Prevalence and Antimicrobial Susceptibility Pattern of Microorganisms in Cell Phones and the Behavioral Pattern Associated with the Demographic of Dhaka South City Corporation

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

Submitted by

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Declaration

I hereby declare that the thesis project titled **"Prevalence of Microorganisms in Cell Phones and the behavioral pattern associated with the demographic of Dhaka City (South)"** has been written and submitted by me, Mohammed Zawad Reza 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

Mobile phones are currently one of the mostly used electronic devices and it is used by people from almost every walk of life. Along with that, usage of mobile phone is increasing with time passing by due to its availability and efficiency. As a result, the developing nations of the world are also embracing this technological wonder, and since Bangladesh has also found the golden touch of technology, mobile phones have climbed up to the top spots of our priority list. We take everywhere with us, use it all the time. All these occurrences raise the question of potential threats that come from mobile phones. One of those threats would be the transmission of pathogenic microorganisms from cell phones and their potentiality to act as vectors. With this in mind, the demography of Dhaka South City Corporation was selected to observe the prevalence of organism amongst the cell phones of the population and isolate and identify a group of potentially disease-causing organisms. A total of 110 mobile phone samples were included in this study for isolation of bacteria. 95% of the cell phones were found to be contaminated with bacteria and 216 bacterial samples were isolated from the mobile phones. Out of these colonies, we found *Staphylococcus* spp.(47.6%), *Bacillus* spp.(10.1%), *Micrococcus* spp.(19.09%), *E. coli* (20.3%) and fecal coliforms (12.03%). It was found that the participants who used cell phones while eating, used the cell phones inside washrooms, shared their cell phones with other persons and used the cell phones while being sick had more potentially pathogenic microorganisms than the participants who did not. It was also found that the participants who cleaned their cell phones everyday 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. Also, it was found that personal hand hygiene and cell phone hygiene is very important and also washing of hands 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 cell phones, thus keeping it clean.

Table of Contents

Chapter 1

Introduction

1.1Normal Flora of Human Body:

The 'normal microflora' is the term most commonly used when referring to the microbial collection that consistently inhabits the bodies of healthy animals. Other terms used are 'normal flora', 'commensals' and 'indigenous microbiota' (Tannock, 1999).

1.2 What Role Does the Normal Microflora play In the Body's Defense System?

The microflora in the adult human body consist of an enormous biomass of more than 100 000 billion bacteria of over 400 different species, which generate intense metabolic activity, mainly in the colon, and play an important physiologic role in the host (Bourlioux *et a*l., 2003).

Table1.1: 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.,

1.3 Factors Associated With Microbial Flora Infection:

Even though no significant harm is caused by our body‟s normal microbiota, presence of the following factors may result in various infections:

Individual Susceptibility: Susceptibility to some infections is higher in the very young and the very old and in immuno-suppressed patients (Peterson, 1996). Malnutrition, irradiation, indiscriminate use of antibiotics can lower the patient‟s immunity thereby making them more vulnerable to the infection (Ducel *et al.,* 2002). Susceptibility to bacterial infections depends on the physiologic and immunologic condition of the host and on the virulence of the bacteria. An individual becomes susceptible to infection with a variety of bacteria if the skin or mucosa is breached, particularly in the case of severe wounds such as burns or contaminated surgical wounds (Peterson, 1996).

Environmental Factors: It was concluded from this study that bacterial skin populations in certain areas of the body, namely the back, the groin, and the hands, increase with increasing temperature and humidity (Duncan *et al.,* 1968). 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).

1.4 Sources and Mode of Transmission of Infection:

In reference to transmission, „mode‟ should refer to the method that a pathogen uses to get from starting point to destination, whereas the „route‟ is the path taken using the chosen mode and includes a starting point (site of pathogen presentation, or portal of exit), a specific pathway used, and a destination (where the pathogen enters) (Antonovics *et al.,*2017). 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 (Ducel *et al.*, 2002).

1.5 Transmission of Pathogens by Hands:

Bacteria recovered from the hands could be divided into two categories, namely resident or transient. The resident flora (resident microbiota) consists of microorganisms residing under the superficial cells of the stratum corneum and can also be found on the surface of the skin. *Staphylococcus epidermidis* is the dominant species, other resident bacteria include *S. hominis* and other coagulase-negative *Staphylococci* (CONS), followed by *Coryneform* bacteria (*Propionibacteria, Corynebacteria, Dermobacteria,* and *Micrococci*). Among fungi, the most common genus of the resident skin flora, when present, is *Pityrosporum (Malassezia)* spp. Resident flora has two main protective functions: microbial antagonism and the competition for nutrients in the ecosystem.In general, resident flora is less likely to be associated with infections, but may cause infections in sterile body cavities, the eyes, or on non-intact skin. Transient flora (transient microbiota), which colonizes the superficial layers of the skin, is more amenable to removal by routine hand hygiene. Transient microorganisms do not usually multiply on the skin, but they survive and sporadically multiply on skin surface. They are often acquired by HCWs during direct contact with patients or contaminated environmental surfaces adjacent to the patient

and are the organisms most frequently associated with HCAIs. Some types of contact during routine neonatal care are more frequently associated with higher levels of bacterial contamination of HCWs‟ hands: respiratory secretions, nappy/diaper change, and direct skin contact. The transmissibility of transient flora depends on the species present, the number of microorganisms on the surface, and the skin moisture. The hands of some HCWs may become persistently colonized by pathogenic flora such as *S. aureus*, Gram-negative *Bacilli*, or yeast

1.6 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 cell phones, 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. The major source of spread of community-acquired infections is fomites.

1.6.1 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 (Rusin *et al.,* 2002). 40% transfer rate was evaluated for *Escherichia coli* from a nonporous surface to hands in one study (Scott & Bloomfield, 1990). Bacterial transfer rates were observed to be 38.5% to 41.8% from an average cell phone and 27.6% to 40.0% from a plastic bodied cell phone to a person‟s hand with minimal contact times (Rusin *et al.,* 2002).

1.6.2 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

1.7 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. Bacteria such as *Escherichia coli, Shigella dysenteriae, Streptococcus pneumoniae, Klebsiella pneumonia* and *Staphylococcus aureus* as well as *Corynebacterium diphtheriae* cause diarrhea, dysentery, pneumonia, skin infections, food poisoning and intoxication as well as whooping cough respectively. The organism and the diseases that can be transmitted through the use and sharing of cell phones include boil and food borne diseases (*Staphylococcus aureus* and *Escherichia coli*), and diarrhea (*Escherichia coli, Pseudomonas aeruginosa*) and sore throat (*Streptococcus pyogenes*) (Schmidt and Brubaker, 2004).

1.8 Cell Phones 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. 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 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. 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 cell phone results in increased health problems and how to most effectively reduce this problem.

1.9 Hand Hygiene Programs:

Hand hygiene applies to hand washing, antiseptic hand wash, antiseptic hand rub, or surgical hand antisepsis. 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 food borne illness showed that poor personal hygiene, primarily ineffective hand washing is an important contributor to food borne 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 cell phone contamination and to raise awareness about phone cleaning programs among the people of Dhaka.

1.10 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%), MSCoNS09 (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, (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* spp., *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 species such as *Alternaria* spp. (28.0%), *Aspergillus niger* (32.0%), *Cladosporium* spp. (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.

1.11 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 cell phones of the demography of Dhaka South 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 South.

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

- Isolating the bacterial contaminants present in Random samples collected from the 11 thanas of Dhaka City South.
- 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 owners of the cell phones.
- 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 & Methods

2.1 Study Area:

The study was conducted at 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 June 2018.

2.3 Sample Size:

A total of about 110 mobile phone samples were collected from 110 individuals from the area of 11thanas of Dhaka South City Corporation. The samples were taken from people of all walks of life while taking their lifestyle, locality, and personal preferences into consideration. Cell phone models were also considered as a category. Samples are collected from throughout Dhaka City South, in the number of 10 from each thana. The number of samples was constant in the areas and population of the designated zone.

2.4 Materials:

2.4.1 Equipment:

Equipment that was used in this study includes:

- 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
- Glassware, 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 at37ºC of incubation period, *E.coli* and *Klebsiella* will produce pink colonies. Bacteria which cannot 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 pneumonia* 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 pneumonia* 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:

Table2.1: 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

2.6 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. 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 washedoff 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 slidewas 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 withtap 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.

 Forindole 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 thenincubated 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 thenincubated 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 acetylmethylcarbinol 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.

 Thecolour 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 ofSimmons 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 tubecontaining 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 2448 hours the color of both the butt and slant of agar slant cultures were observed.
- Theresults were recorded based on the following observation (Cappuccino & Sherman, 2005).

Table 2.2: 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 filterpapers were labeled according to the sample being tested.

 Using an inoculating loop, a well-isolated colony from pure 24-hour culture waspicked 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).

2.6.2.4.10 Antibiotic Susceptibility Test of the Isolates

The antimicrobial susceptibility test of the isolates was performed according to the national committee for clinical laboratory standards (NCCLS) method using Kirby-Bauer disk diffusion test on Muller-Hinton agar. In short the isolated bacterium was suspended in a nutrient broth and incubated for 30minutes to make it comparable with 0.5% McFarland standard. After incubation a sterile cotton swab was dipped in to the suspension and bacteria were inoculated on to the Muller-Hinton Agar. Antibiotic discs were placed by using disc dispenser and the plate was incubated for 24 hrs at 37∘C. Results were interpreted after measuring the zone of inhibition and being compared with the standards. The isolates were tested against GEN, K, P, CL, OX, VA, IMI, CIP and TE; these 9 antibiotics.

2.7 Questionnaire

The data was collected using 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 September 2017 to June 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 cell phone, while the independent variables were age, occupation, cell phone brand, type etc. The occupation and area of the collected sample were nominal measurements and the other variables were scale. Occupation had 10 strings 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 was 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.

Chapter 3

Results

3.1 Analysis of the Survey according to the Questionnaire

In our study, most the people who volunteered were 21-25 years old, 44% of the total volunteers. Other than that, people aging between 16 and 20 and between 26 and 30 were high in number as well; 26% and 20% respectively. People above 30 were the least with 10% of the total (Table 3.1.1). In table 3.1.2, we can see that most of the volunteers were students (65%) and the least were people from „others" category, who are mostly Health Care Workers, housewives etc. Table 3.1.3 depicts that most of the people are medium users of their phones (58%), they do not use it too much or too less. In table 3.1.4, we can see that 53% of the people sometimes use their phones while eating and 31% of them use it always. The people who use their phones too much are in second place (28%). Most of the people use phones inside washrooms on average (54%) and 26% of them always use their phones inside washrooms (Table 3.1.5). A lot of people use their phones during public gatherings at the frequency of always and sometimes with 40% and 36% respectively (3.1.6). Around 55% of people always share their phones physically with others and 26% of them share it sometimes (3.1.7). On an average, 55% of the people sometimes use their phones during sickness and 33% of them use always (Table 3.1.8). Mobile phones are mostly kept in pockets and purses; 34% and 25% respectively (Table 3.1.9). People usually keep money (22%), keys (17%), IDs (16%) and headphones (12%) along with their mobile phones (Table 3.1.10). Table 3.1.11 represents that around 65% of the volunteers do not clean their cell phones at all, whereas only 16% of them clean once a month. From Table 3.1.12, we can see that 71% of the people use protective covers for their cell phone, while 29% do not. Lastly, table 3.1.13 shows that 10 samples were taken from 11 thanas each and the thanas were Motijheel, Sutrapur, Dhanmondi, Azimpur, Shahbag, Bongshal, Wari, Malibagh, Hazaribag, Jatrabari and Moghbazar.

Table3.1.1: Age Groups of the Volunteers

Age Group	Frequency	Percentage (%)	Cumulative Percentage (%)
16-20	29	26	26
21-25	48	44	70
26-30	22	20	90
Above 30	11	10	100
Total	110	100	

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

Table3.1.2: Occupation of the Volunteers

Occupation	Frequency	Percentage (%)	Cumulative Percentage (%)
Student	72	65	65
Daily Workers	12	12	77
Job Holders	18	16	93
Others	8	7	100
Total	110	100	

Students are the dominant group sitting at 62%.

Table3.1.3: How Often Do You Use Your Cell Phone?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	31	28	28
Average	64	58	86
Not more than necessary	15	14	100
Total	110	100	

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

Table3.1.4: Do You Use Your Cell Phone While Eating?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	34	31	31
Average	58	53	84
Not more than necessary	18	16	100
Total	110	100	

53% of the participants sometimes use their cell phones while eating.

Table3.1.5: Do You Use Your Cell Phone Inside Washroom?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	29	26	26
Average	59	54	80
Not more than necessary	22	20	100
Total	110	100	

Most people use cell phones in the washroom at an average rate

Table3.1.6: Do You Use Your Cell Phone during Public Gatherings?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	44	40	40
Average	40	36	76
Not more than necessary	26	24	100
Total	110	100	

The usage of cell phones during public gatherings ranges from a lot to average

Table3.1.7: Do You Share Your Cell Phone Physically With Other People?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	28	26	26
Average	61	55	81
Not more than necessary	21	19	100
Total	110	100	

Around 55% people share their phones physically with others at a moderate range

Table3.1.8: Do You Use Your Cell Phone While You Are Sick?

	Frequency	Percentage (%)	Cumulative Percentage (%)
A lot	36	33	33
Average	60	55	88
Not more than necessary	14	12	100
Total	110	100	

Cell phone usage while being sick is mostly sometimes, sitting at 55%

Table3.1.9: Where Do You Usually Keep Your Cell Phone When It Is With You?

