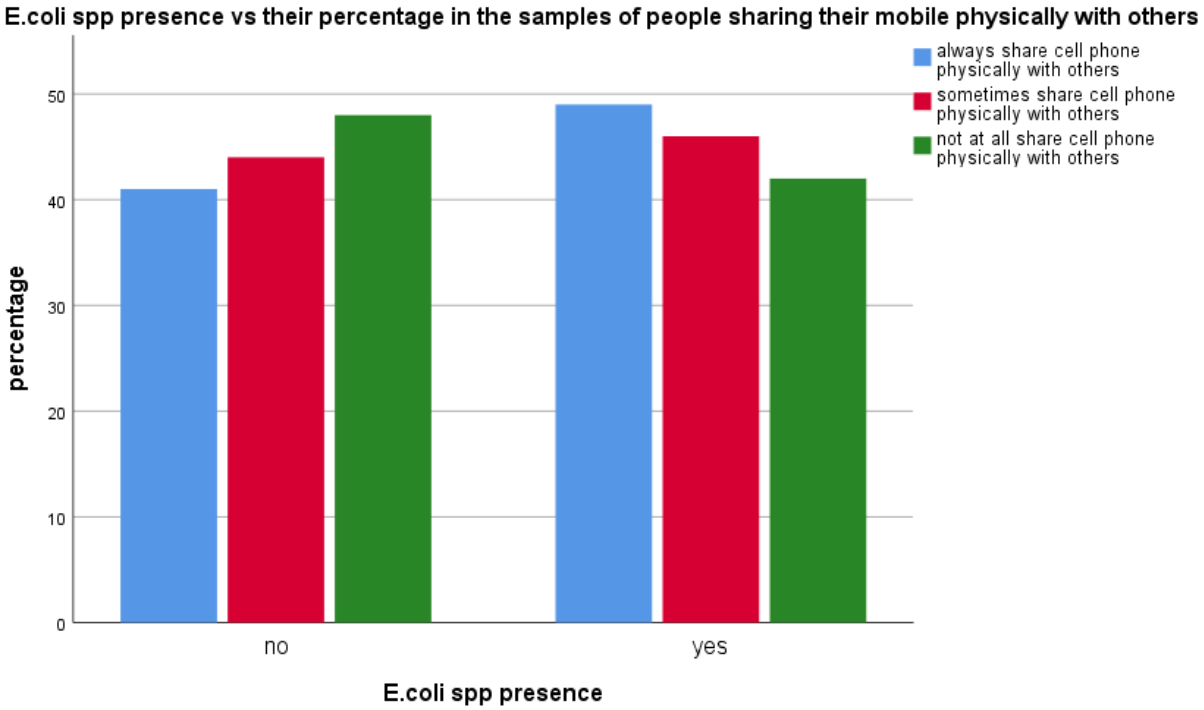


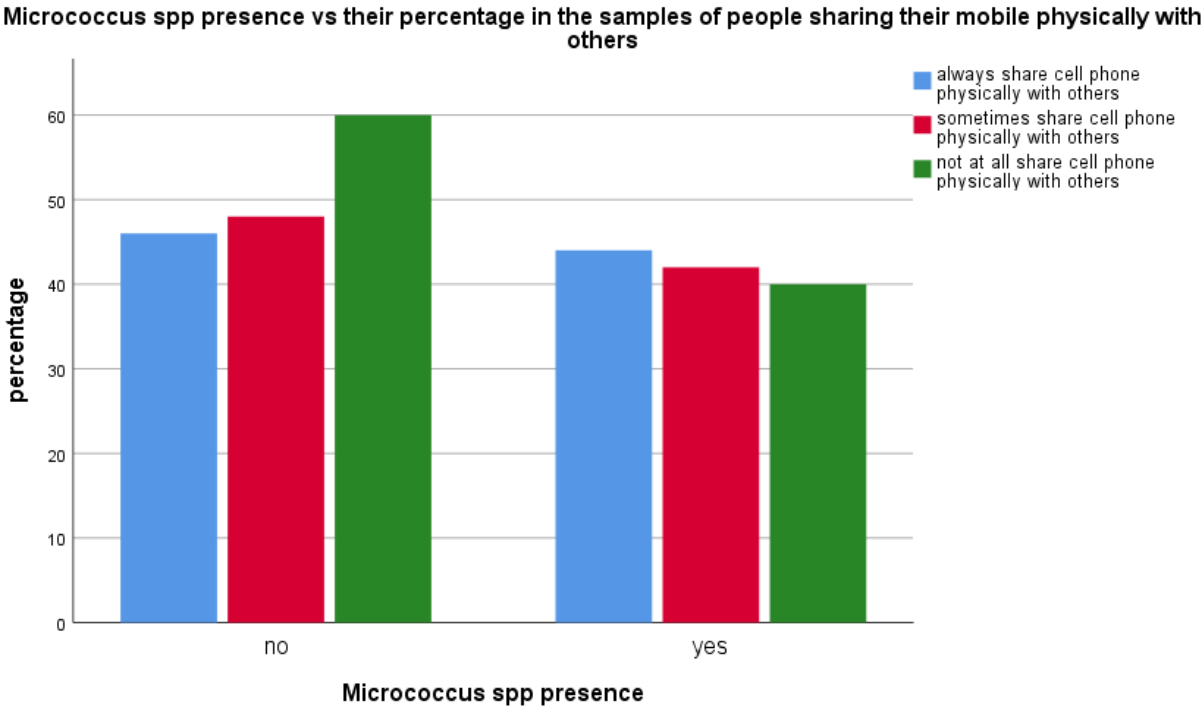
Graph 3.4.2 Shows the presence of Fecal Coliform in people who used their cellphone inside the washroom. The entirety of participants who always uses their cellphone inside the washroom showed Fecal Coliform Presence at 100%. Those who tended to use their cellphone sometimes in the washroom showed 70% presence of Fecal Coliform while those who opt not to use their cellphone inside the washroom showed less than 2% Fecal Coliform Contamination amongst the overall Fecal Coliform Isolates. Presence of E. coli amongst the participants who use their cellphone inside the washroom shows a similar result to Fecal Coliform in the highest concentration of E.coli being present in the phones whose owners used them inside the washroom and less than 3 % presence in those who did not. *Staphylococcus spp*, *Bacillus spp*, and *Micrococcus Spp* show on average almost equal chances of being present in the sample regardless of cell phone usage inside the washroom. With less than 5% difference between their presence, it is observed that cell phone usage in washroom does not affect their presence significantly.

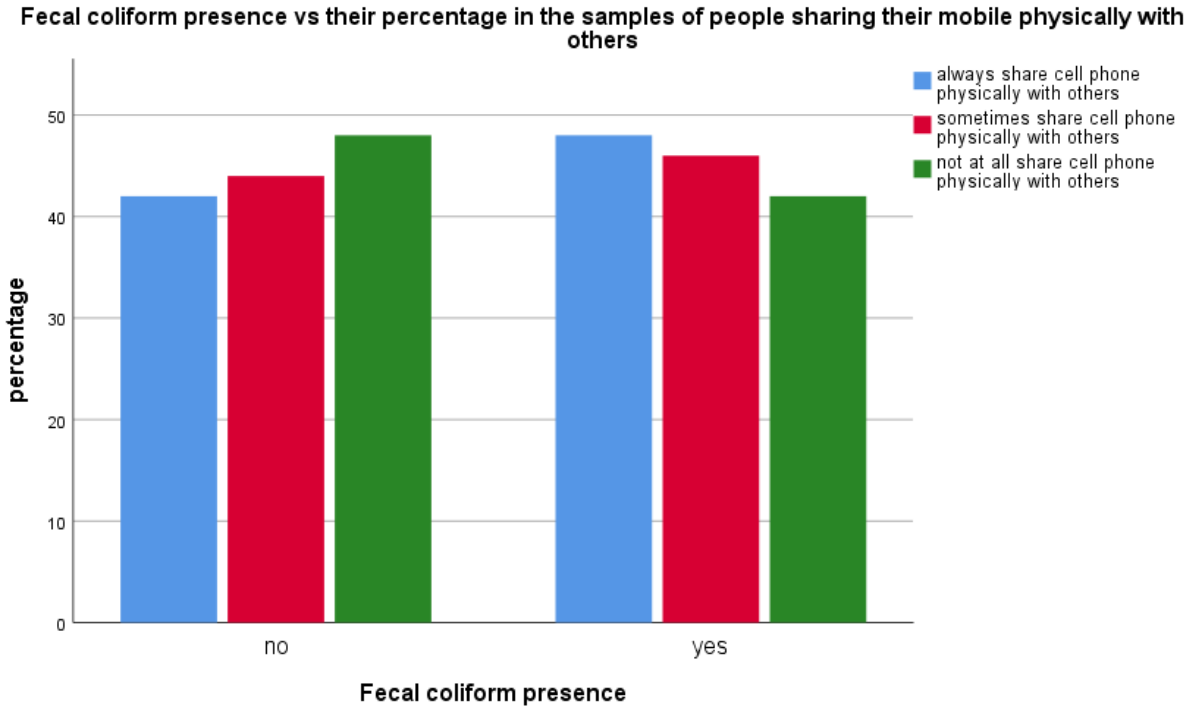
3.4.3 Presence of organism in the samples of people based on whether they share their cellphone physically with others.





