ISOLATION AND IDENTIFICATION OF BACTERIA FROM MOBILE PHONES OF STUDENTS AND EMPLOYEES OF HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY

A THESIS

BY

ZAKARIE AHMED HUSSEIN

REGISTRATION NO. 1705192

SEMESTER: JANUARY-JUNE, 2018

SESSION: 2017

MASTER OF SCIENCE (MS)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

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Approved as to style and content by

______________________________	________________________
(Prof. Dr. Md. Khaled Hossain)	**(Dr. Nazmi Ara Rumi)**
Research Supervisor	Research Co- Supervisor

____

(Prof. Dr. Md. Mostafizer Rahman)
Chairman
Department of Microbiology

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2018

DEDICATED
TO
MY BELOVED
PARENTS, SISTER
AND BROTHERS

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ABSTRACT

Mobile phones are increasingly used by professionals university staff and health care professionals for communication these may harbor in various potential pathogens. The ability of the microbes to survive on the surface of mobile phone makes it as one of the important fomites in the spread of microorganism between users. Present study was designed to isolate and identify of bacteria from mobile phones of students and employees of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh. A total of 32 swab samples of mobile phones were collected in different academic staffs, nonacademic staffs, students and cleaners. In this study bacteria were identified on the basis of morphological, cultural and biochemical characterization. The total viable count (TVC) of different swab samples of mobile phone in different categories (academics, nonacademic, students and cleaners) were studied. samples were ranged from 73×10^{-6} CFU/ml to 260×10^{-6} CFU/ml. Among 32 swab samples the bacterial percentage isolation frequency and occurrence were *Staphylococcus spp* 21 (25.6%), *Bacillus spp* 17(20.7%), *Klebsiella spp* 16(19.5%), *Pseudomonas spp* 15(18%) and *Salmonella spp* 13(15.85%). The vitro antibiotic sensitivity test of isolated bacteria *Staphylococcus spp, Bacillus spp, Klebsiella spp, Pseudomonas spp and Salmonella spp* were resistance in randomly Penicillin, Amoxicillin, Cefaclor, Ofloxacin and Ciprofloxacin.

The finding of this research indicates that all mobile phones under consideration were infected by several microbes most of which belongs to the natural flora of the human body. Therefore sharing of mobiles, usage of mobile during eating should be discouraged. Personal hand hygiene is very important and also washing of hand before and after handling phones since it is a source of disease transmission. The purpose of this study was to investigate microbial colonization on mobile phones and suggested a cost effective solution for the emerging nosocomial infection.

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CHAPTER 1

INTRODUCTION

CHAPTER 1
INTRODUCTION

A mobile phone or cellular phone is a long range, portable electronic device that can make and receive telephone calls over a radio link whilst moving around a wide geographic area. It does so by connecting to a cellular network provided by a mobile phone operator, allowing access to the public telephone network. Mobile phones have become an integral and indispensable part of daily life. In many countries, most adult and many children now own mobile phones. At present, Asia has the fastest growth rate of cellular phone subscribers in the world. (TRAI, 2009-10). A mobile phone can spread infectious diseases by its frequent contact with hands (Kilic *et al.,* 2009).

In recent times there has been an increase in the use of mobile phones by academic and non-academic staff of educational institutions. Innovations in mobile phones have led to better strategic life with good communication (Adetona *et al.,* 2011). Therefore the use of mobile phones in the course of a working day has made mobile phones potential agents of microbial transmission (Soto *et al.***,** 2006). The increase use of mobile phones is seen as responsible for rise in community infection rates reported by ecological findings (Brady *et al*., 2006).

Mobile phones could be contaminated through various sources such as human skin or hand, bag, phone pouch, pockets, environment and food particles; these sources are linked through which microorganisms colonized the phone, thus causing diseases that range from mild to chronic. Although, microorganisms isolated so far by health researchers are mostly normal flora of the source of contamination, they may serve as reservoirs of infection, allowing the transportation of the contaminating bacteria to many different clinical environments (Brady *et al*., 2007). Mobile phones could be a health hazard with tens of thousands of microbes living on each square inch of the phone. *Staphylococci,* particularly *S.epidermidis* are members of the normal flora of the human skin, respiratory and gastrointestinal tracts (Jayachandra *et al.,* 2011).