	Frequency	Percentage (%)	Cumulative Percentage (%)
Pocket	37	34	34
Purse	28	25	59
Backpack	21	19	78
Hands	15	14	92
Others	9	8	100
Total	110	100	

Most of the people keep their phones in either pockets or purses

Table3.1.10: What Are the Other Things That Reside With Your Cell Phone?

	Frequency	Percentage (%)	Cumulative Percentage (%)
Headphones	13	12	12
Wallet	13	12	24
Keys	19	17	41
ID's	18	16	57
Money	24	22	79
Cosmetics	10	9	88
Others	13	12	100
Total	110	100	

Table3.1.11: How Often Do You Clean Your Cell Phone?

	Frequency	Percentage (%)	Cumulative Percentage (%)
Everyday	8	7	7
Once a week	12	11	18
Once a month	18	16	34
Not at all	72	65	100
Total	110	100	

A whopping 65% of the individuals do not their phones at all

Table3.1.12: Do You Use A Cover For Your Cell Phone?

	Frequency	Percentage (%)	Cumulative Percentage (%)
Yes	78	71	71
No	32	29	100
Total	110	100	

Most of them use covers

Table13.1.13: Area

Thanas	Frequency	Percentage (%)	Cumulative Percentage (%)
Motijheel	10	9.09	9.09
Sutrapur	10	9.09	18.18
Dhanmondi	10	9.09	27.27
Hazaribag	10	9.09	36.36
Jatrabari	10	9.09	45.45
Moghbazar	10	9.09	54.54
Bongshal	10	9.09	63.63
Shahbag	10	9.09	72.72
Wari	10	9.09	81.81
Malibagh	10	9.09	99.99
Azimpur	10	9.09	100
Total	110	100	

The areas of Dhaka South City Corporation were 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 110, 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 cell phones, which were collected from 11 thanas of Dhaka South City Corporation, 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 216 isolates, *Staphylococci* spp. was found in 103 isolates which indicates 47.6% of the total colony. Moreover, *E. coli* were seen in 44 cases and *Bacillus* spp. was seen in 22 cases. 21 and 26 colonies were identified as *Micrococcus* spp. and fecal coliform respectively. 5 samples overall showed no colonies.

Motijheel thana (table 3.2.1) is dominated by *Staphylococcus* spp. with 10 colonies and the others being *E .coli* and *Bacillus* spp. numbering at 4 and 1 colonies respectively. Sutrapur thana (Table 3.2.2) had 9 colonies of *Staphylococcus* spp., 6 colonies of *E. coli*, 4 fecal coliform colonies and 3 colonies of *Micrococcus* spp. However, 1 sample was free of contamination. Dhanmondi thana (Table 3.2.3) had 8 colonies of *Staphylococcus* spp., 6 *E. coli* colonies, 3colonies of fecal coliform and *Bacillus* spp. each and a single colony of *Micrococcus* with 2 of the samples being free of contamination. Hazaribag thana (Table 3.2.4) contained 10 colonies of *Staphylococcus* spp., 5 fecal coliform colonies, 2 colonies of *Bacillus* spp., 7 colonies of *E. coli* and 2 *Micrococcus* colonies. Jatrabari thana (Table 3.2.5) contained 10, 6 and 3 colonies of *Staphylococcus* spp., *E. coli,* fecal coliform respectively. Moghbazar thana (Table 3.2.6) had *Staphylococcus* spp., *Bacillus* spp., fecal coliforms and *Micrococcus* spp. 10, 2, 3 and 1 colonies respectively. Bongshal thana (Table 3.2.7) had the highest number of fecal coliform presence in the entire study with 6 colonies along with the highest *Micrococcus* spp. present numbering in 5 colonies. Others organisms were 10 colonies of *Staphylococcus* spp., 3 colonies of *Bacillus* spp. and *E. coli* each. Shahbag thana (Table 3.2.8) showed 2 colonies of *E. coli,* 3 colonies of *Bacillus* spp. and 10 colonies of *Staphylococcus* spp. Wari thana (Table 3.2.9) had *Staphylococcus* spp., fecal coliforms, *E. coli, Bacillus* spp. and *Micrococcus* spp. numbering in 10, 4, 5, 3 and 2

colonies respectively. Malibagh thana (Table 3.2.10) contained 8 colonies of *Staphylococcus* spp., 4 colonies of *Bacillus* spp., 4 colonies of *E. coli*, 3 fecal coliform colonies with 2 of the samples being free of contamination. Finally, Azimpur thana (Table 3.2.11) showed 10 *Staphylococcus* spp. colonies and 1 colony of *Bacillus* spp., *E. coli, Micrococcus* spp. and fecal coliforms each.

Table3.2.1: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Motijheel.

Isolates ID	**Mac-Conkey Agar**	**Mannitol Salt Agar**	**Membrane Fecal**	**Eosine Methylene Blue**	**Bacillus Cereus Agar**	**Hi- Chrome Agar**	**Blood Agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	**Suspected organism**
1.MJ1		Small, Yellow coloured				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.MJ2		Small, Yellow coloured colonies				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
3.MJ3a		Small, Yellow coloured colonies				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
4.MJ3b					Blue Colour colonies	Green Colour	Beta	Large	White	Circular	Entire	Convex	***Bacillus* spp.**

12.MJ8b	11.MJ8a	10.MJ7	9.MJ6b	8.MJ6a	7.MJ5	6.MJ4b	5.MJ4a
Small, Pink Colour			Small, Pink Colour			Small, Pink Colour	
	Small, Yellow coloured	Small, Yellow coloured		Small, Yellow coloured	Small, Yellow coloured		Small, Yellow coloured
Metallic Green Sheen			Metallic Green Sheen			Metallic Green Sheen	
Purple Colour	Golden Yellow colour colonies	Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies	Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies
Gamma	Beta Hemolysis	Beta Hemolysis	Gamma	Beta Hemolysis	Beta Hemolysis	Gamma	Beta Hemolysis
Small	Small	Small	Small	Small	Small	Small	Small
Creamy	Yellow	Yellow	Creamy	Yellow	Yellow	Creamy	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Raised	Convex	Convex	Raised	Convex	Convex	Raised	Convex
E. coli	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**

15.MJ10	14.MJ10a	13.MJ9
Small, Pink Colour		
	Small, Yellow coloured	Small, Yellow coloured
Metallic Green Sheen		
Purple Colour	Golden Yellow colour colonies	Golden Yellow colour colonies
Gamma	Beta Hemolysis	Beta Hemolysis
Small	Small	Small
Creamy	Yellow	Yellow
Circular	Circular	Circular
Entire	Entire	Entire
Raised	Convex	Convex
E. coli	***Staphylococcus* spp.**	***Staphylococcus* spp.**

Table3.2.2: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Sutrapur.

4.SP2	3.SP1c	2.SP1b	1.SP1a	Isolates ID
		Small, Pink		**Mac-Conkey**
			Small, Yellow coloured	**Mannitol Salt Agar**
	Blue Colour Colonies			**Membrane Fecal Coliform**
		Metallic Green Sheen		**Eosine Methylene Blue**
				Bacillus Cereus Agar (BC)
		Purple Colour	Golden Yellow colour colonies	**Hi-Chrome**
	Gamma	Gamma	Beta Hemolysis	**Blood Agar**
	Medium	Small	Small	**Size**
	White	Creamy	Yellow	**Color**
	Circular	Circular	Circular	**Form**
	Entire	Entire	Entire	**Margin**
	Convex	Raised	Convex	**Elevation**
None	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**Suspected organism**

13.SP5d	12.SP	11.SP5b	10.SP5a	9.SP4b	8.SP4a	7.SP3c	6.SP3b	5.SP3a
	Small, Pink			Small, Pink			Small, Pink	
Small, White Colonies			Small, Yellow coloured		Small, Yellow coloured	Small, White Colonies		Small, Yellow coloured
		Blue Colour Colonies						
	Metallic Green			Metallic Green Sheen			Metallic Green Sheen	
Yellow Colour	Purple Colour		Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies	Yellow Colour	Purple Colour	Golden Yellow colour colonies
Gamma	Gamm	Gamma	Beta Hemolysis	Gamma	Beta Hemolysis	Gamma	Gamma	Beta Hemolysis
Small	Small	Medium	Small	Small	Small	Small	Small	Small
Yellow	Cream	White	Yellow	Creamy	Yellow	Yellow	Creamy	Yellow
Circular	Circul	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Raised	Convex	Convex	Raised	Convex	Convex	Raised	Convex
***Micrococcus* spp.**	***E. coli***	**Fecal coliform**	***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	***Micrococcus* spp.**	***E. coli***	***Staphylococcus* spp.**

21.SP9	20.SP8b	19.SP8a	18.SP7c	17.SP7	16.SP7a	15.SP6b	14.SP6a
				Small, Pink			
Small, Yellow coloured	Small, White Colonies	Small, Yellow coloured			Small, Yellow coloured		Small, Yellow coloured
			Blue Colour Colonies			Blue Colour Colonies	
				Metallic Green Sheen			
Golden Yellow colour colonies	Yellow Colour	Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies		Golden Yellow colour colonies
Beta Hemolysis	Gamma	Beta Hemolysis	Gamma	Gamma	Beta Hemolysis	Gamma	Beta Hemolysis
Small	Small	Small	Medium	Small	Small	Medium	Small
Yellow	Yellow	Yellow	White	Cream	Yellow	White	Yellow
Circular	Circular	Circular	Circular	Circula	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Raised	Convex	Convex	Convex
***Staphylococcus* spp.**	***Micrococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**

23.SP1	22.SP10a
Small, Pink	
	Small, Yellow coloured
Metallic Green Sheen	
Purple Colour	Golden Yellow colour colonies
Gamma	Beta Hemolysis
Small	Small
Cream	Yellow
Circula	Circular
Entire	Entire
Raised	Convex
E. coli	***Staphylococcus* spp.**

Table3.2.3: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Dhanmondi.

5.DM	**4.DM2c**	**3.DM2**	**2.DM2a**	**1.DM**	**Isolates ID**
		Small, Pink Colour			**Mac- Conkey Agar**
			Small, Yellow coloured		**Mannitol Salt Agar**
	Blue Colour Colonies				**Membrane Fecal Coliform**
		Metallic Green Sheen			**Eosine Methylene Blue**
					Bacillus Cereus Agar
		Purple Colour	Golden Yellow colour colonies		**Hi- Chrome Agar**
	Gamma Hemolysi	Gamma Hemoly	Beta Hemolysis		**Blood Agar**
	Medium	Small	Small		**Size**
	White	Cream	Yellow		**Color**
	Circular	Circula	Circular		**Form**
	Entire	Entire	Entire		**Margin**
	Convex	Raised	Convex		**Elevation**
None	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**None**	**Suspected organism**

14.DM7a	13.DM6	12.DM6a	11.DM5	10.D	9.DM5a	8.DM4c	7.DM4	6.DM4a
				Small, Pink Colour			Small, Pink Colour	
Small, Yellow coloured		Small, Yellow coloured			Small, Yellow coloured			Small, Yellow coloured
	Blue Colour Colonies							
				Metallic Green			Metallic Green Sheen	
			Blue Colour			Blue Colour		
Golden Yellow colour colonies		Golden Yellow colour colonies	Green Colour	Purple Colour	Golden Yellow colour colonies	Green Colour	Purple Colour	Golden Yellow colour colonies
Beta Hemolysis	Gamma Hemolysi	Beta Hemolysis	Beta Hemolys	Gamma	Beta Hemolysis	Beta Hemolys	Gamma Hemoly	Beta Hemolysis
Small	Medium	Small	Large	Small	Small	Large	Small	Small
Yellow	White	Yellow	White	Cream	Yellow	White	Cream	Yellow
Circular	Circular	Circular	Circular	Circul	Circular	Circular	Circula	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Raised	Convex	Convex	Raised	Convex
***Staphylococcus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**	***Bacillus* spp.**	***E. coli***	***Staphylococcus* spp.**	***Bacillus* spp.**	***E. coli***	***Staphylococcus* spp.**

23.DM1	22.DM10a	21.DM9c	20.DM9b	19.DM9a	18.DM8c	17.DM8b	16.DM8a	15.DM
Small, Pink Colour			Small, Pink Colour					Small, Pink Colour
	Small, Yellow coloured			Small, Yellow coloured		Small, White Colonies	Small, Yellow coloured	
					Blue Colour Colonies			
Metallic Green Sheen			Metallic Green Sheen					Metallic Green Sheen
		Blue Colour						
Purple Colour	Golden Yellow colour colonies	Green Colour	Purple Colour	Golden Yellow colour colonies		Yellow Colour	Golden Yellow colour colonies	Purple Colour
Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemoly
Small	Small	Large	Small	Small	Medium	Small	Small	Small
Creamy	Yellow	White	Creamy	Yellow	White	Yellow	Yellow	Cream
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circula
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Raised	Convex	Convex	Raised	Convex	Convex	Convex	Convex	Raised
E. coli	***Staphylococcus* spp.**	***Bacillus* spp.**	***E. coli***	***Staphylococcus* spp.**	**Fecal coliform**	***Micrococcus* spp.**	***Staphylococcus* spp.**	***E. coli***

Table3.2.4: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Hazaribag.