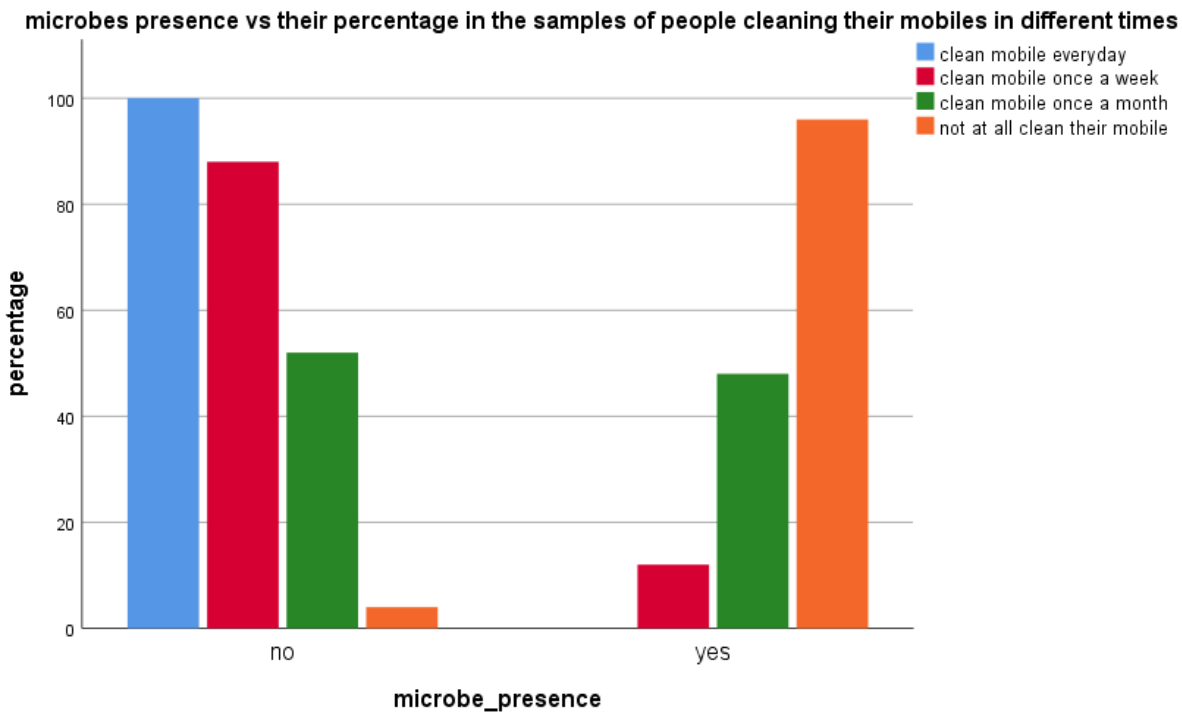

E.coli spp presence vs their percentage in the samples of people sharing their mobile physically with others
always share cell phone physically with others
sometimes share cell phone physically with others
not at all share cell phone physically with others
percentage
0
10
20
30
40
50
no
yes
E.coli spp presence


Micrococcus spp presence vs their percentage in the samples of people sharing their mobile physically with others
always share cell phone physically with others
sometimes share cell phone physically with others
not at all share cell phone physically with others
percentage
0
10
20
30
40
50
60
no
yes
Micrococcus spp presence



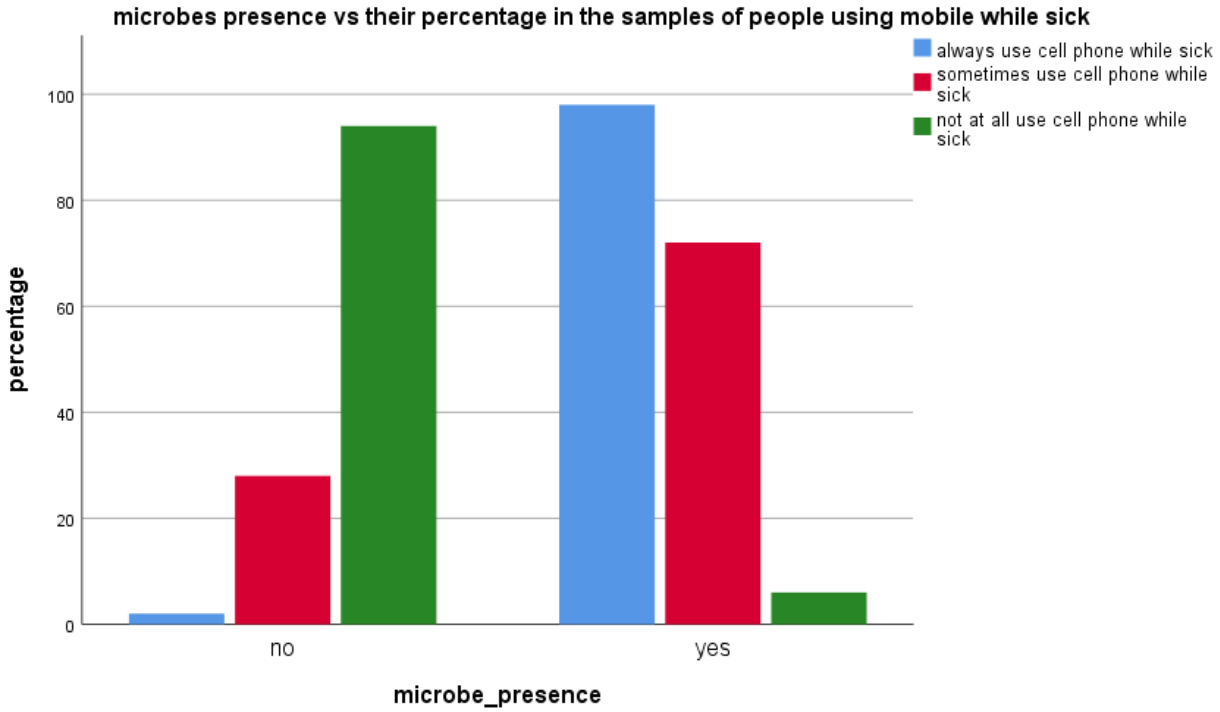
Graph 3.4.3 shows the presence of organisms based on the behavioral pattern associated with sharing the cell phone physically with other people. It is observed that the presence of *Staphylococci spp* is slightly higher at 60% in the ones who share their cellphone sometimes with other people, while those who always share their cellphone physically had slightly lower chance of having *staphylococci spp* presence at 48%.Those who don't share at all is at 42%. *Micrococcus spp* presence is lowest amongst those who do not share their phone, at 40%. *Bacillus spp, E.coli,* and Fecal coliform show similar presence regardless of whether the cell phone is shared physically amongst others or not.