A number of studies have consistently reported that 5– 21% of healthcare workers with mobile phones provide a reservoir of bacteria known to cause nosocomial infections (Brady et al., 2006; Jeske *et al*., 2007; Brady *et al*., 2009 and Sadat *et al*., 2010). Hands play a major role in the transmission of infection in healthcare institutions, in industrial

settings such as food industries and also in all community and domestic setting (Aiello *et al*., 2002). Hands and instrument used by workers serve as vectors for the transmission of micro-organisms (Brady *et al*., 2006).

Improper practice of hand hygiene and use of mobile phones both together play significant role in spread of some human harmful pathogens. So mobile phones may transmit more than just information in today's busy work places and other environments. They may also be involved in the transmission of infections. In spite that mobile phones are used in close contact with the body, the hygiene risk involved in using these instrument has not yet been determined. Previous studies demonstrated that the hands of healthcare workers can become contaminated by bacteria from their mobile phones (Khivsara *et al*., 2006; Jeske *et al*., 2007; Sadat *et al*., 2010).

Due to benefits of the mobile phone, its hazard to health is often overlooked. The constant handling of the phone by different users exposes it to an array of microorganisms and thus makes it a good carrier for microbes. The cell phones have been identified as one of the media by which bacterial pathogen could be transmitted. (Mofolorunsho *et al*., 2013).The cell phones can harbour various potential pathogens and serves as exogenous source of nosocomial infection among hospitalized patients (Chawla *et al*., 2009). The pathogenic bacteria are present on approximately 40% of mobile phones belonging to patients in a hospital (Tagoe *et al.,* 2011).

Microbiologists say that the combination of constant handling with the heat generated by the phones creates a prime breeding ground for many microorganisms that are normally found on the skin. Nasal carriage of *S.aureus* occurs in 20-50% of human beings. *Staphylococci* are also found regularly on clothes, bed linen, and other human environments. (Melnick *et al.,* 2004). *Staphylococcus aureus*, a common bacterium found on the skin and in the noses of up to 25% of healthy people and animals can cause illnesses from pimples and to pneumonia and meningitis, and is a close relative of methicillin Resistant *Staphylococcus aureus* (MRSA). The main reservoir of *S.aureus* is the hand from where it is introduced into food during preparation. (Hui *et al* .,2001).Bacterial cells could readily adhere to mobile phone surfaces and could form organized colonies (Beveridge *et al*., 1997). A study to determine the transfer efficiency of micro-organisms by fomites suggests that the Gram-positive bacteria are transmitted most readily followed by viruses and Gram-negative bacteria (Rusin *et al*., 2002). It has

been shown that a significant number of germs could be transferred between the hands of the users of mobile phones, and vice versa (Ulger *et al.*, 2009).

Mobile phones have also been reported to be a reservoir for microorganisms. Most of 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 some body, calling and picking up calls on their mobile phones. Then as soon as 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. (Deepak *et al*., 2015).

It has been reported that a mobile phone can harbor more microorganisms than a man's lavatory seat, the sole of a shoe or the door handle (Brady *et al*., 2006). Further, sharing of mobile phones between people may directly facilitate the spread of potentially pathogenic bacteria to the community. The potential of mobile phones as vectors to nosocomial infection has been studied before (Rafferty and Pancoast, 1984; Brady *et al*, 2006; Brady *et al*., 2007). Studies reported that the most commonly found bacterial isolate was *coagulase negative Staphylococcus* (CONS) as a part of normal skin flora. Potentially pathogenic bacteria found were methicillin sensitive *S.aureus* (MSSA), coliforms, methicillin resistant *S.aureus* (MRSA), *Corynebacterium spp., Enterococcus faecalis, Clostridium perfringens, Klebsiella spp., Enterobacter spp., Pseudomonas spp., Aeromonas spp, Acinetobacter* and *Stenotrophonmonas maltophilia.* They can cause opportunistic infections (Soto *et al.,* 2006).