Isolates ID	**1.HB1a**	**2.HB1b**	**3.HB2a**	**4.HB2b**	**5.HB3a**	**6.HB3b**
Mac-Conkey Agar		Small, Pink Colour colonies				Small, Pink Colour
Mannitol Salt Agar	Small, Yellow coloured		Small, Yellow coloured		Small, Yellow coloured	
Membrane Fecal Coliform						
Eosine Methylene Blue		Metallic Green Sheen colonies				Metallic Green Sheen
Bacillus Cereus Agar (BC)				Blue Colour colonies		
Hi- Chrome Agar	Golden Yellow colour colonies	Purple Colour colonies	Golden Yellow colour colonies	Green Colour colonies	Golden Yellow colour colonies	Purple Colour colonies
Blood Agar Hemo- Lysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis
Size	Small	Small	Small	Large	Small	Small
Color	Yellow	Creamy	Yellow	White	Yellow	Creamy
Form	Circular	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Raised	Convex	Convex	Convex	Raised
Suspected organism	***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***E. coli***

14.HB6b	13.HB6a	12.HB5c	11.HB5b	10.HB5a	9.HB4c	8.HB4b	7.HB4a
Small, Pink Colour colonies					Small, Pink Colour		
	Small, Yellow coloured	Small, White Colonies		Small, Yellow coloured			Small, Yellow coloured
			Blue Colour Colonies			Blue Colour Colonies	
Metallic Green Sheen colonies					Metallic Green Sheen		
Purple Colour colonies	Golden Yellow colour colonies	Yellow Colour colonies		Golden Yellow colour colonies	Purple Colour colonies		Golden Yellow colour colonies
Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Small	Small	Medium	Small	Small	Medium	Small
Creamy	Yellow	Yellow	White	Yellow	Creamy	White	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Raised	Convex	Convex	Convex	Convex	Raised	Convex	Convex
E. coli	***Staphylococcus* spp.**	***Micrococcus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**	***E. coli***	**Fecal coliform**	***Staphylococcus* spp.**

22.HB9c	21.HB9b	20.HB9a	19.HB8c	18.HB8b	17.HB8a	16.HB7b	15.HB7a
Small, Pink Colour			Small, Pink Colour				
		Small, Yellow coloured			Small, Yellow coloured		Small, Yellow coloured
	Blue Colour Colonies			Blue Colour Colonies			
Metallic Green Sheen			Metallic Green Sheen				
						Blue Colour colonies	
Purple Colour colonies		Golden Yellow colour colonies	Purple Colour colonies		Golden Yellow colour colonies	Green Colour colonies	Golden Yellow colour colonies
Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis
Small	Medium	Small	Small	Medium	Small	Large	Small
Creamy	White	Yellow	Creamy	White	Yellow	White	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Raised	Convex	Convex	Raised	Convex	Convex	Convex	Convex
E. coli	**Fecal coliform**	***Staphylococcus* spp.**	***E. coli***	**Fecal coliform**	***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**

26.HB10d	25.HB10c	24.HB10	23.HB10a
	Small, Pink Colour		Small, Yellow coloured colonies
Small, White Colonies		Blue Colour Colonies	
	Metallic Green Sheen		
Yellow Colour colonies	Purple Colour colonies		Golden Yellow colour colonies
Gamma Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Small	Medium	Small
Yellow	Creamy	White	Yellow
Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire
Convex	Raised	Convex	Convex
***Micrococcus* spp.**	***E. coli***	**Fecal coliform**	***Staphylococcus* spp.**

Table3.2.5: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Jatrabari.

2.JB1b	1.JB1a	Isolates ID
Small, Pink Colour		**Mac-Conkey**
	Small, Yellow coloured	**Mannitol Salt Agar**
		Membrane Fecal Coliform
Metallic Green Sheen		**Eosine Methylene Blue**
		Bacillus Cereus Agar (BC)
Purple Colour	Golden Yellow colour colonies	**Hi-Chrome**
Gamma Hemolysis	Beta Hemolysis	**Blood Agar**
Small	Small	**Size**
Creamy	Yellow	**Color**
Circular	Circular	**Form**
Entire	Entire	**Margin**
Raised	Convex	**Elevation**
E. coli	***Staphylococcus* spp.**	**Suspected organism**

10.JB6	**9.JB5b**	**8.JB5a**	**7.JB4**	**6.JB3**	**5.JB2c**	**4.JB2b**	**3.JB2a**
	Small, Pink Colour					Small, Pink Colour	
Small, Yellow coloured		Small, Yellow coloured	Small, Yellow coloured	Small, Yellow coloured			Small, Yellow coloured
					Blue Colour Colonies		
	Metallic Green Sheen					Metallic Green Sheen	
Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies
Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Small	Small	Small	Small	Medium	Small	Small
Yellow	Creamy	Yellow	Yellow	Yellow	White	Creamy	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Raised	Convex	Convex	Convex	Convex	Raised	Convex
***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**

18.JB10a	**17.JB9c**	**16.JB9b**	**15.JB9a**	**14.JB8b**	**13.JB8a**	**12.JB7b**	**11.JB7a**
		Small, Pink Colour		Small, Pink Colour			
Small, Yellow coloured			Small, Yellow coloured		Small, Yellow coloured		Small, Yellow coloured
	Blue Colour Colonies					Blue Colour Colonies	
		Metallic Green Sheen		Metallic Green Sheen			
Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies		Golden Yellow colour colonies
Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Medium	Small	Small	Small	Small	Medium	Small
Yellow	White	Creamy	Yellow	Creamy	Yellow	White	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Raised	Convex	Raised	Convex	Convex	Convex
***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**

19.JB10	Small, Pink Colour			Metallic Green Sheen		Purple Colour	Gamma Hemolysis	Small	Creamy	Circular	Entire	Raised	*E. coli*

Table3.2.6: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Moghbazar.

Isolates ID	**Mac-Conkey Agar**	**Mannitol Salt Agar**	**Membrane Fecal Coliform**	**Eosine Methylene Blue**	**Bacillus Cereus (BC)**	**Hi-Chrome**	**Blood Agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	**Suspected organism**
1.MB1		Small, Yellow coloured				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.MB2a		Small, Yellow coloured				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
3.MB2b			Blue Colour Colonies				Gamma Hemolysis	Medium	White	Circular	Entire	Convex	**Fecal coliform**
4.MB3		Small, Yellow coloured				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
5.MB4		Small, Yellow coloured				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

12.MB8b	**11.MB8a**	**10.MB7**	**9.MB6**	**8.MB5c**	**7.MB5b**	**6.MB5a**
Small, White Colonies	Small, Yellow coloured	Small, Yellow coloured	Small, Yellow coloured			Small, Yellow coloured
				Blue Colour Colonies		
					Blue Colour colonies	
Yellow Colour	Golden Yellow colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies		Green Colour	Golden Yellow colour colonies
Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis
Small	Small	Small	Small	Medium	Large	Small
Yellow	Yellow	Yellow	Yellow	White	White	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Convex	Convex	Convex
***Micrococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***Bacillus* spp.**	***Staphylococcus* spp.**

16.MB10	15.MB10	14.MB10a	13.MB9
		Small, Yellow coloured	Small, Yellow coloured
	Blue Colour Colonies		
Blue Colour colonies			
Green Colour		Golden Yellow colour colonies	Golden Yellow colour colonies
Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis
Large	Medium	Small	Small
White	White	Yellow	Yellow
Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex
***Bacillus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**	***Staphylococcus* spp.**

Table3.2.7: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Bongshal.

2.BS1b	1.BS1a	Isolates ID
		Mac-Conkey Agar
	Small, Yellow coloured	**Mannitol Salt Agar**
		Membrane Fecal Coliform
		Eosine Methylene Blue
Blue Colour colonies		**Bacillus Cereus Agar (BC)**
Green Colour	Golden Yellow colour colonies	**Hi-Chrome**
Beta Hemolysis	Beta Hemolysis	**Blood Agar**
Large	Small	**Size**
White	Yellow	**Color**
Circular	Circular	**Form**
Entire	Entire	**Margin**
Convex	Convex	**Elevation**
***Bacillus* spp.**	***Staphylococcus* spp.**	**Suspected organism**

10.BS4b	**9.BS4a**	**8.BS3c**	**7.BS3b**	**6.BS3a**	**5.BS2b**	**4.BS2a**	**3.BS1c**
	Small, Yellow coloured		Small, White Colonies	Small, Yellow coloured	Small, White Colonies	Small, Yellow coloured	
		Blue Colour Colonies					Blue Colour Colonies
Blue Colour colonies							
Green Colour	Golden Yellow colour colonies		Yellow Colour	Golden Yellow colour colonies	Yellow Colour	Golden Yellow colour colonies	
Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis
Large	Small	Medium	Small	Small	Small	Small	Medium
White	Yellow	White	Yellow	Yellow	Yellow	Yellow	White
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
***Bacillus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***Micrococcus* spp.**	***Staphylococcus* spp.**	***Micrococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**

18.BS7b	17.BS7a	16.BS6c	15.BS6b	14.BS6a	13.BS5c	12.BS5b	11.BS5a
		Small, Pink Colour				Small, Pink Colour	
	Small, Yellow coloured		Small, White Colonies	Small, Yellow coloured			Small, Yellow coloured
					Blue Colour Colonies		
		Metallic Green Sheen				Metallic Green Sheen	
Blue Colour colonies							
Green Colour	Golden Yellow colour colonies	Purple Colour	Yellow Colour	Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies
Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Large	Small	Small	Small	Small	Medium	Small	Small
White	Yellow	Creamy	Yellow	Yellow	White	Creamy	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Raised	Convex	Convex	Convex	Raised	Convex
***Bacillus* spp.**	***Staphylococcus* spp.**	***E. coli***	***Micrococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**

26.BS10b	**25.BS10a**	**24.BS9c**	**23.BS9b**	**22.BS9a**	**21.BS8c**	**20.BS8b**	**19.BS8a**
			Small, Pink Colour				
Small, White Colonies	Small, Yellow coloured			Small, Yellow coloured		Small, White Colonies	Small, Yellow coloured
		Blue Colour Colonies			Blue Colour Colonies		
			Metallic Green Sheen				
Yellow Colour	Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies		Yellow Colour	Golden Yellow colour colonies
Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Small	Medium	Small	Small	Medium	Small	Small
Yellow	Yellow	White	Creamy	Yellow	White	Yellow	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Raised	Convex	Convex	Convex	Convex
***Micrococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**Fecal coliform**	***Micrococcus* spp.**	***Staphylococcus* spp.**

27.BS10c			Blue Colour Colonies				Gamma Hemolysis	Medium	White	Circular	Entire	Convex	**Fecal coliform**

Table3.2.8: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Shahbag.

Isolates ID	**Mac-Conkey Agar**	**Mannitol Salt Agar**	**Membrane Fecal Coliform**	**Eosine Methylene Blue**	**Bacillus Cereus Agar (BC)**	**Hi-Chrome**	**Blood Agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	**Suspected organism**
1.SB1a		Small, Yellow coloured colonies				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
2.SB1b	Small, Pink Colour			Metallic Green Sheen		Purple Colour	Gamma Hemolysis	Small	Creamy	Circular	Entire	Raised	***E. coli***
3.SB2a		Small, Yellow coloured colonies				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
4.SB2b					Blue Colour colonies	Green Colour	Beta Hemolysis	Large	White	Circular	Entire	Convex	***Bacillus* spp.**
5.SB3		Small, Yellow coloured colonies				Golden Yellow colour colonies	Beta Hemolysis	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

12.SB8	**11.SB7b**	**10.SB7a**	**9.SB6b**	**8.SB6a**	**7.SB5**	**6.SB4**
Small, Yellow coloured colonies		Small, Yellow coloured colonies		Small, Yellow coloured colonies	Small, Yellow coloured colonies	Small, Yellow coloured colonies
	Blue Colour colonies		Blue Colour colonies			
Golden Yellow colour colonies	Green Colour	Golden Yellow colour colonies	Green Colour	Golden Yellow colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies
Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis
Small	Large	Small	Large	Small	Small	Small
Yellow	White	Yellow	White	Yellow	Yellow	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Convex	Convex	Convex
***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**

15.SB10	14.SB9b	13.SB9a
	Small, Pink Colour	
Small, Yellow coloured colonies		Small, Yellow coloured colonies
	Metallic Green Sheen	
Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies
Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Small	Small
Yellow	Creamy	Yellow
Circular	Circular	Circular
Entire	Entire	Entire
Convex	Raised	Convex
***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**

Table3.2.9: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Wari.

3.WR2a	2.WR1b	1.WR1a	Isolates ID
	Small, Pink Colour colonies		**Mac-Conkey Agar**
Small, Yellow coloured colonies		Small, Yellow coloured colonies	**Mannitol Salt Agar**
			Membrane Fecal Coliform
	Metallic Green Sheen		**Eosine Methylene Blue**
			Bacillus Cereus Agar (BC)
Golden Yellow colour colonies	Purple Colour	Golden Yellow colour colonies	**Hi-Chrome**
Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	**Blood Agar**
Small	Small	Small	**Size**
Yellow	Creamy	Yellow	**Color**
Circular	Circular	Circular	**Form**
Entire	Entire	Entire	**Margin**
Convex	Raised	Convex	**Elevation**
***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	**Suspected organism**

11.WR5a	10.WR4c	9.WR4b	8.WR4a	7.WR3b	6.WR3a	5.WR2c	4.WR2b
		Small, Pink Colour colonies				Small, Pink Colour colonies	
Small, Yellow coloured colonies			Small, Yellow coloured colonies		Small, Yellow coloured colonies		
	Blue Colour Colonies			Blue Colour Colonies			Blue Colour Colonies
		Metallic Green Sheen				Metallic Green Sheen	
Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies		Golden Yellow colour colonies	Purple Colour	
Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis
Small	Medium	Small	Small	Medium	Small	Small	Medium
Yellow	White	Creamy	Yellow	White	Yellow	Creamy	White
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Raised	Convex	Convex	Convex	Raised	Convex
***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**Fecal coliform**	***Staphylococcus* spp.**	***E. coli***	**Fecal coliform**

19.WR8a	18.WR7c	17.WR7b	16.WR7a	15.WR6c	14.WR6b	13.WR6a	12.WR5b
		Small, Pink Colour colonies					Small, Pink Colour colonies
Small, Yellow coloured colonies			Small, Yellow coloured colonies	Small, White Colonies		Small, Yellow coloured colonies	
	Blue Colour Colonies						
		Metallic Green Sheen					Metallic Green Sheen
					Blue Colour colonies		
Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies	Yellow Colour	Green Colour	Golden Yellow colour colonies	Purple Colour
Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis
Small	Medium	Small	Small	Small	Large	Small	Small
Yellow	White	Creamy	Yellow	Yellow	White	Yellow	Creamy
Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Raised	Convex	Convex	Convex	Convex	Raised
***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	***Micrococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***E. coli***

24.WR10	23.WR9b	22.WR9a	21.WR8c	20.WR8b
Small, Yellow coloured colonies		Small, Yellow coloured colonies	Small, White Colonies	
	Blue Colour colonies			Blue Colour colonies
Golden Yellow colour colonies	Green Colour	Golden Yellow colour colonies	Yellow Colour	Green Colour
Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis
Small	Large	Small	Small	Large
Yellow	White	Yellow	Yellow	White
Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Convex
***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***Micrococcus* spp.**	***Bacillus* spp.**

Table3.2.10: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Malibagh.