3.4.4 Presence of organism in the samples of people based on whether they clean their cellphones



Graph 3.4.4 shows the impact cellphone hygiene had on the presence of microorganisms on the said sample. The mobile phones that were cleaned every day show an astounding 0% microorganism presence. Amongst those who cleaned their phone once a week, showed around 13% presence of Microorganisms. Those who cleaned their phone on average once a month showed the reduced chance of microorganism presence. Finally, those who opted not to clean their cellphone at all shows microorganism presence in 97% of the cases. Cellphone cleaning here focuses on using commercially available cleaning solution like 70% ethanol, hand sanitizers, and liquid soaps.

3.4.5 Presence of organism in the samples of people based on whether they use their cellphone while being sick

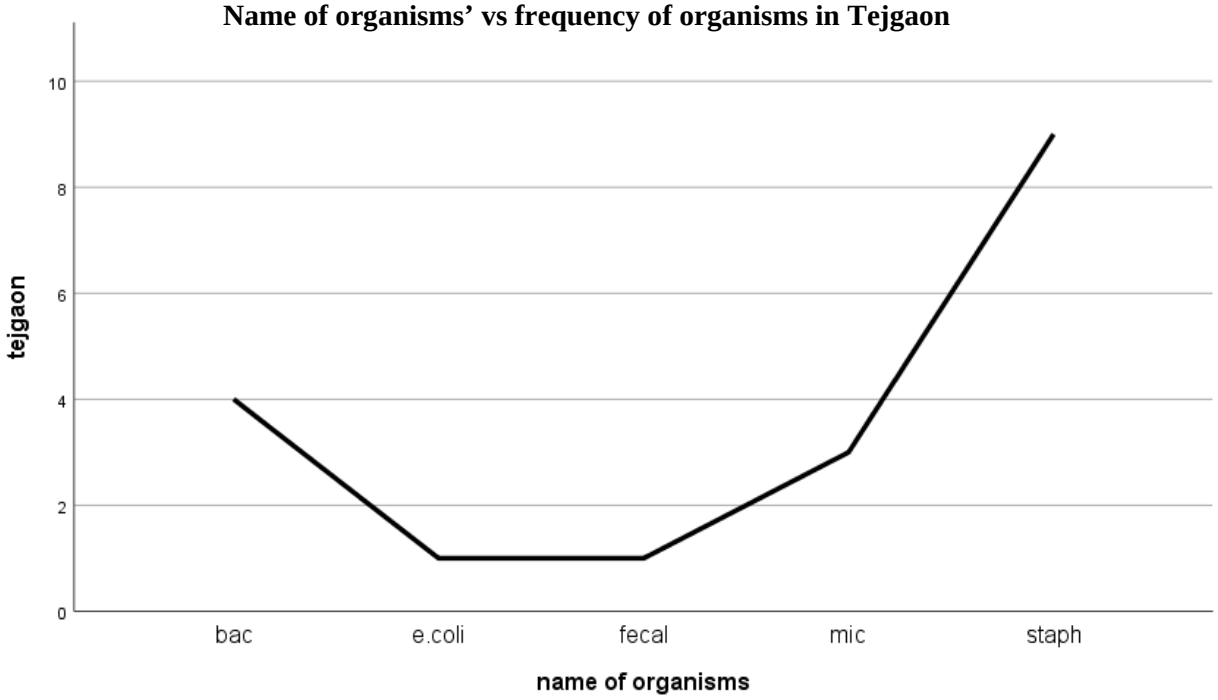


Graph 3.4.5 focuses on the presence of microorganisms on cellphones based on whether they were used while their owner was sick or not. Those who did not use their cellphone while being sick showed less than 5% microorganism present in their cellphones while those who opted to use their cellphone while sick showed microorganism presence in 98% case.

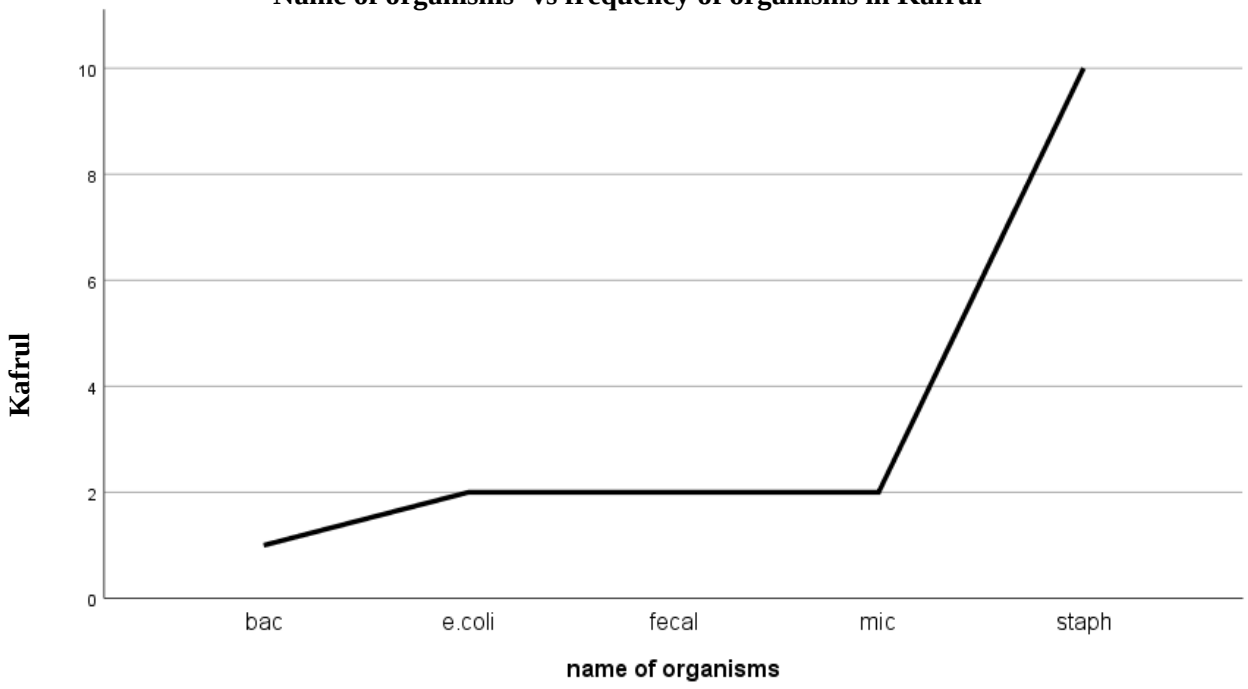
3.5 Name of the organism and the Frequency of its Presence in the Thanas of Dhaka City North

This Graph shows the Frequency of Organisms present in the Thanas of Dhaka City (North). All the graphs show their peak at Staphylococcus spp, showing the abundance of Staphylococcus spp. Highest concentration of E.coli is observed in Mirpur and Gulshan (3.5.3) area, peaking at 6 colonies. Micrococcus spp is observed in the most number at Khilgaon(3.5.7), peaking at number 5. Fecal Coliform also present in the highest number at Khilgaon (3.5.7), peaking at number 7. Bacillus spp is found 4 in number in both Tejgaon (3.5.1) and Khilgaon (3.5.7).