The objective of this research work was to isolate and identify of bacteria from mobile phone in students and employees of Hajee Mohammad Danesh Science and Technology University. With a great consideration given to the above facts in view, the aims of the present study were:

1) To isolate and identify bacteria that regularly associated with mobile phones.
2) To check the contamination of bacteria pathogens on the cell phone.
3) To determine antimicrobial susceptibility pattern of the identified bacteria.

LITERATURE REVIEW

CHAPTER 2
LITERATURE REVIEW

Shahaby *et al*., (2012) reported that bacterial contamination rate of mobile phones in the university setting that are in frequent contact with faculty members, personnel, students and/or physicians and nurses in the university clinic. A total of 101 mobile phones belonging to different categories working in various departments at Taif University, KSA were screened for microorganisms' contamination. Out of the total 101 mobile phones, growth was obtained in 78 (77.2%) mobile phones; 70 (89.7%) from staffs, personnel, students and 8 (10.3%) from clinical workers. Staphylococcus spp and Bacillus spp were the most commonly isolated organisms. Coagulase negative Staphylococcus was the most frequently isolated; 60 (27.12%). The efficacy of decontamination with 70% isopropyl alcohol was found to be 71.3%, as only 29 mobile phones showed growth after decontamination. It was found that around 61.5% of the mobile phones of health care workers at university clinic were contaminated and thus acted as a potential source of nosocomial infections. According to morphological, physiological characteristics, APi profiles and sequencing of 16S-rRNA gene, the selected eight isolates were identified as Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Staphylococcus hominis, Staphylococcus succinus, Staphylococcus xylosus and Staphylococcus saprophyticus. Based on random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), 32 unique RAPD fragments were identified among the selected isolates. Such unique fragments could be considered as specific markers and might be utilized in tracking the bacterial isolates.

Haider *et al*., (2016) Observed that high bacterial contaminate the main source for many pathogenic disorders. In general the rate of bacterial contamination in mobile phones 100%. The bacterial percentages of isolation frequency and occurrence were Staphylococcus aureus (5 Staphylococcus epidermidis (84%), Bacillus spp. (30%), Escherichia coli (43%) and Proteus spp. (11%). The res findings indicate that Staphylococcus epidermidis is dominant bacteria associated with mobile phones, due to direct contact with the human skin.

Deepak Kumar *et al*., (2015) isolated and characterized bacteria from mobile phones of University of Gondar students and employees to show that mobile phones are potential reservoir for number of bacteria. Total 50 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, we found E. coli, E. aerogenes, Streptococcus spp. and S. aureus in the percentage of 23.53%, 23.53%, 17.65% and 35.30% respectively. The finding of this research indicates that bacteria isolated and characterized from mobile phones are known to cause infections in human beings; therefore sharing of mobiles, usage of mobile during eating should be discouraged. Personal hand hygiene is very important and also washing of hand before and after handling of food and phone decontamination should be adopted by people to prevent cross and self-contamination by these bacteria.

Amira and Al-Abdalall, (2010) determined microbial contamination of mobile phones in the city of Dammam, in the eastern region of Saudi Arabia, and identify the most important microbial species associated with these phones in order to take the necessary remedial measures. Materials and Methods: The analysis of a total of 202 samples was done to identify fungal and pathogenic bacteria isolates. Sterile swabs were frmly passed on the handset, the butons and the screens of mobile phones, then inoculated into media of bacteria and fungi. Frequency distributions of isolates were calculated. Results: There were 737 isolated of the following bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Neisseria sicca, Micrococcus luteus, Proteus mirabilis, Bacillus subtilis, and Enterobacter aerogenes at the rate of 56.58, 13.57, 8.01, 7.73, 6.51, 3.66, 2.85 and 1.09% respectively. There were fungal isolates as follows: Alternaria alternata, Aspergillus niger, Cladosporium sp., Penicillium spp., Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer, Aspergillus ochraceus at the rate of 29.07, 26.74, 20.93, 10.47, 6.98, 2.33, 2.33, 1.16%, respectively. Conclusions: 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. This means that it is necessary to sterilize hands afer contact with a phone since it is a source of disease transmission.