1.ML1a	Isolates ID
	Mac-Conkey Agar
Small, Yellow coloured	**Mannitol Salt Agar**
	Membrane Fecal Coliform
	Eosine Methylene Blue
	Bacillus Cereus Agar (BC)
Golden Yellow colour colonies	**Hi-Chrome**
Beta Hemolysis	**Blood Agar**
Small	**Size**
Yellow	**Color**
Circular	**Form**
Entire	**Margin**
Convex	**Elevation**
***Staphylococcus* spp.**	**Suspected organism**

10.ML5b	9.ML5a	8.ML4c	7.ML4b	6.ML4a	5.ML3	4.ML2b	3.ML2a	2.ML1b
			Small, Pink Colour					Small, Pink Colour
	Small, Yellow coloured			Small, Yellow coloured			Small, Yellow coloured	
		Blue Colour Colonies						
			Metallic Green Sheen					Metallic Green Sheen
Blue Colour colonies						Blue Colour colonies		
Green Colour	Golden Yellow colour colonies		Purple Colour	Golden Yellow colour colonies		Green Colour	Golden Yellow colour colonies	Purple Colour
Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis		Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis
Large	Small	Medium	Small	Small		Large	Small	Small
White	Yellow	White	Creamy	Yellow		White	Yellow	Creamy
Circular	Circular	Circular	Circular	Circular		Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire		Entire	Entire	Entire
Convex	Convex	Convex	Raised	Convex		Convex	Convex	Raised
***Bacillus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**	***E. coli***	***Staphylococcus* spp.**	**None**	***Bacillus* spp.**	***Staphylococcus* spp.**	***E. coli***

18.ML9c	17.ML9b	16.ML9a	15.ML8b	14.ML8a	13.ML7	12.ML6b	11.ML6a
	Small, Pink Colour						
		Small, Yellow coloured		Small, Yellow coloured			Small, Yellow coloured
Blue Colour Colonies							
	Metallic Green Sheen						
			Blue Colour colonies			Blue Colour colonies	
	Purple Colour	Golden Yellow colour colonies	Green Colour	Golden Yellow colour colonies		Green Colour	Golden Yellow colour colonies
Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis		Beta Hemolysis	Beta Hemolysis
Medium	Small	Small	Large	Small		Large	Small
White	Creamy	Yellow	White	Yellow		White	Yellow
Circular	Circular	Circular	Circular	Circular		Circular	Circular
Entire	Entire	Entire	Entire	Entire		Entire	Entire
Convex	Raised	Convex	Convex	Convex		Convex	Convex
Fecal coliform	***E. coli***	***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	**None**	***Bacillus* spp.**	***Staphylococcus* spp.**

21.ML10	20.ML10	19.ML10a
	Small, Pink Colour	
		Small, Yellow coloured
Blue Colour Colonies		
	Metallic Green Sheen	
	Purple Colour	Golden Yellow colour colonies
Gamma Hemolysis	Gamma Hemolysis	Beta Hemolysis
Medium	Small	Small
White	Creamy	Yellow
Circular	Circular	Circular
Entire	Entire	Entire
Convex	Raised	Convex
Fecal coliform	***E. coli***	***Staphylococcus* spp.**

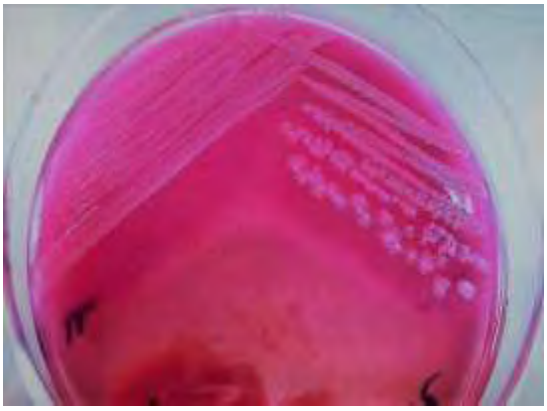
Table3.2.11: Cultural and Morphological Characteristics of the Bacterial Isolates from Cell Phones on various Selective, Differential, Enriched and Nutrient Media on Azimpur.

3.AP2a	2.AP1b	1.AP1a	Isolates ID
	Small, Pink Colour		**Mac-Conkey Agar**
Small, Yellow coloured colonies		Small, Yellow coloured colonies	**Mannitol Salt Agar**
			Membrane Fecal Coliform
	Metallic Green Sheen		**Eosine Methylene Blue**
			Bacillus Cereus Agar (BC)
Golden Yellow colour colonies	Purple Colour colonies	Golden Yellow colour colonies	**Hi-Chrome**
Beta Hemolysis	Gamma Hemolysis	Beta Hemolysis	**Blood Agar**
Small	Small	Small	**Size**
Yellow	Creamy	Yellow	**Color**
Circular	Circular	Circular	**Form**
Entire	Entire	Entire	**Margin**
Convex	Raised	Convex	**Elevation**
***Staphylococcus* spp.**	***E. coli***	***Staphylococcus* spp.**	**Suspected organism**

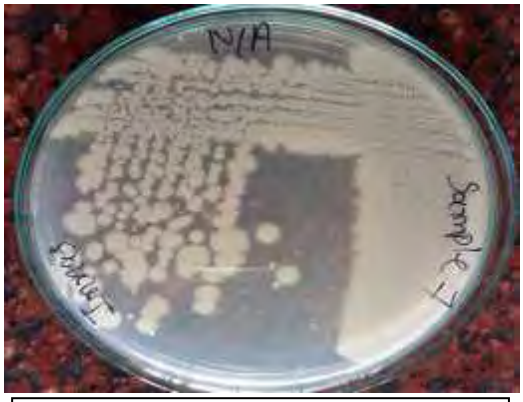
10.AP7a	**9.AP6**	**8.AP5**	**7.AP4b**	**6.AP4a**	**5.AP3**	**4.AP2b**
Small, Yellow coloured colonies	Small, Yellow coloured colonies	Small, Yellow coloured colonies		Small, Yellow coloured colonies	Small, Yellow coloured colonies	Small, White Colonies
			Blue Colour colonies			
Golden Yellow colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies	Green Colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies	Yellow Colour colonies
Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis
Small	Small	Small	Large	Small	Small	Small
Yellow	Yellow	Yellow	White	Yellow	Yellow	Yellow
Circular	Circular	Circular	Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex	Convex	Convex	Convex
***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Bacillus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Micrococcus* spp.**

14.AP10	13.AP9	12.AP8	11.AP7b
Small, Yellow coloured colonies	Small, Yellow coloured colonies	Small, Yellow coloured colonies	
			Blue Colour Colonies
Golden Yellow colour colonies	Golden Yellow colour colonies	Golden Yellow colour colonies	
Beta Hemolysis	Beta Hemolysis	Beta Hemolysis	Gamma Hemolysis
Small	Small	Small	Medium
Yellow	Yellow	Yellow	White
Circular	Circular	Circular	Circular
Entire	Entire	Entire	Entire
Convex	Convex	Convex	Convex
***Staphylococcus* spp.**	***Staphylococcus* spp.**	***Staphylococcus* spp.**	**Fecal coliform**

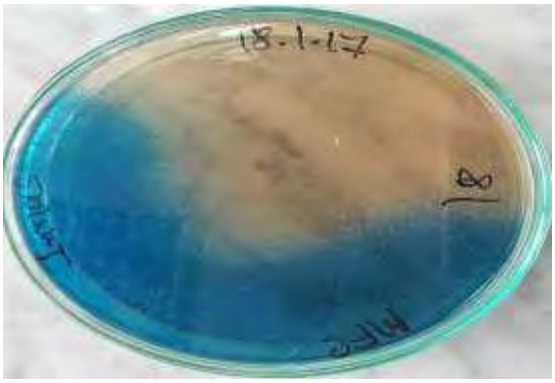
Growth of *Staphylococcus* species on MSA



Growth of *E. coli* on MacConkey agar

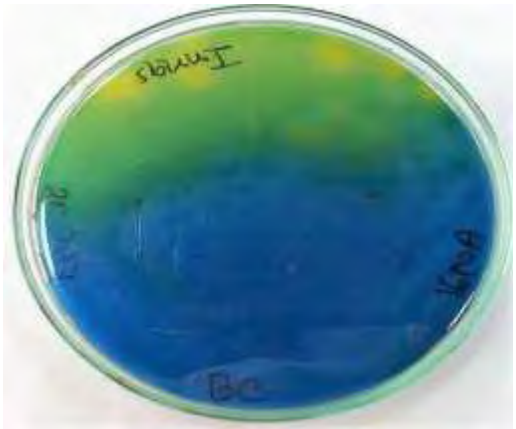


Growth of *Bacillus* species on Nutrient Agar



Growth of fecal coliform on MFC

Growth of *E. coli* on EMB Agar

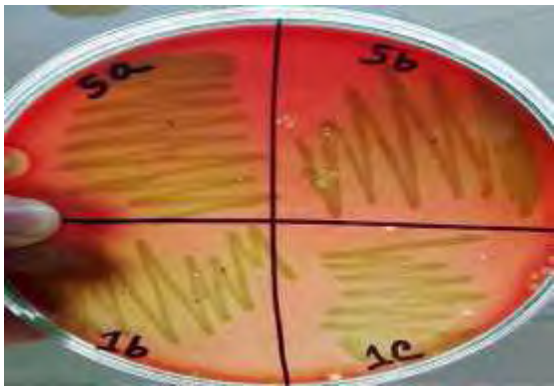


Growth of *Bacillus* on BC Agar

Figure 3.1: Bacterial growth on various selective media

Beta hemolysis

Alpha hemolysis



Gamma Hemolysis

Figure 3.2: Bacterial growth on Blood Agar

3.3 Biochemical Characteristics of the Bacterial Isolates:

Bacterial isolates extracted from Cell Phones 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.

Isolates ID	Gram Reaction	Shape	Indole test	Methyl Red (MR) Test	VP Test	Citrate Test	Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease	Nitrate Reduction Test	Catalase test	Oxidase Test	Suspected organism
MJ1	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

MJ2	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ3a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ3b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
MJ4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ4b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
MJ5	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ6a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ6b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

MJ7	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ8a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ8b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
MJ9	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ10a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MJ10b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SP1a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP1b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

SP1c																		**Fecal coliform**
SP2																		**None**
SP3a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP3b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SP3c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
SP4a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP4b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SP5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP5b																			**Fecal coliform**
SP5c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SP5d	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**

SP6a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP6b																			**Fecal coliform**
SP7a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP7b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SP7c																			**Fecal coliform**
SP8a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP8b	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
SP9	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP10a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SP10b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

DM1																		**None**
DM2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM2b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
DM2c																			**Fecal coliform**
DM3																			**None**
DM4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM4b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
DM4c	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
DM5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM5b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
DM5c	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**

DM6a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM6b																			**Fecal coliform**
DM7a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM7b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
DM8a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM8b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
DM8c																			**Fecal coliform**
DM9a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM9b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
DM9c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**

DM10a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
DM10b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB1a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB1b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB2a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB2b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
HB3a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB3b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

HB4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB4b																			**Fecal coliform**
HB4c	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB5b																			**Fecal coliform**
HB5c	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
HB6a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB6b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB7a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB7b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**

HB8a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB8b																			**Fecal coliform**
HB8c	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB9a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB9b																			**Fecal coliform**
HB9c	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB10a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
HB10b																			**Fecal coliform**
HB10c	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
HB10d	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
JB1a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

JB1b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
JB2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB2b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
JB2c																			**Fecal coliform**
JB3	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB4	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB5b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
JB6	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

JB7a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB7b																			**Fecal coliform**
JB8a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB8b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
JB9a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB9b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
JB9c																			**Fecal coliform**
JB10a	+	Coccin cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
JB10b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

MB1	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB2b																			**Fecal coliform**
MB3	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB4	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB5b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
MB5c																			**Fecal coliform**
MB6	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

MB7	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB8b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
MB9	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
MB10b																			**Fecal coliform**
MB10c	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
BS1a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS1b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
BS1c																			**Fecal coliform**

BS2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS2b	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
BS3a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS3b	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
BS3c																			**Fecal coliform**
BS4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS4b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
BS5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS5b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
BS5c																			**Fecal coliform**