3.5.1 Graph – Frequency of Organisms in Tejgaon.

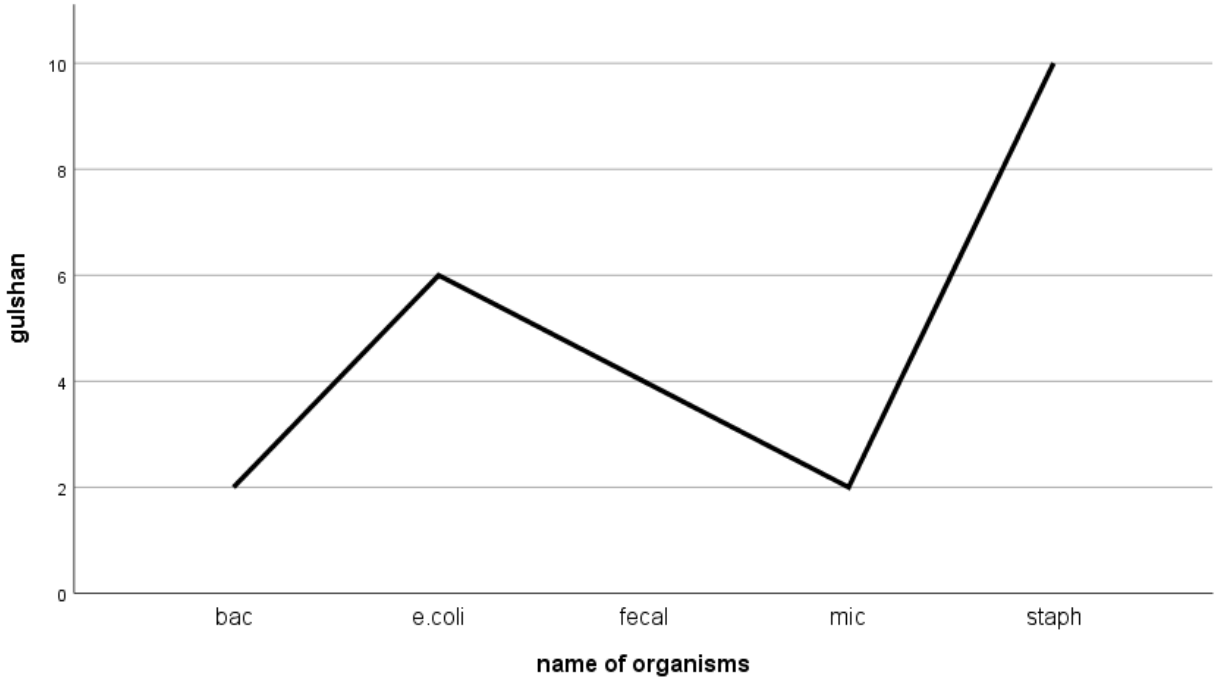


3.5.2 Graph – Frequency of Organisms in Kafrul.



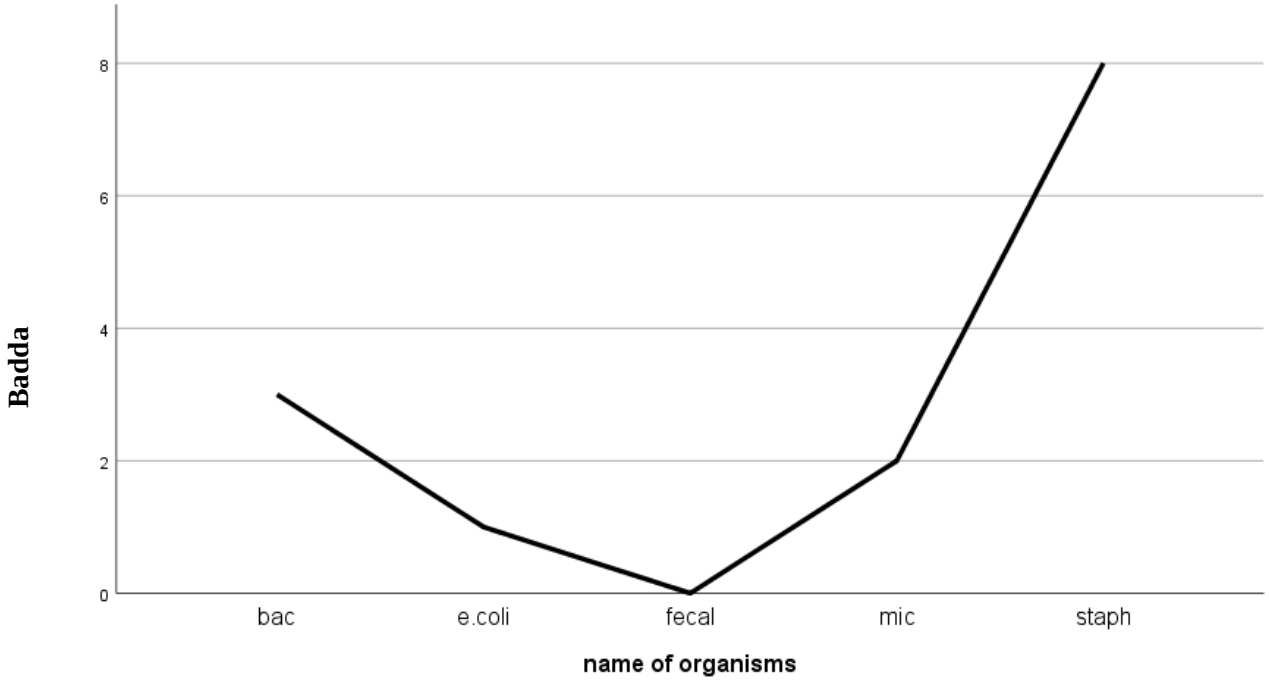
3.5.3 Graph – Frequency of Organisms in Gulshan.

Name of organisms' vs frequency of organisms in Gulshan



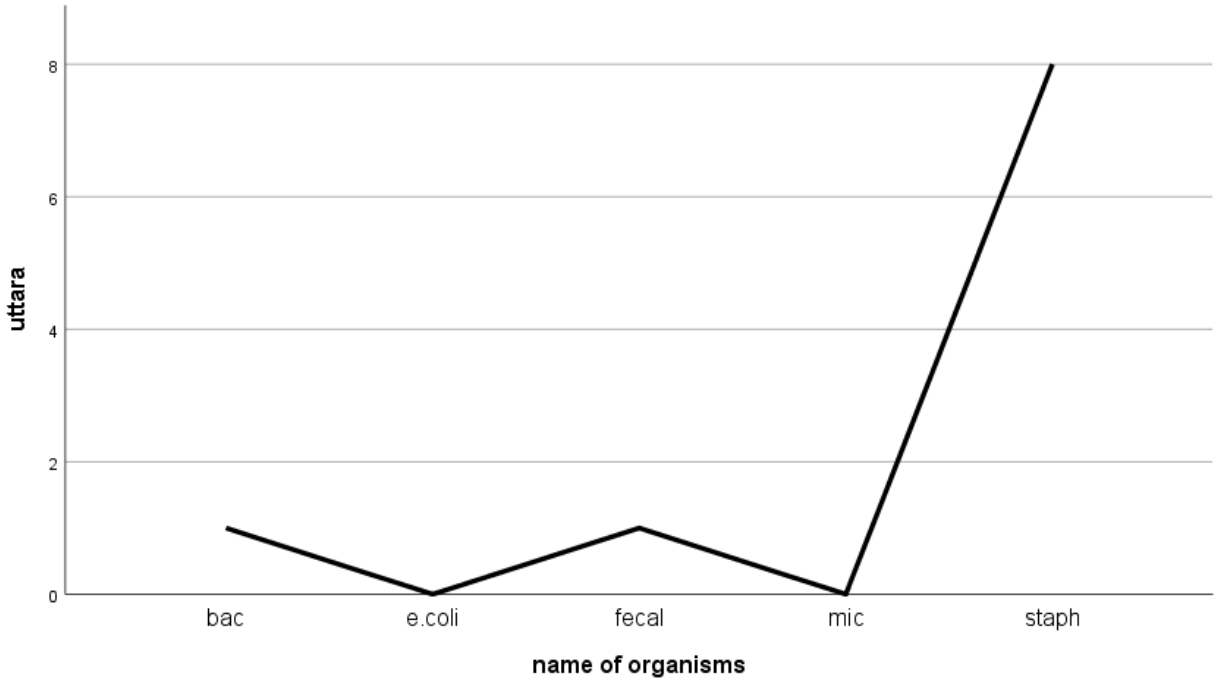
3.5.4 Graph – Frequency of Organisms in Badda