Famurewa and David, (2009) isolated bacteria from 150 cell phone of the volunteers in the university premises, commercial centres, hospital personnel (doctors and nurses) and hospitalized patients. Organism encountered include: Escherichia coli, (28.2%), Pseudomonas aeruginosa (22.6%), Klebsiella sp (14.5%), Serratia sp (13.7), Staphylococcus aureus (12.9%) and Proteus vulgaris (8.1%). Antibiotic susceptibility test carried out on the isolated organisms using agar diffusion method show that all the isolates were resistant to augment in while resistance to common antibiotics tested was equally high. E. coli and P. aeruginosa which were the predominant organisms were equally the most resistant against the antibiotic tested. Multiple antibiotic resistance was observed among the isolates. All the isolates were resistant to more than three antibiotics. This revealed that cell phone may have notable role in the transmission of multidrug resistant nosocomial pathogens.

PURNIMA, (2014) determined contamination of cell phones by pathogenic microorganisms: Comparison between hospital staff and college students. Nusantara Bioscience 6: 203-206. Cell phone (CP) is a long range portable electronic device. The cell phone is constantly exposed to arrays of microorganisms, making it a harbor and breeding ground for microbes especially those associated with skin. The adult human is covered with approximately 2m2 of skin with area supporting about 106 bacteria. To check whether the cell phone act as a vector for transmission of various pathogens, a potential study was carried out in Microbiology Department of Shri Radhakisan Laxminarayan Toshniwal College of Science, Akola, Maharashtra, India. Total 20 cell samples were screened. Two parameters were considered: College students and hospital staff. The isolated bacteria Staphylococcus aureus, Escherichia coli, Pseudomonas sp., Bacillus subtilis, Aerobacter aerogenes, Salmonella, Shigella, Streptococci, P. vulgaris were identified on the basis of morphological and cultural characteristics. The main aim of present study was to check the contamination by bacterial pathogens on cell phones and also to check role of cell phone for transmission of pathogens from person to person or not.

Mohamed and Ismail, (2014) reported mobile phones are harbor various potential pathogens and become an exogenous source of nosocomial infections. A total of 91 mobile phones belonging to staff members in Taif University screened for bacterial isolates using bacteriological methods. Bacteriological analysis revealed that about

(85.1 %) of mobile phone samples were contaminated with bacteria. Some bacterial species were isolated from mobile phone samples. They identified as Gordonia, Pantoea, Ochrobactrum, Staphylococcus and Bacillus spp. Genetic diversity of these bacteria was investigated by Random Amplified Polymorphic DNA (RAPD) analysis. The fingerprinting patterns revealed two main clusters of strains with a similarity level of approximately 55.8%. Phylogenetic analysis of the partial 16S rRNA sequences of bacterial strains were divided them into five species with similarity value ranged from 97 to 100 % comparing with bacterial sequences in NCBI database. These results recommend that mobile phones can be heavily colonized by high quantities of pathogenic bacteria and thus potential sources of disease transmission requiring application of sound personal hygiene as preventive methods.

Ibrahim *et al*., (2013) investigated the bacterial density of mobile phones of volunteered technologies inthe Food Science Department of Rufus Giwa Polytechnic, by using standard bacteriological methods. A total of 174 colonies belonging to 10 genera were isolated from the mobile phones. The isolated genera were Staphylococcus sp(12(24.14), Klebsiella sp(23(13.22%), Enterococcus sp (08(4.59%). Bacillus sp(14(8.05%), Acinetobacter sp(13(7.47%), Corynebacterium sp(10(5.75%), Pseudomonas sp(24(13.79%), Proteus sp (13(7.47%), Serratia sp(10(5.75%) and E.coli (17(9.75%) when their morphological, gram staining and biochemical characteristics were compared with known taxa. This study showed that all mobile phones under consideration were infected by several microbes, most of which belong to the natural flora of the human body. This means that it is necessary to sterilize hands after contact with phones since it is a source of disease transmission.