BS6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS6b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
BS6c	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
BS7a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS7b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
BS8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS8b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
BS8c																			**Fecal coliform**
BS9a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS9b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
BS9c																			**Fecal coliform**

BS10a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
BS10b	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
BS10c																			**Fecal coliform**
SB1a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB1b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
SB2a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB2b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
SB3	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB4	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

SB5	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB6b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
SB7a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB7b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
SB8	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB9a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
SB9b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***

SB10	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR1a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR1b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
WR2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR2b																			**Fecal coliform**
WR2c	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
WR3a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR3b																			**Fecal coliform**
WR4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

WR4b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
WR4c																			**Fecal coliform**
WR5a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR5b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
WR6a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR6b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
WR6c	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
WR7a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR7b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
WR7c																			**Fecal coliform**

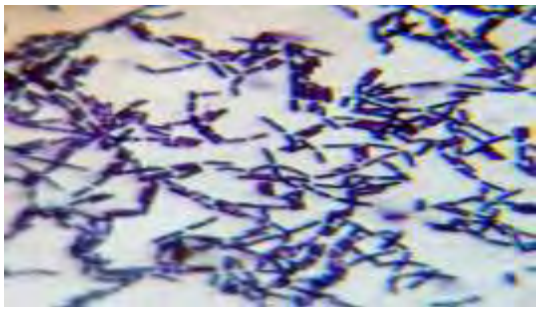
WR8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR8b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
WR8c	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**
WR9a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
WR9b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
WR10	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML1a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML1b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
ML2a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

ML2b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
ML3																			**None**
ML4a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML4b	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
ML4c																			**Fecal coliform**
ML5a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML5b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
ML6a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML6b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
ML7																			**None**
ML8a	+	Cocci in cluster	-	+	+	+	Y/Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML8b	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**

ML9a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML9b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
ML9c																			**Fecal coliform**
ML10a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
ML10b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
ML10c																			**Fecal coliform**
AP1a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP1b	-	Short rods	+	+	-	-	Y / Y	+	+	+	-	+	+	+	-	+	+	-	***E. coli***
AP2a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP2b	+	Cocci	-	-	-	-	R/ R	-	-	-	-	-	-	-	-	+	+	-	***Micrococcus* spp.**

AP3	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP4a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP4b	+	Long rods	-	-	+	-	R/ Y	+	-	-	-	-	-	-	+	+	+	-	***Bacillus* spp.**
AP5	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP6	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP7a	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP7b																			**Fecal coliform**
AP8	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**

AP9	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**
AP10	+	Cocci in cluster	-	+	+	+	Y / Y	+	+	+	-	-	-	-	+	+	+	-	***Staphylococcus* spp.**



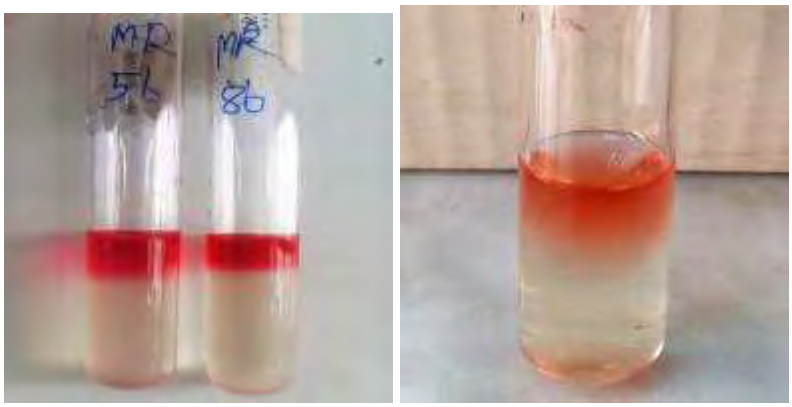
Gram positive rods

Gram negative rods

Figure: Gram staining of bacterial isolates

Indole test (positive) Indole test (negative)

Figure: Biochemical test results of bacterial isolates



Methyl red test (positive) Methyl red test (negative)

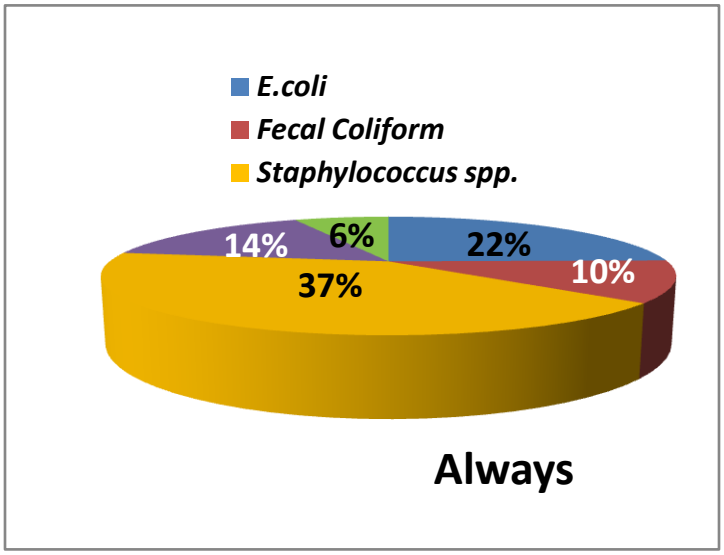
Voges-Proskauer test (positive) Voges-Proskauer test (negative)

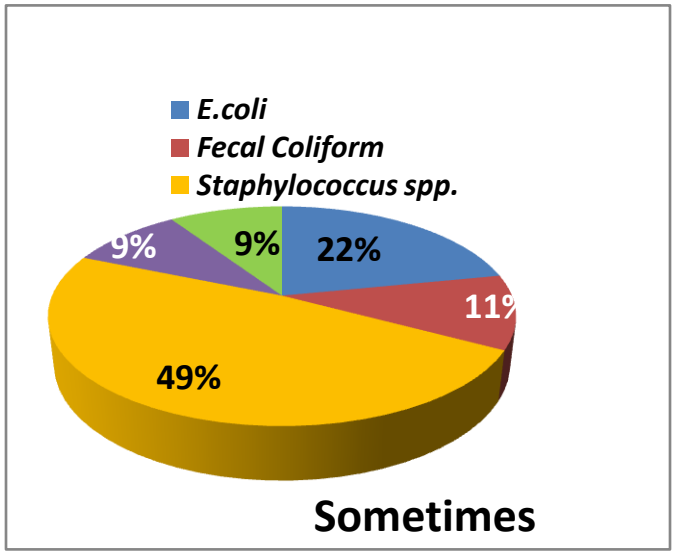
TSI test

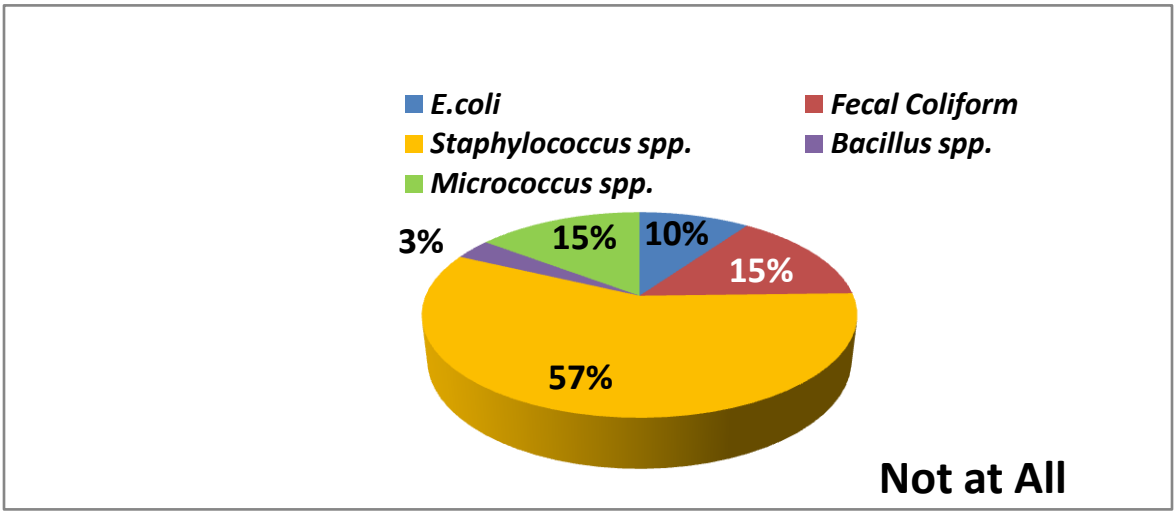
Figure: Biochemical test results of bacterial isolates

3.4 Correlation between the Behaviors of Cell Phone Users and the Organisms Present in the Cell Phone

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

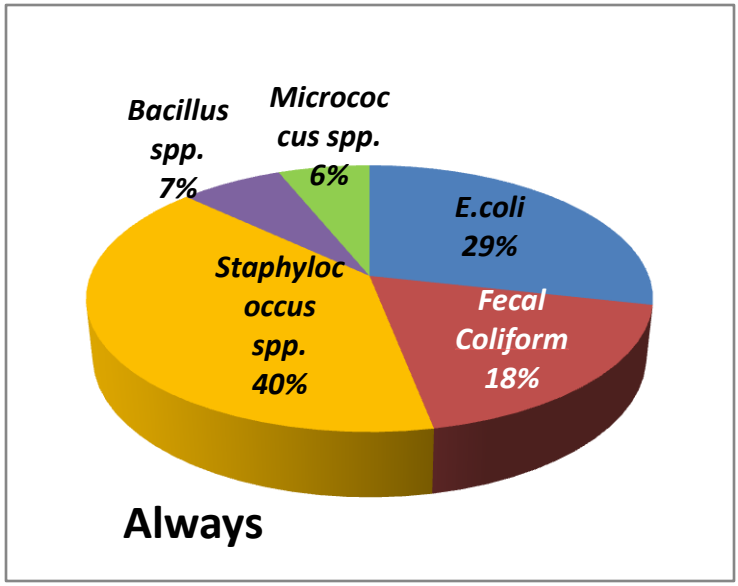


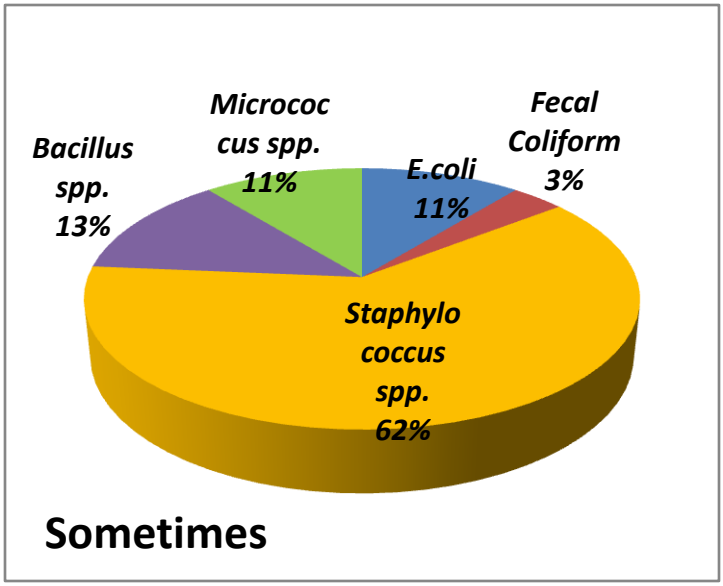


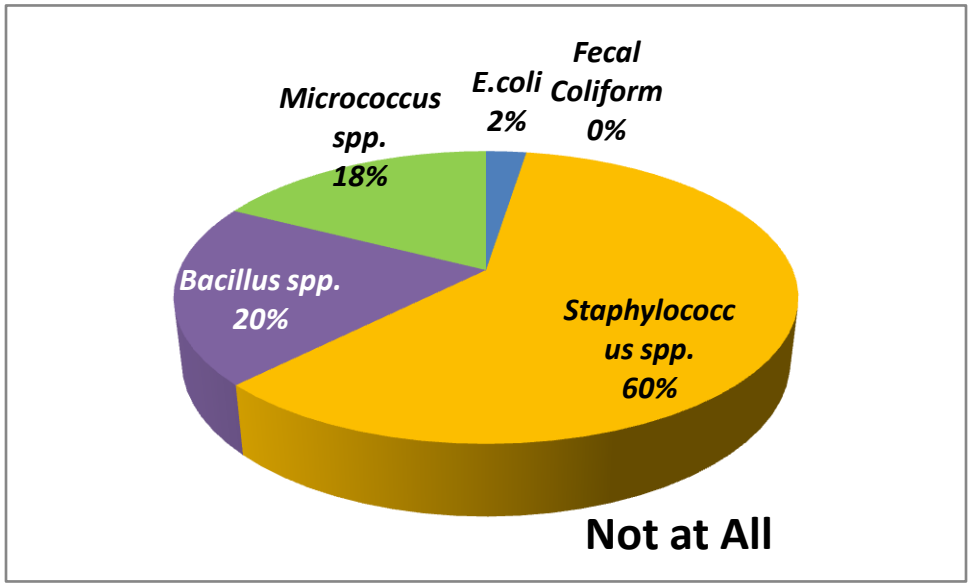


In graph 3.4.1 it is observed that the phones that were always used while eating had the highest concentration of *Bacillus* spp. and *E. coli* present in the study with 14% and 22% respectively of the total organisms of "Always" category. While the ones that were not used at all while eating had the least amount of *Bacillus* spp. *E. coli* presence; of around 3% and 10% respectively of the total organisms "Not at All" category.