Name of organisms' vs frequency of organisms in Badda



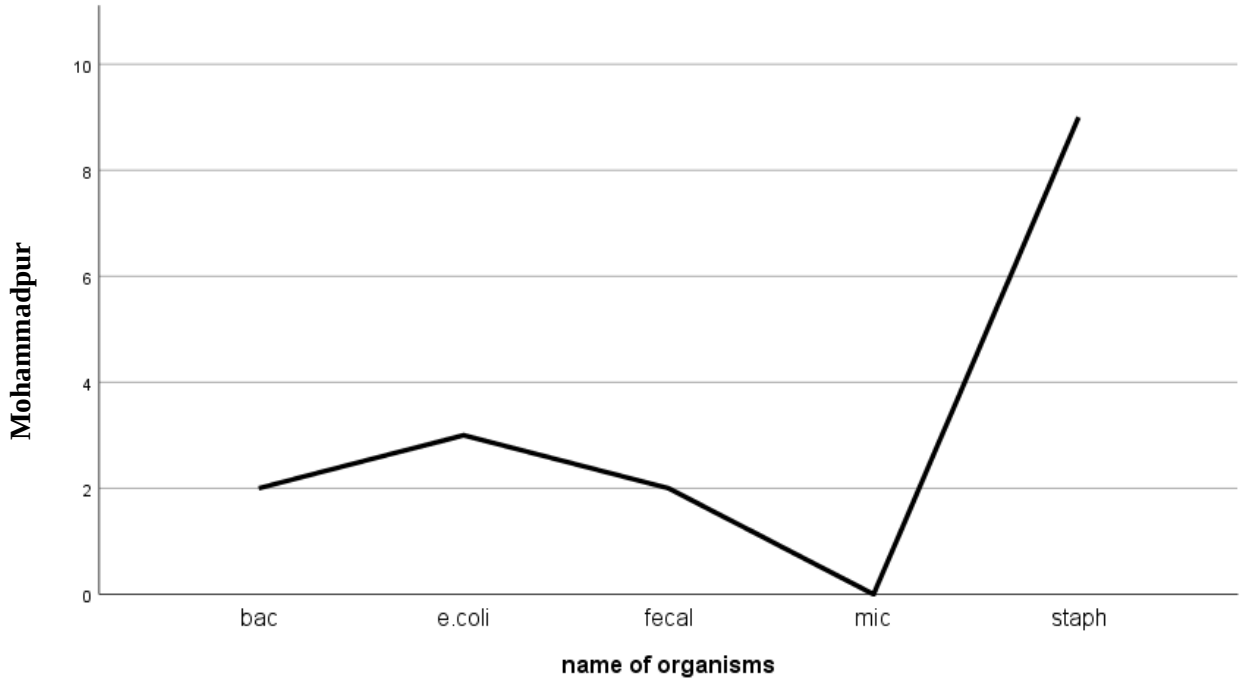
3.5.5 Graph – Frequency of Organisms in Uttara

Name of organisms' vs frequency of organisms in Uttara



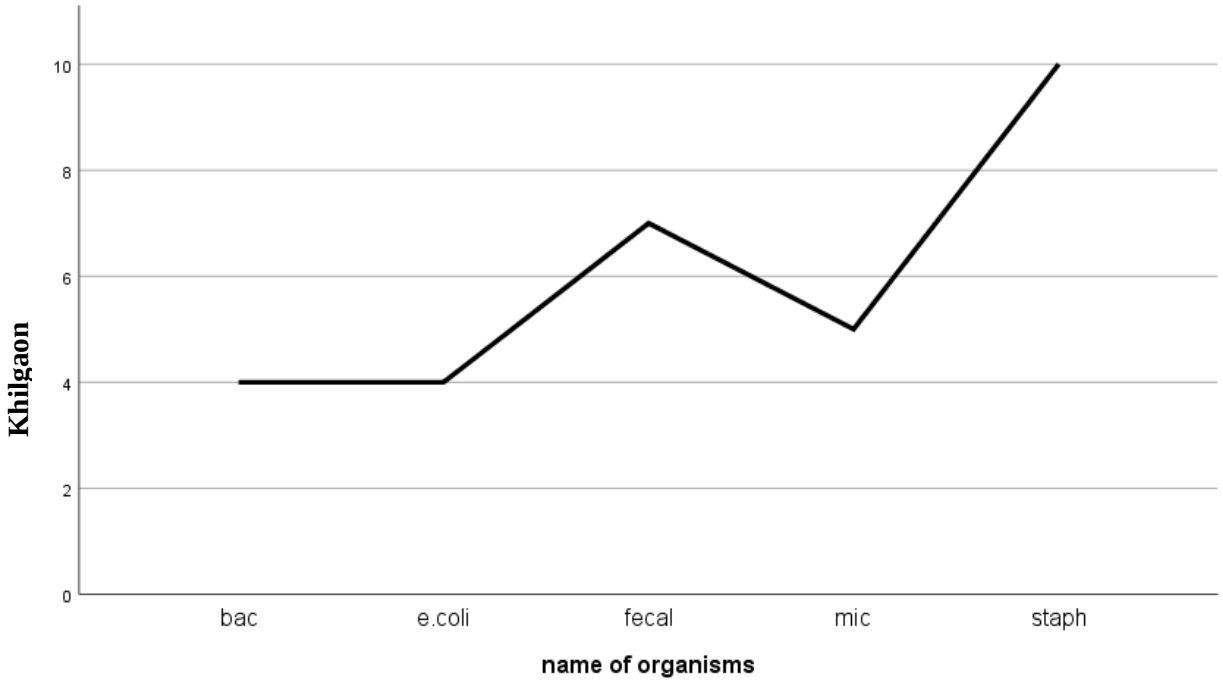
3.5.6 Graph – Frequency of Organisms in Mohammadpur

Name of organisms' vs frequency of organisms in Mohammadpur



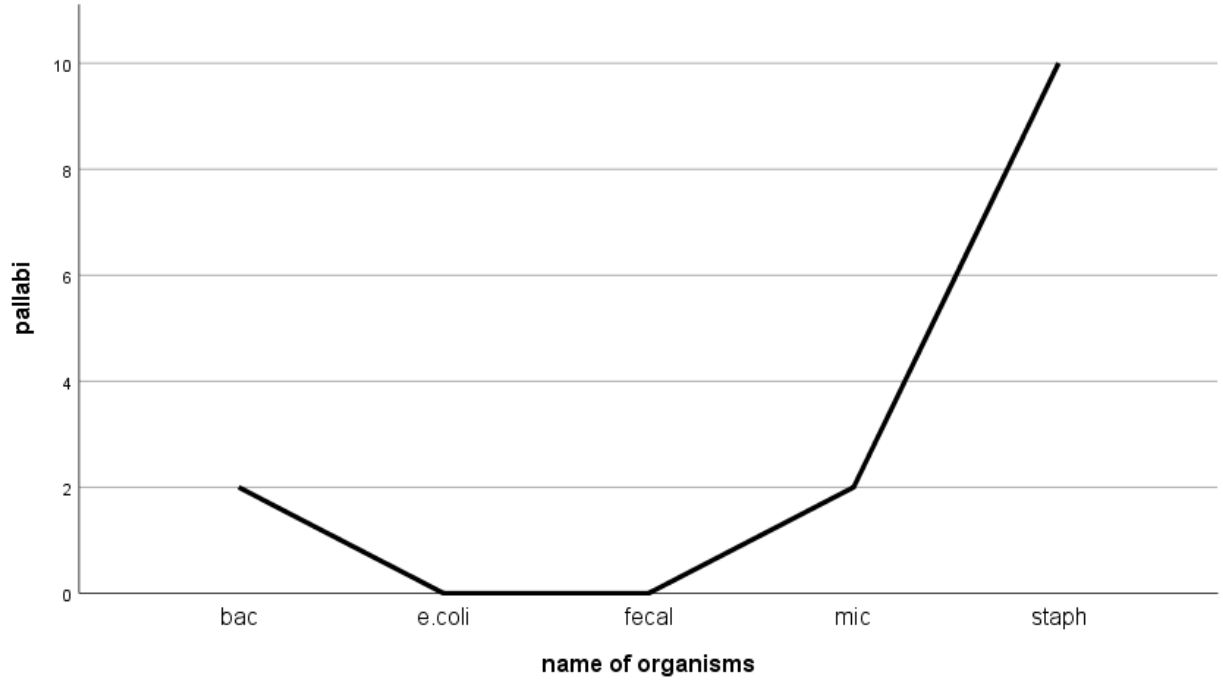
3.5.7 Graph – Frequency of Organisms in Khilgaon.

Name of organisms' vs frequency of organisms in Khilgaon



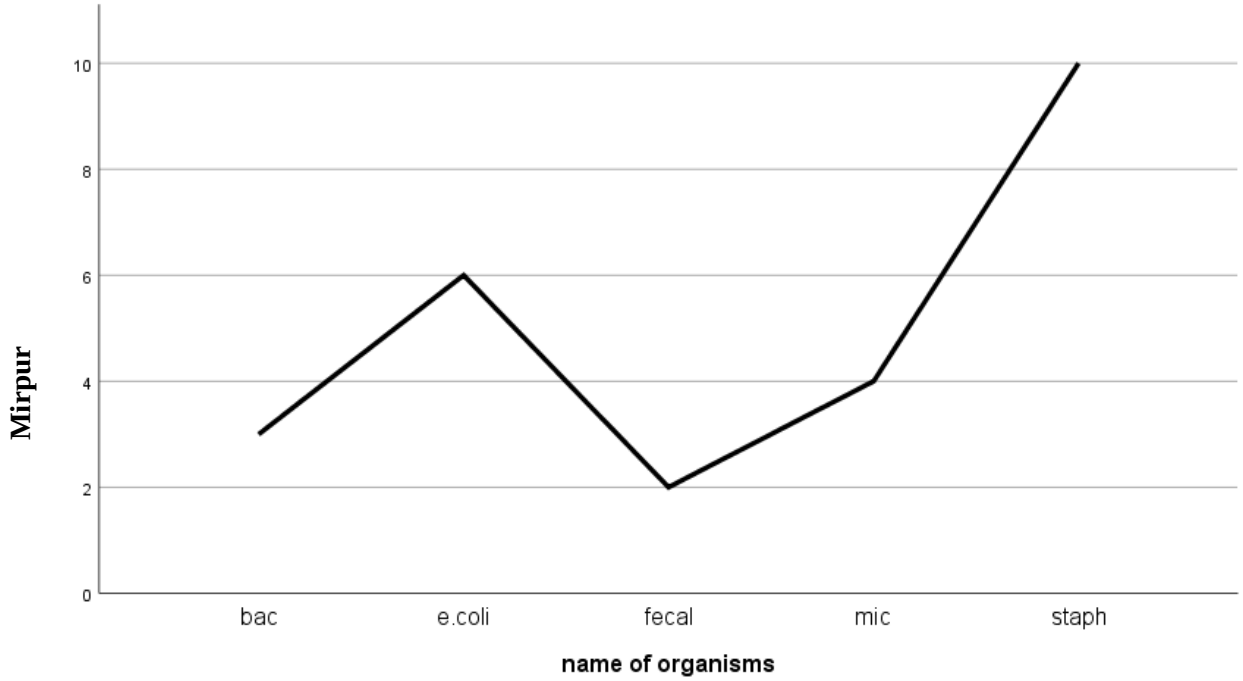
3.5.8 Graph – Frequency of Organisms in Pallabi.

Name of organisms' vs frequency of organisms in Pallabi



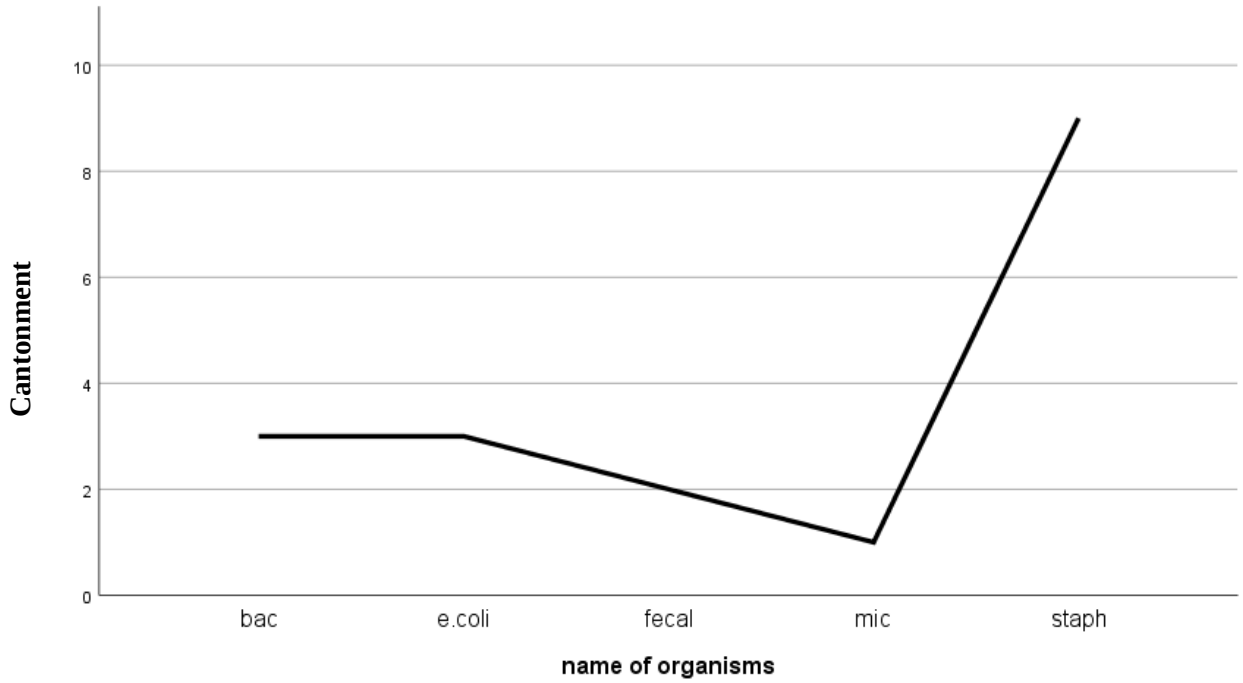
3.5.9 Graph – Frequency of Organisms in Mirpur.

Name of organisms' vs frequency of organisms in Mirpur



3.5.10 Graph – Frequency of Organisms in Cantonment.

Name of organisms' vs frequency of organisms in Cantonment



Chapter 4

Discussion

We are living in a world, which is full of microbes, it is not possible to make this world microbe free but microbiological standards and hygiene practices should be adopted by the society for a healthy life. Cellphones, due to technological advances has become a part and parcel of our everyday lives. They are now used not only for simple voice communication but to access the internet, take photos, communicate, share, purchasing of products, online banking etc. They have become an integral part of our modern society, truly becoming our own personal assistant. This, in turn, has turned a cell phone into an available source of the vector for pathogenic and non-pathogenic microorganisms to spread and propagate, which poses a unique health risk not present in any of the items we use in our daily lives. Bangladesh, being a rapidly developing country and possessing one of the most highly dense population counts in the world, is at most risk in case of an epidemic caused by microorganisms which can use a cellphone as its potential vector. This investigation aimed to isolate and identify bacteria from the cellphones of Dhaka City North and create awareness that mobile could also serve as a vector for transfer these bacteria from one individual to another, therefore personal hygiene and mobile decontamination are very important. Microbiological standards in hygiene are necessary for a healthy life. It is not uncommon, however, to observe practices that deviate from normal standards of hygiene in both the developing and the developed world. This investigation confirms such a deviation, as a variety of microbes, were found on mobile phones.

This type of work focusing on such a diverse and large demographic has not been attempted in Bangladesh before. The survey work, which was based around the north City corporation of Dhaka city was concentrated around the 10 thanas. The samples that were collected were chosen from people from all walks ranging from student to government officers and so forth.