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

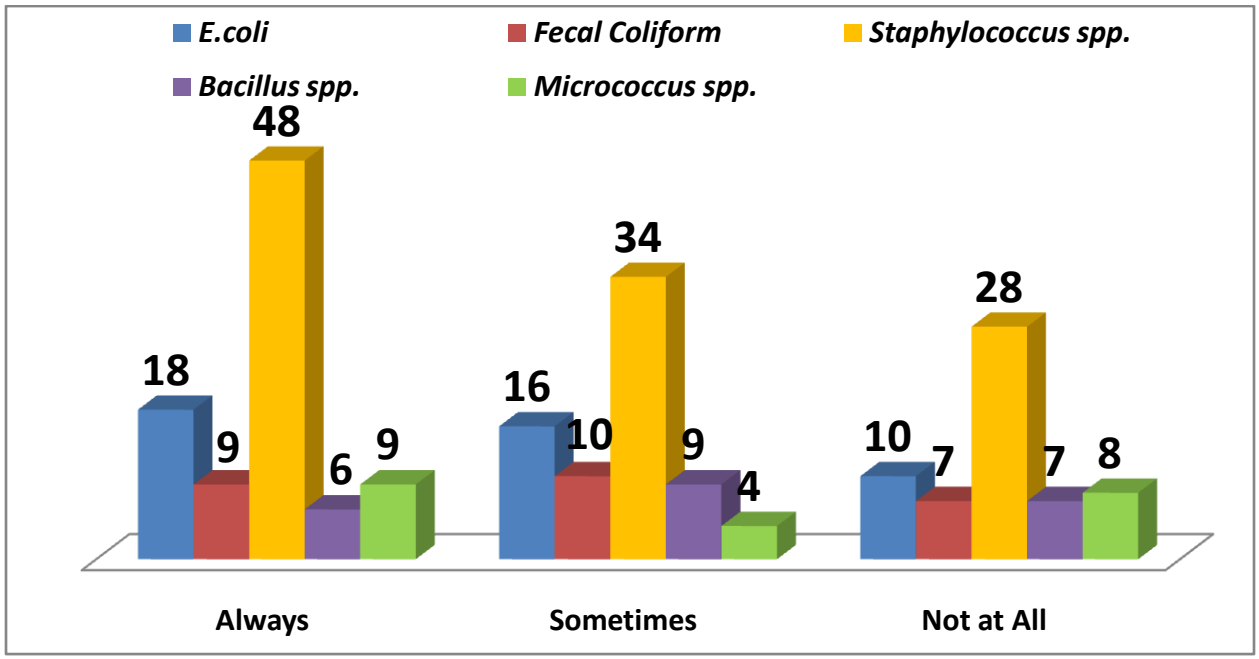






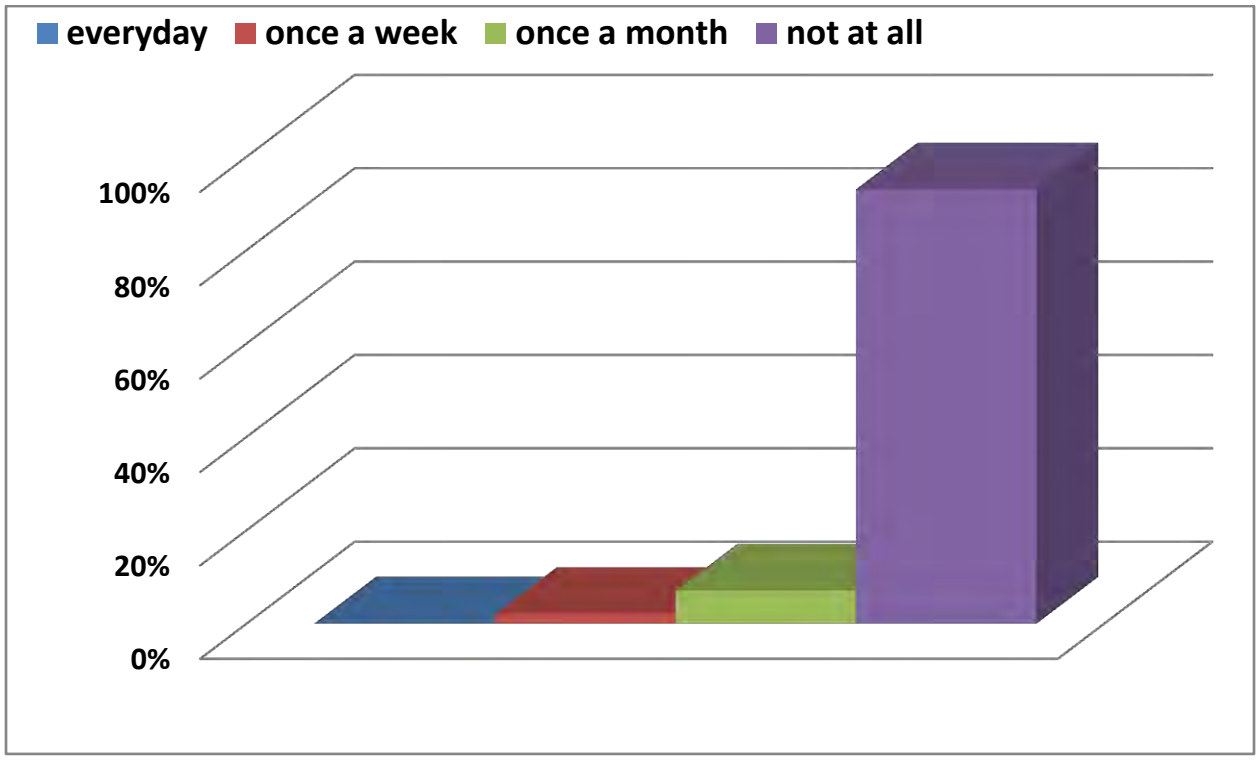
Graph 3.4.2 shows high percentages of fecal coliform and *E.coli* in the phones that were always used inside washrooms with 18% and 29% of the total organism in "Always" category. Whereas. The phones that were not used at all inside washroom showed a drastic drop in the percentages of the same two species; falling down at 0% and 2% respectively. The lesser the phones were used inside washrooms, the lesser was the contamination with these two microorganisms.

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



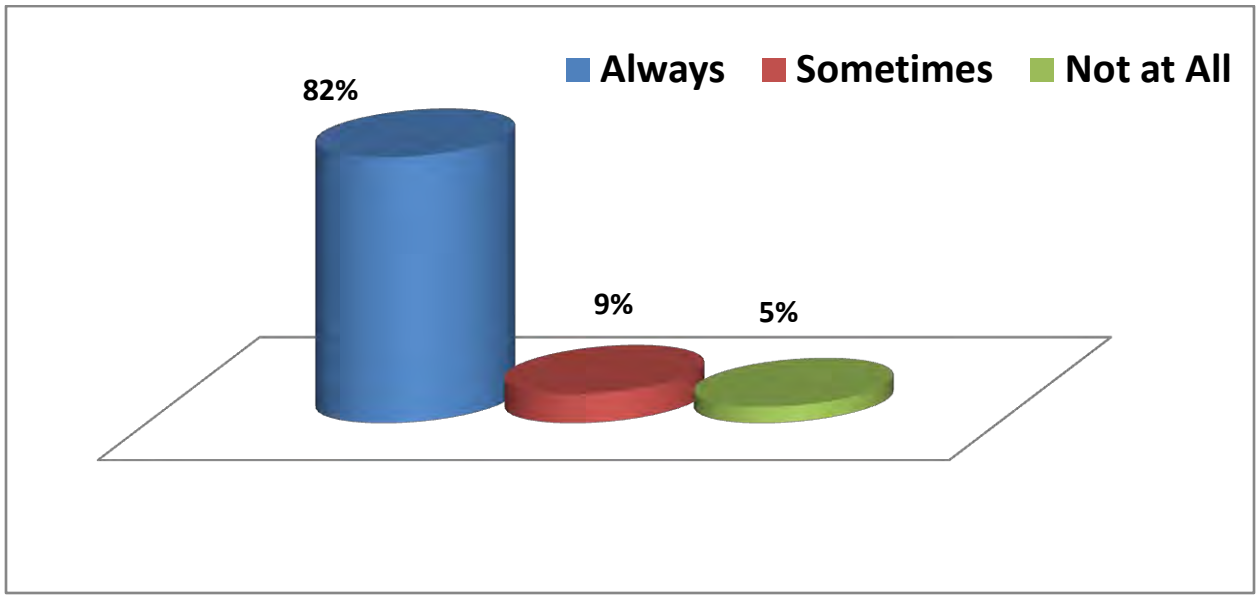
Graph 3.4.3 shows that the phones that were always shared physically with others were high in *Staphylococcus* spp. concentration. 48 colonies of *Staphylococcus* spp. were found in such samples. With the decreasing frequency of sharing phones with others, the *Staphylococcus* spp. presence had also decreased. The phones that were sometimes shared with others contained 34 colonies of the same organism and the ones that were not used at all contained 28 colonies of *Staphylococcus* spp.

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



Graph 3.4.4 shows the impact of cell phone hygiene 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 phones at least once a week, showed around less than 10% presence of microorganisms. And those who cleaned their phone on average once a month showed less than 20% microbial contamination. Lastly, the ones who had never cleaned their cell phones at all showed above 90% microorganism presence. Cell phone cleaning here focuses on using commercially available cleaning solution like 70% ethanol, hand sanitizers, alcohol pads and liquid soaps.

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



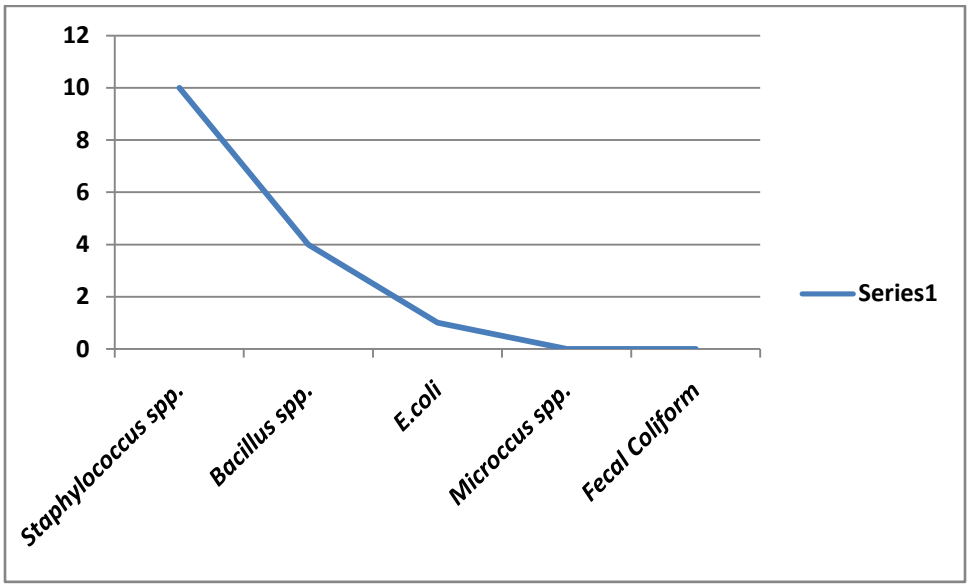
Graph 3.4.5 focuses on the presence of microorganisms on cell phones based on whether they were used while their owner was sick. Those who did not use their cell phones, while being sick showed around 5% microorganism present in their cell phones while the ones who opted to use their cell phones while sick showed microorganism presence in 82% of the case.

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

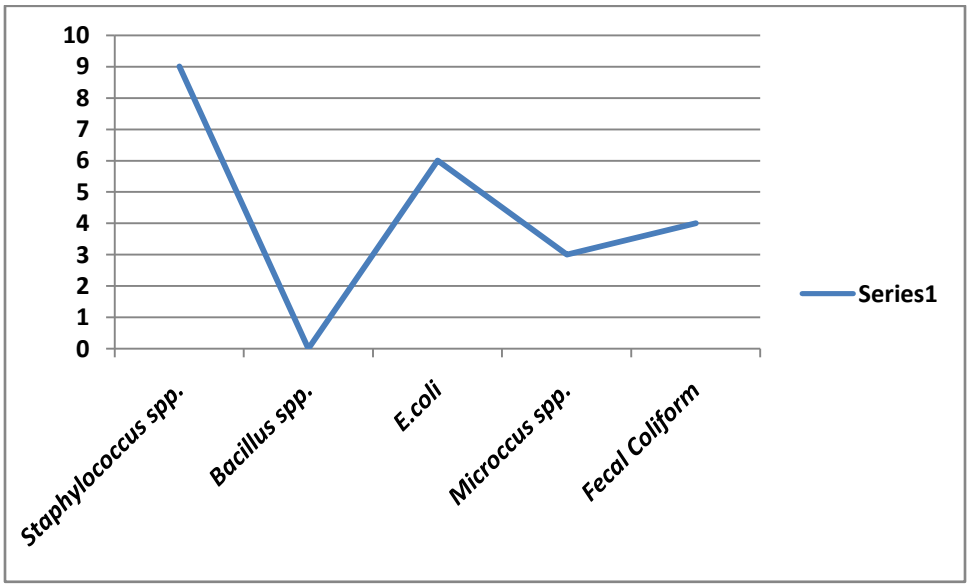
This Graph shows the frequency of organisms present in the thanas of Dhaka City (South). All the graphs show their peak at *Staphylococcus* spp., showing the abundance of *Staphylococcus* spp.