In the survey that was conducted, the overall age of the selected population ranged from 16 to above 30, with the age group of 21- 25 being dominant at being 40% of the participants, closely followed by the age group 16-20 at 32% (Graph 3.1.1). The occupation category is dominated by students, at 62% while the other jobs and occupation is 38% (Graph 3.1.2). The usage of the cell phone is observed on average at 53%, while 32% claimed to use their cellphone a lot. Only 15% is found to not use the cell phone more than out of necessity (Graph 3.1.3). 49% of the participants said that they use their cellphone sometimes while eating along with 20% which

claimed to use their cellphone always while eating (Graph 3.1.4). In the behavioral pattern of using a cellphone in the washroom, 20% claimed to always use the cellphone inside washroom, while 42% uses cellphone sometimes in a washroom and 38% not using mobile phones in the washroom (Graph 3.1.5). Cellphone usage in public Gatherings peaks at usage sometimes at 54%, always at 21% and not at all at 25% (Graph 3.1.6). 60% of the participants claimed to share their cell phone physically with other people sometimes, with 11% claiming to share always and 29% not sharing at all. While being sick, 56% use their cellphone sometime, 10% use their cellphone always even while being sick while 34% doesn't use their cellphone while sick (Graph 3.1.8). Cellphone storage during everyday life is diversified among the participants, ranging from pocket, purse, backpack, hands at 28%, 22%, 20% and 25% respectively (Graph 3.1.9). Other everyday items that are present alongside the cellphone in their storage is mostly Headphones, wallet, keys, Id's, Money, Cosmetics etc. (Graph 3.1.10). Cellphone cleaning was found to be at an alarmingly low level, with only 10% cleaning their cellphones every day, 15% once a week, 20% once a month and consisting of more than half the participants, 54% claimed to not clean their phone at all (Graph 3.1.11). Using a particular cellphone ranged from relatively new at 1 month from 4 years (Graph 3.1.12). 66% of cellphone users used a cover on their cellphone while 34% preferred not to (Graph 3.1.13).

From this 100 Samples, 187 organisms were isolated from 5 different types. The Growth of colonies in Membrane Fecal Coliform Agar (MFC) indicates the possibility of the presence of the fecal contamination on the mobile phone. The presence of Gram-negative rods indicated fecal contamination of mobile phones. Gram-negative sepsis is most commonly caused by *E. coli, Klebsiella* spp *Enterobacter* spp and *Pseudomonas aeruginosa* (Bone *et al.,* 1993). It has also been advanced that the endotoxin or lipopolysaccharide (LPS) produced by members of this group has been implicated as a primary initiator of the pathogenesis of septic shock. Out of these four bacteria used to cause sepsis, we isolated and identified *E. coli* from mobile and the percentage of both the bacteria found to be 14%% in our investigation. The percentage of *E. coli* isolated from a mobile phone was found 28.2% in another study, which is slightly higher than our study (Famurewa and David *et al.,* 2009). In the previous study, it is already reported that mobile phones may get contaminated with such bacteria such as *E. coli*, *Enterococci*, *S. aureus.* They reported that 16.7% of the samples were positive for pathogens known to cause

nosocomial infections. The percentage all the bacteria identified in our study were found higher than this study.

Al-Abdalall *et al*., (2010) analysis of a total of 202 samples was done to identify fungal and pathogenic bacteria isolates. There were 737 isolated of the following bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Neisseria sicca, Micrococcus luteus, Proteus mirabilis, Bacillus subtilis,* and *Enterobacter aerogenes* at the rate of 56.58, 13.57, 8.01, 7.73, 6.51, 3.66, 2.85 and 1.09% respectively. *S. aureus* is a common bacterium found on the skin and the noses of up to 25% of the healthy human beings and animals can cause illness from pimples and boils to pneumonia and meningitis and is a close relative of methicillin-resistant *S. aureus* (MRSA). The main reservoir of *S. aureus* is the hand from where it is introduced into food during preparation. In the previous study, the percentage of *Streptococcus spp.* and *Staphylococcus* from the personal mobile phone was reported 1% and 19% respectively (Yusha'u *et al.,* 2010). In the study of Morubagal *et al*., (2017) a total of 175 samples were examined from which 203 bacteria were isolated. Out of which, 90 (43.68%) were *Staphylococcus* species, in the present course of investigation percentage of *Staphylococcus* was found to be on 93.6% of the cellphone, which was very much higher than the previous report. The number of the colony itself was 94, which is 50.8% of the overall colony count. Phones of students had the largest variety of bacteria in this study. This may be as a result of long-time exposure to the environment and very high usage during everyday work. Multiple usage and exposure of mobile phones to environmental microbes on the hand and skin of the users may have contributed to the level of isolation of bacteria from commercial phones in the present study. This agrees with the previous findings Rusin *et al., (*2000). Dave and Shendes *et al.,* (2015) research findings in urban India indicate that *Staphylococcus aureus* (52.7%), *Staphylococcus epidermidis* (17.06%), *Pseudomonas aeruginosa* (12.2), *Micrococcus luteus* (9.1), *Enterobacter aerogenes* (1.8%) and *Bacillus subtilis* (7.07%) are the main bacterial isolates frequently associated with mobile phones. The results showed that mobile phones were contaminated with different types of bacteria mentioned above. Therefore, due to personal nature of individuals and proximity to the sensitive part of our bodies in usage such as faces, ears, lips, and hands of users could become veritable reservoirs of pathogens that could result in infections. *Bacillus subtilis* with a 100% frequency of occurrence has been identified as an important organism in food spoilage. This undoubtedly contributes a great deal to food spoilage and the contamination of food if food is prepared or eaten with infected hands.

The percentage of bacterial contamination on the tested cell phones was 92%, of which the most abundant isolates were coagulase-negative *Staphylococci spp*, which accounted for > 90% of the total samples. Gram-positive bacilli were isolated from 25 (13.5%) samples. Although most cell phones tested were contaminated with one or more microorganisms, contamination with *Staphylococcus spp* was found in 94 cell phones. This represents a high percentage of contamination with this pathogenic organism that is commonly found in toilets. Evidence from the previous study conducted by Kotris *et al*., (2016) revealed that ~20% of cell phones belonging to doctors and nurses are contaminated with pathogenic bacteria. Given that medical students are present in health-care settings, mobile devices belonging to this group might as vehicles for the transmission of infection to patients if these devices are not used cautiously. The concern about cell phone contamination in medical settings is increased due to the possibility of cross-contamination of these devices that act as an environmental reservoir and source of bacterial cross-contamination. Two-thirds of the cell phones examined in our study had never been decontaminated. One of the most recommended methods of decontamination is cleaning the cell phone with 70% alcohol, which showed a significant decrease in the number of bacterial contaminants. Continuous visual reminders such as leaflets and posters about cell phone restrictions and hand hygiene can be included in good infection control practices. Although hand hygiene is one of the basic infection control measures, many authors strongly recommend further focus on this issue, providing more evidence about its importance in this context. The ability of pathogens to survive on the surface of cellphones, the survival time, and the risk of transmitting these pathogens to patients should be examined. Therefore, more studies are required to guarantee that they are aligned with the guidelines on infection control, to decrease the potential of transmitting pathogenic organisms found on cell phones.

The overall implication of these results is that mobile phones which make communication easy and accessible also form good carriers of pathogenic agents of disease transmission. If care is not taken, they could be vehicles for the transmission of biological weapons. *Karabay et al.*, (2007) reported that mobile phones may get contaminated with such bacteria as *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, which cause severe infections, and may serve as a vehicle for the spread of nosocomial pathogens. Users of mobile phones are found everywhere: in the market, the home, hospitals, and schools. They could, therefore, be the cause of the spread of the infection in the community. Our results indicate that isolates were associated

with various strata of society. In addition, people should be informed that these devices may be a source for transmission of hospital-acquired infections to and from the community. Further studies for the possible means of decontamination of mobile phones, such as the use of alcohol and/or disinfection tissues, should be found and employed in safety. Micro-organisms can be transferred from person to person or from inanimate objects (such as pagers, ballpoint pens, hospital charts, computer keyboards, mobile phones and fixed telephones) to hands and vice versa.

Karabay et al., (2007) found that most of the organisms isolated were skin flora. However, in his paper, 16.7% of the samples were positive for pathogens known to be associated with nosocomial transmissions, such as *Enterococci spp*, *S. aureus*, and *K. pneumonia*. Other investigators reported that telephones, intercoms and bedpan flusher handles may be contaminated with potentially pathogenic bacteria. *Jeske et al., (2010)* also reported that bacterial contamination of anesthetists' hands by personal mobile phones occurred, (38/40 physicians, 4/40 with human pathogen bacteria) in the operating theatre.

More than half of our population own mobile phones based on telecommunication information ministry, which estimates the number at a whopping 80 million, and increasing technological applications have led to increased use of these devices to provide better communication between people. However, the increased use of mobile phones is seen against a background rise in the rate of nosocomial infections. Since the restriction of the use of mobile phones is not effective for the prevention of the spread of nosocomial infections it is necessary to develop effective preventive strategies that will include environmental decontamination, hand hygiene, surveillance, and contact isolation for the prevention of these nosocomial infections. Simple cleaning of computers and telephones with 70% isopropyl alcohol may decrease the bacterial load. Further studies may be warranted to check the antimicrobial capability of the microorganisms found on the cellphone, identification of other microorganisms' present, Plasmid profiling of identified organism and pathogenicity of the organisms found.

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Appendix

Appendix- I

Media compositions

The composition of all media used in the study is given below:

Nutrient Agar

Component	Amount (g/L)
Peptone	5.0
Sodium chloride	5.0
Beef extract	3.0
Agar	15.0
Final pH	7.0

Saline

Component	Amount (g/L)
Sodium Chloride	9.0

Nutrient broth

Component	Amount (g/L)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH	7.4±0.2 at 25ºC

Mannitol Salt Agar

Component	Amount (g/L)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	75.0
D-mannitol	10.0
Phenol red	0.025
Agar	15.0
Final pH	7.4 ± 0.2 at 25°C

MacConkey Agar

Component	Amount (g/L)
Peptic digest of animal tissue	1.5
Casein enzymatic hydrolysate	1.5
Pancreatic digest of gelatin	17.0
Lactose	10.0
Bile salt	1.50
Crystal violet	0.001
Neutral red	0.03
Agar	15.0
Final pH	7.1 ± 0.2 at 25°C

Blood Agar Base

Component	Amount (g/L)
Beef heart infusion from (beef extract)	500.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0
Final pH	6.8 ± 0.2 at 25°C

Eosine Methylene Blue Agar (EMB):

Component	Amount (g/L)
Peptone	10.0
Dipotassium Phosphate	2.0
Lactose	5.0
Sucrose	5.0
Eosin yellow	0.14
Methylene Blue	0.065
Agar	13.50
Final pH	7.1 ± 0.2 at 25°C

Bacillus cereus Agar (BC Agar):

Component	Amount (g/L)
Peptic digest of animal tissue	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium phosphate	2.5
Monopotassium phosphate	0.25
Sodium pyruvate	10.0
Bromo thymol blue	0.12
Agar	15.0
Final pH	7.12± 0.2 at 25°C

Muller Hinton Agar

Component	Amount (g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH	7.3± 0.1 at 25°C

HiCrome UTI Agar:

Component	Amount (g/L)
Peptic digest of animal tissue	15.0
Chromogenic mixture	26.80
Agar	15.0
Final pH	7.1 ± 0.2 at 25°C

Simmon's Citrate Agar

Component	Amount (g/L)
Magnesium sulphate	0.2
Ammoniun dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bacto agar	15.0
Bacto bromo thymol blue	0.08

Methyl Red -Voges Proskauer (MR-VP) Media

Component	Amount (g/L)
Peptone	7.0
Dextrose	5.0
Dipotassium hydrogen phosphate	5.0
Final pH	7.0

Triple Sugar Iron Agar (TSI)

Component	Amount (g/L)
Bio-polytone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Phenol red	0.0125
Agar	13.0
Final pH	7.3

Motility Indole Urease (MIU) Agar

Component	Amount (g/L)
Tryptone	10
Phenol red	0.1
Agar	2.0
Sodium chloride	5.0
pH (at 25°C)	6.8 ± at 25°C

Nitrate Reduction Broth

Component	Amount (g/L)
Beef extract	3.0
Gelatin peptone	5.0
Potassium nitrate	1.0

Indole broth

Component	Amount (g/L)
Peptone	10.0
Sodium chloride	5.0

Appendix – II

Reagents and buffers

Gram's iodine (300 ml)

To 300 ml distilled water, 1 g iodine and 2 g potassium iodide was added. The solution was mixed on a magnetic stirrer overnight and transferred to a reagent bottle and stored at room temperature.

Crystal Violet (100 ml)

To 29 ml 95% ethyl alcohol, 2 g crystal violet was dissolved. To 80 ml distilled water, 0.8 g ammonium oxalate was dissolved. The two solutions were mixed to make the stain and stored in a reagent bottle at room temperature.

Safranin (100ml)

To 10 ml 95% ethanol, 2.5 g safranin was dissolved. Distilled water was added to the solution to make a final volume of 100 ml. The final solution was stored in a reagent bottle at room temperature.

Malachite green (100 ml)

To 20 ml distilled water, 5 g malachite green was dissolved in a beaker. The solution was transferred to a reagent bottle. The beaker was washed two times with 10 ml distilled water separately and a third time with 50 ml distilled water and the solution was transferred to the reagent bottle. The remaining malachite green in the beaker was washed a final time with 10 ml distilled water and added to the reagent bottle. The stain was stored at room temperature.

Kovac's Reagent (150 ml)

To a reagent bottle, 150 ml of reagent grade isoamyl alcohol, 10 g of p-dimethylaminobenzaldehyde (DMAB) and 50 ml of HCl (concentrated) were added and mixed. The reagent bottle was then covered with an aluminum foil to prevent exposure of reagent to light and stored at 4°C.

Methyl Red (200 ml)

In a reagent bottle, 1 g of methyl red powder was completely dissolved in 300 ml of ethanol (95%). 200 ml of destilled water was added to make 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol and stored at 4°C.

Barrit's Reagent A (100 ml)

5% (wt/vol) a-naphthol was added to 100 ml absolute ethanol and stored in a reagent bottle at 4°C.

Barrit's Reagent B (100 ml)

40% (wt/vol) KOH was added to 100 ml distilled water and stored in a reagent bottle at 4°C.

Oxidase Reagent (100 ml)

To 100 ml distilled water, 1% tetra-methyl-*p*-phenylenediamine dihydrochloride was added and stored in a reagent bottle covered with aluminum foil at 4°C to prevent exposure to light.

Catalase Reagent (20 ml 3% hydrogen peroxide)

From a stock solution of 35 % hydrogen peroxide, 583 µl solution was added to 19.417 ml distilled water and stored at 4°C in a reagent bottle.

Urease Reagent (50 ml 40% urea solution)

To 50 ml distilled water, 20 g pure urea powder was added. The solution was filtered through a HEPA filter and collected into a reagent bottle. The solution was stored at room temperature.

Nitrate Reagent A (100 ml)

5N acetic acid was prepared by adding 287 ml of glacial acetic acid (17.4N) to 713 ml of deionized water. In a reagent bottle, 0.6 g of N,N-Dimethyl-α-naphthylamine was added along with 100 ml of acetic acid (5N) and mixed until the colour of the solution turned light yellow. The reagent was stored at 4°C.

Nitrate Reagent B (100 ml)

In a reagent bottle, 0.8 g of sulfalinic acid was added along with 100 ml acetic acid (5N)[a] to form a colorless solution and stored at 4°C.

2N NaOH:

In a small Durham bottle 4 g NaOH was added. Then 50 ml distilled water was added to prepare 50 ml of 2N NaOH. Then it was stored at room temperature.

X