Highest concentration of *E. coli* is observed in Hazaribag (3.5.4) area, peaking at 7 colonies. Sutrapur (3.5.2) and Dhanmondi (3.5.3) are in second spot with 6 colonies each. *Micrococcus* spp. is observed in the most number at Bongshal (3.5.7), peaking at number 5. Fecal coliform is also present in the highest number at Bongshal (3.5.7), peaking at number 6. *Bacillus* spp. is found with 4 colonies each in both Motijheel (3.5.1) and Malibagh (3.5.10).

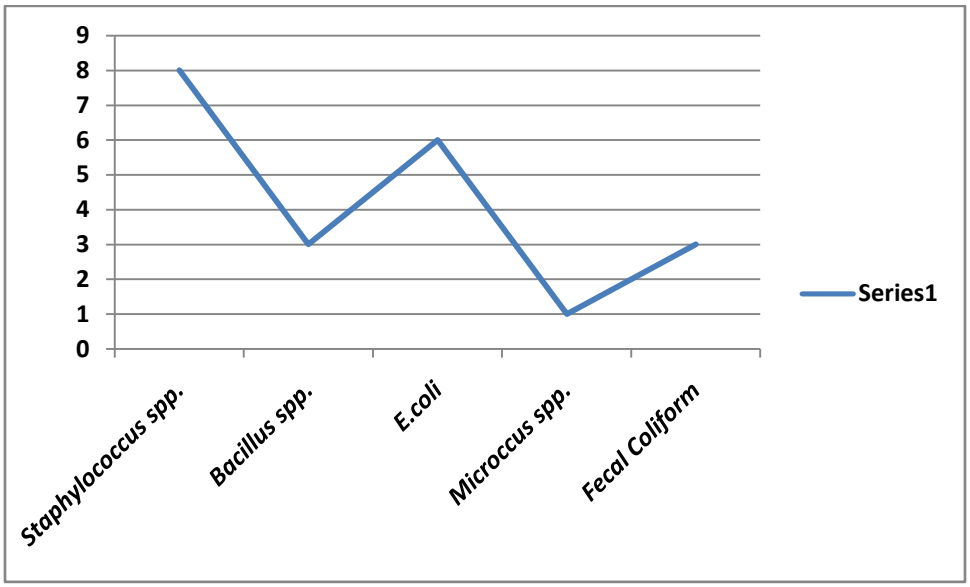
3.5.1 Graph – Frequency of Organisms in Motijheel



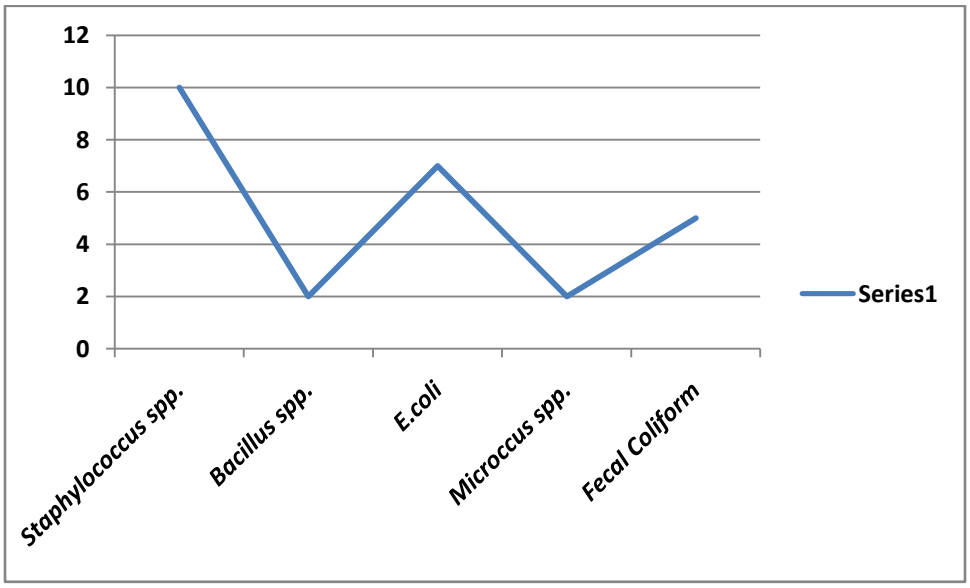
3.5.2 Graph – Frequency of Organisms in Sutrapur



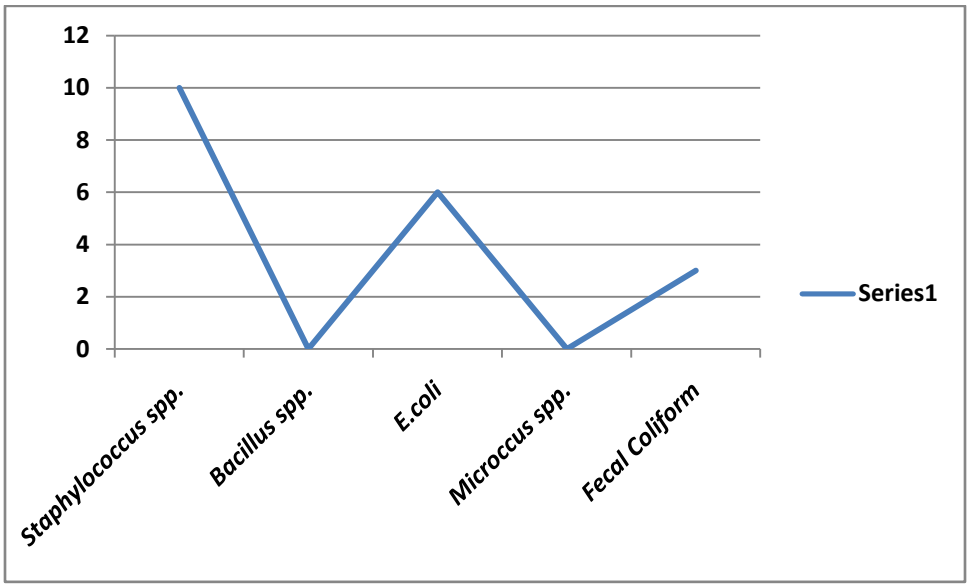
3.5.3 Graph – Frequency of Organisms in Dhanmondi



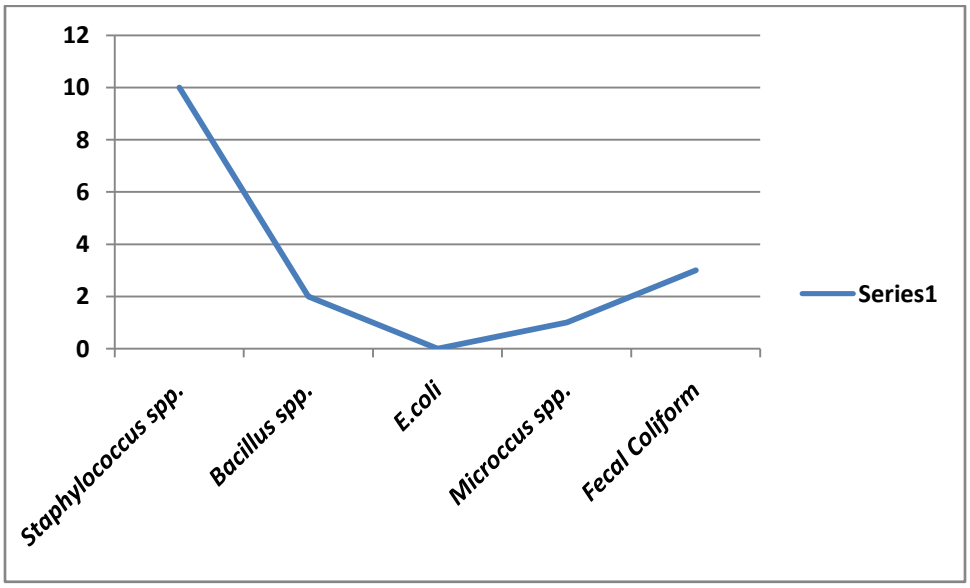
3.5.4 Graph – Frequency of Organisms in Hazaribag



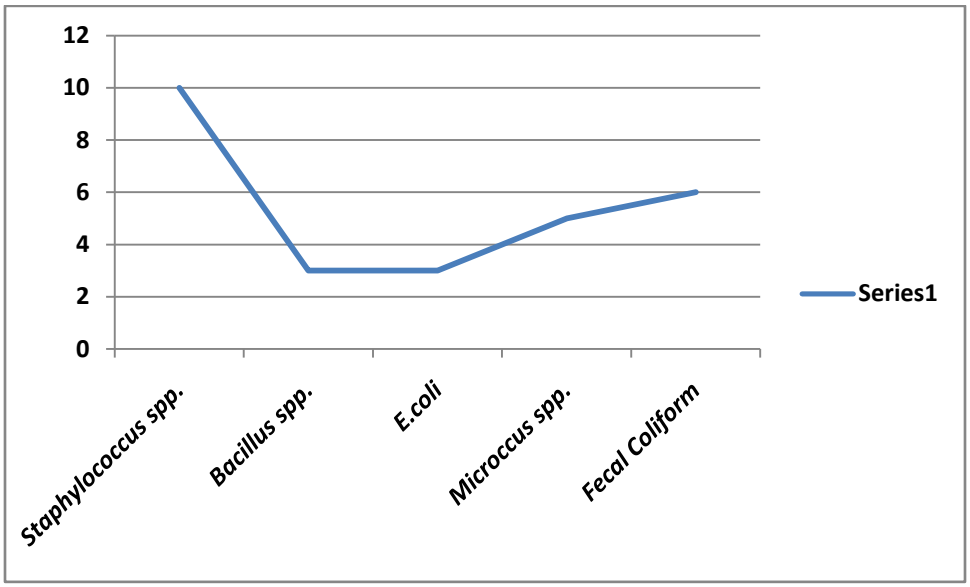
3.5.5 Graph – Frequency of Organisms in Jatrabari



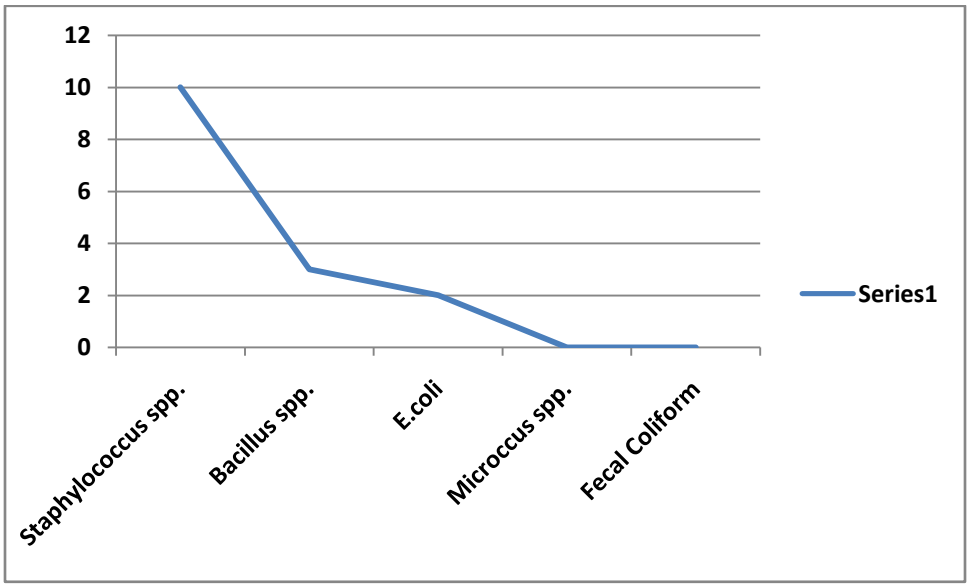
3.5.6 Graph – Frequency of Organisms in Moghbazar



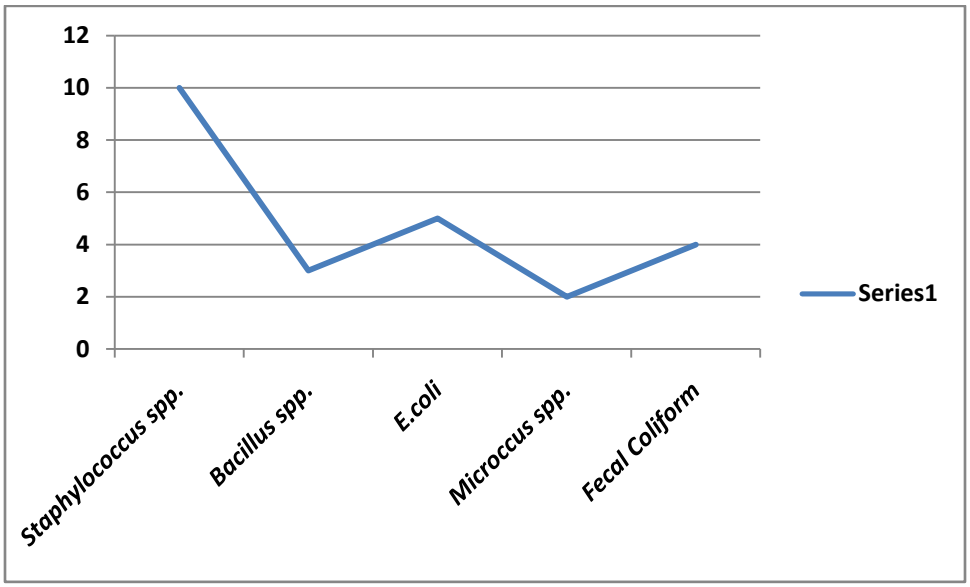
3.5.7 Graph – Frequency of Organisms in Bongshal



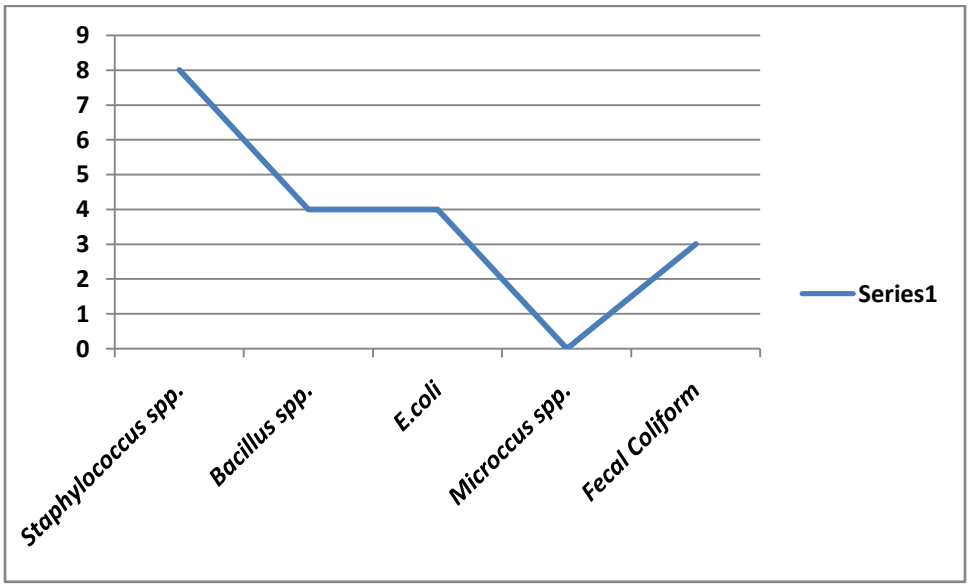
3.5.8 Graph – Frequency of Organisms in Shahbag



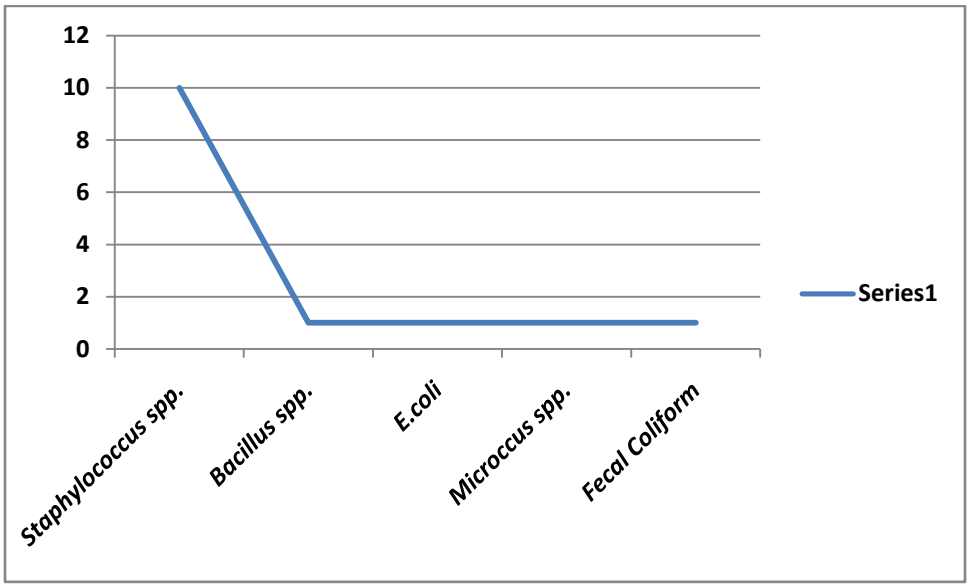
3.5.9 Graph – Frequency of Organisms in Wari



3.5.10 Graph – Frequency of Organisms in Malibagh



3.5.11 Graph – Frequency of Organisms in Azimpur



3.6 Kirby-Bauer Disc Diffusion Antibiotic Susceptibility Test Results

Table 3.6

	GEN	P	K	TE	VA	IMI	CIP	OX	CL	NX	R
Staphylococcus spp.	S	S	S	R	S	R	S	S	S	S	S
E.coli	R	R	S	R	R	S	S	R	R	R	S
Bacillus spp.	S	R	S	S	S	I	S	R	S	S	S
Fecal Coliform	S	R	S	S	S	I	S	S	S	R	S
Micrococcus spp.	S	S	S	S	S	S	S	S	S	S	S

The 12 selected colonies were tested against 11 different antibiotics. The antibiotics were: Gentamicin, Penicillin, Kanamycin, Tetracyclin, Vancomycin, Imipenem, Ciprofloxacin, Oxacillin, Chloramphenicol, Norfloxacin and Rifampicin.

From Table 3.6 we can see that *Staphylococcus* spp. were susceptible to Gentamicin, Penicillin, Kanamycin, Vancomycin, Ciprofloxacin, Oxacillin, Chloramphenicol, Norfloxacin and Rifampicin; resistant against Tetracyclin, Imipenem. *E. coli* were susceptible Kanamycin, Imipenem, Ciprofloxacin and Rifampicin, and resistant against Gentamicin, Penicillin, Tetracyclin, Vancomycin, Oxacillin, Chloramphenicol, Norfloxacin. *Bacillus* spp. were susceptible to Gentamicin, Kanamycin, Tetracyclin, Vancomycin, Ciprofloxacin, Chloramphenicol, Norfloxacin and Rifampicin; resistant against Penicillin and Oxacillin, and intermediate against Imipenem. *Micrococcus* spp. were susceptible to all the antibiotics. Lastly, fecal coliforms were susceptible to Gentamicin, Kanamycin, Tetracyclin, Vancomycin, Ciprofloxacin, Oxacillin, Chloramphenicol and Rifampicin, but resistant against Penicillin and Norfloxacin and also intermediate against Imipenem.

Chapter 4

Discussion

This study took place in order to identify and isolate bacteria found from the mobile phones of civilians residing in Dhaka South City Corporation and it has also produced eye-catching results. The amount of isolated bacterial colonies is more than 200 and variety in the isolated microbes was noticeable as well. Thus, such interpretation supports the claim that mobile phones can serve as a potential vector for different varieties of microbes, whether pathogenic and nonpathogenic; and along the way, emphasizes on the need of creating awareness against the transfer of microbes from one individual to another through mobile phones. Moreover, the importance of hand hygiene and cleanliness of everyday accessories has also been expressed through this study.

Since our world is becoming a larger reservoir of microbes with every passing second, the methods and ways of transmission is in an ever-increasing and ever-alarming situation; and mobile phones being one of the mostly used accessories of present time, is playing a great role in the transmission of microbes by serving as a vector. The blessings of technological advancement have not missed out on Bangladesh as well and as a result, the usage of mobile phones in this country is increasing day by day, which in turn raises the concern of microbial transmission from these devices. Therefore, the necessity of strict microbiological standard and personal hygiene programs are more urgent than ever. Taking all the situations into account, this investigation took place in order to identify the prevalence of microbes in the mobile phones of Dhaka South City Corporation (DSCC) dwellers and for a call up towards personal hygiene.

11 thanas of DSCC were chosen for this study with 10 mobile phone samples from each thana. The volunteers were diverse in age, occupation and habit; ranging from students to job-holders. However, this study was different from other similar studies because a large demographic was chosen for this purpose and the regions showed a lot of diversity amongst themselves.

A total of 110 samples were collected for the study and 105 of them were found to be contaminated with bacteria, which represents around 95% of total sample. Along with that, total number of bacterial colonies isolated was 216 and contained 5 different genera with *Staphylococcus* spp. being the most prominent (47.6%) and *Bacillus* spp. being the least (10.1%). Others were *E. coli*, fecal coliforms and *Micrococcus* spp. with 20.3%, 12.03% and 19.09% respectively. Presence of fecal coliforms suggests that the samples contained fecal contamination. Due to release of endotoxin or lipopolysaccharide (LPS) by *E. coli*, it is regarded as the most common cause of gram-negative sepsis, which in turn, results in septic shock (Bone

et al., 1993). In our study, *E.coli* has proved to be the second most prominent microbial group with around 20.3% prevalence. Also, the presence of fecal coliforms was confirmed by the growth of bacterial colonies in Membrane Fecal Coliform Agar (MFC). In the studies that took place before this, the prevalence of microbes varied both in numbers and in type of the bacteria. For example, Dave and Shende, (2015) found *Pseudomonas aeruginosa* and *Enterobacter aerogenes* along with *Staphylococcus* spp., *Bacillus* spp. However, the prevalence of *Staphylococcus* spp. came out as the highest (69.76%). They have also claimed that *Staphylococcus aureus* may cause several diseases ranging from pimples and boils to pneumonia and meningitis. In another report, Zakai *et al*., (2015) found *Staphylococcus* spp. to be most prominent bacteria as well. They conducted their investigation on 105 mobile phones. However, they also identified *Viridans Streptococci* and *Pantoea* species on lower levels. Moreover, Al-Abdalall *et al*., (2010) investigated a total of 202 samples in order to identify both fungal and pathogenic bacteria isolates. 737 isolates of the following bacteria were found in total, which are: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Neisseria sicca*, *Micrococcus luteus*, *Proteus mirabilis*, *Bacillus subtilis*, and *Enterobacter aerogenes* at the rates of 56.58%, 13.57%, 8.01%, 7.73%, 6.51%, 3.66%, 2.85% and 1.09% respectively. According to their claim, *S. aureus* can evolve into methicillin-resistant *S. aureus* (MRSA). The main reservoir of *S. aureus* is the hand through which the bacteria are transferred to foods. Also, Verma *et al*., (2015) concluded that bacteria isolated and characterized from mobile phones are responsible for causing infections in humans, and thus discouraged the sharing of mobiles phones and the use of mobiles while eating. They also emphasized on personal hand hygiene in order to prevent self and cross contamination by bacteria. Nevertheless, they had isolated *E. coli, E. aerogenes, Streptococcus* spp. and *S. aureus* in the percentage of 23.53%, 23.53%, 17.65% and 35.30% respectively from their research.

However, the names of the individuals who agreed to volunteer in this study has been kept undisclosed and will remain so, but the statistics collected from our questionnaire showed that the highest number of volunteers fall into students category (65%) and job-holders and daily workers coming in on second (16%) and third position (11%), while people from other fields being the least in number. Now, if we look at the age groups of the volunteers, we can see that most of the volunteers are aged 21-25 years and least of them are aged above 30 years. The above two stats depict that young people and students are more vulnerable to bacterial infection

through mobile phones. Other than that, it is quite astonishing that around 26% of the volunteers have claimed to use their phones while in washrooms regularly even though most of them (54%) have claimed to sometimes use their phones in washrooms. Around 20% of them have denied using phones in washrooms. About 58% have claimed to use their cell phones moderately, while 28% and 14% of the volunteers use phone a lot and not more than necessary respectively. About 26% of the total volunteers have claimed to share their cell phones with other people a lot, while 55% of them claiming to share it sometimes and the rest (19%) assuring that they do not share their phones at all. While eating, almost 31% of the volunteers use their cell phones a lot and 53% of them use sometimes. A mere 16% do not use their cell phones at all while eating. It was quite disappointing even though expected that not many people give proper emphasis on cleaning their cell phones. In our study, only 7% of the volunteers claimed that they clean their cell phones everyday while the percentage of those who do not clean it at all is a mammoth 65%. A few have claimed to clean it once a week (11%) and once a month (16%). During public gatherings, around 40% of the volunteers use their phones a lot, 36% use them moderately and 24% do not use at all. During sickness, 33% of them use cell phones regularly, 55% use it moderately, while 12% have claimed to not using cell phones at all. Most of our volunteers keep their cell phones in their pockets (34%), while 25% of them keep it in purses, 19% in their backpacks, 14% prefer to keep them in their hands and 8% of them keep them in other places. Money is the most common item that is kept along with cell phones as 22% have claimed so. Keys and ID‟s taking the second and third spot; 17% and 16% respectively. Wallets and headphones share the fourth spot (12%) while cosmetic accessories coming in on the last position with 9%. Most the volunteers of the study use cell phone covers for extra protection, around 71% and 29% of them do not do so.

One of the primary purposes of this study was to present the prevalence of bacteria in comparison to the behavioral patterns of the volunteers and because of that we emphasized on the questionnaire‟s results and related those to the samples‟ isolates. For example, those who claimed to have always used their cell phones in the washrooms, showed higher amounts of *Escherichia coli* and other fecal coliforms than the ones who claimed to have used phones in the washrooms sometimes. To add to that, we could not find any fecal coliforms in the phones of the individuals who did not used their phones inside washrooms at all. So it shows a relationship between the prevalence of certain microorganisms and the behavioral patterns of the volunteers.

Likewise, those who claimed to have cleaned their cell phones everyday did not any bacterial contamination whereas, the ones who had never cleaned their phones showed around 86% bacterial contamination. This signifies the importance of cleaning mobile devices in order to stay free of contamination thus creating public awareness which was one of the primary goals of the study. 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 (Zakai *et al*., 2015).

The primary goal of the demographic representation was to compare the prevalence of microbes of one thana with another and to see if there was any significant change in the types of microbe across those thanas. Even though the thanas showed difference in amount of the microbial isolates, the types of microorganisms did not differ much. *Staphylococcus* spp. was the most prominent in every thana and *E.coli* was present in every thana as well. The antibiotic susceptibility test was conducted to look for the response of the microorganisms against certain antibiotics. 12 colonies of the 5 types of organisms were tested against 11 different antibiotics and no irregular response was found.

However, since the study was based only on 10 samples from each thana, the results based on demographic could not differentiate much among themselves, but if in future it is carried out with a larger number of samples, then the proper depiction of area based prevalence of microorganisms may become possible.

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