Tagoe *et al*., (2011) revealed that Mobile phones have become one of the most indispensable accessories of professional and social life. However, several researches have indicated the potential colonization of surfaces and their ability to transmit diseases (fomites) of which the mobile phone is no exception. Thus this present study investigates bacterial contamination of mobile phones and their antibiotic susceptibility patterns. Surfaces of 100 mobile phones of randomly selected university students were aseptically swabbed. Serial dilution was used in quantification of bacterial with blood and MacConkey agars used in bacterial isolation. Gram reaction and biochemical reactions were applied in identification and the Kirby Buaer method employed in Antibiotic Sensitivity Testing. There was 100% contamination of all the mobile phones surfaces

with a mean bacterial count of 9.915x107cfu/ml with a total of 11 bacteria spp. isolated. The higher isolates include Bacillus cereus (23%) and Proteus mirabilis (19%), whilst the least isolate were Salmonella spp. (3%) and Shigella. spp. (2%). Pathogenic isolates made up 81.8% of all isolates 18.2%. Salmonella spp. and Shigella spp. showed the most resistance to the antibiotics (87.5%) each whilst Escherichia coli was the most susceptible bacteria to the antibiotics (75%). Amikacin (71.4%) and Gentamicin (63.6%) were the most effective antibiotics whilst Ampicillin, Penicillin, Cloxacillin showed the least effectiveness with 100% bacteria resistance. Thus mobile phones can be heavily colonized by high quantities of pathogenic bacteria and thus potential sources of disease transmission requiring application of sound personal hygiene as preventive methods.

Roberta *et al*., (2014) reported that potential sources of many pathogens are transmitted by hand and contaminated medical devices. There's extensive literature on the survival of organisms on inanimate objects, and studies suggest that commonly used patient-care items may serve as reservoirs and vectors for health care associated infections. For example, vancomycin-resistant enterococci (VRE) are capable of prolonged survival on hands, gloves, and environmental surfaces. Mobile communication devices can act as reservoirs for bacteria associated with HAIs and are routinely transported into the operating environment by medical staff. Cross-contaminations occur between healthcare workers' hands and patients including transmission of multidrug-resistant strains of bacteria. Contaminated mobile phones are hazardous to patients and may also pose a threat of spreading infections into the community. In one study, 88% to 89.5% of study participants never cleaned their mobile phones and pagers were often touched during or after the examination of patients without hand washing. Microbial contamination is a risk associated with the infrequent cleaning of phones. The good news is that there is a significant reduction in contaminated pagers with the use of several prepackaged disinfecting agents. Alcohol wipes with 0.5% chlorhexidine gluconate in 40% ethyl alcohol were significantly more efficacious in eliminating all bacterial growth than the other agents. In a study on the use of alcohol-based hand foam, results showed that the foam simultaneously disinfected the hands and a stethoscope head, which significantly reduced the number of bacterial colonies, including methicillinresistant Staphylococcus aureus (MRSA). These studies suggest that cleaning mobile phones may significantly decrease bacterial colonies and the threat of device-related bacterial cross-contamination.

Vivekanandan Annet Viveka. (2017) reported the ability of the microbes to survive on the touch-pads of the smart phone makes it as one of the important fomites in the spread of microorganisms between users. The objectives of this study were to identify the microorganisms present on the touch screens of smart phones, to determine the possible factors that could influence the bacterial contamination of mobile phone surfaces and to study the usage pattern of mobile phones by veterinary undergraduates. The sample of this study included 40 mobile phones owned by veterinary undergraduates representing all four batches (n=10 per batch) with equal proportion of male and female students. The bacteria from touch screens were isolated and identified using conventional bacteriological techniques. At least one or more species of bacteria were found in all 40 mobile phones sampled. Eleven species of bacteria, such as Coagulase negative Staphylococcus spp. (87.5%), Bacillus spp. (60%), Pseudomonas (50%), Coagulase positive Staphylococcus spp. (22.5%), Klebsiella (22.5%), Acinetobacter (15%), Proteus (12.5%), Staphylococcus aureus (5%), Flavobacterium (5%), Enterobacter (2.5%), Citrobacter (2.5%) and Escherichia coli (2.5%) were identified from the phones sampled.There were no difference in the occurrence of bacterial species in phones obtained from different batches of students (Chi Square Test: $P>0.05$). The occurrence of Pseudomonas (28.6%) and Bacillus (28.6%) was significantly low (Chi Square Test: $P<0.05$) in the touch pads that were said to be cleaned regularly**.** Further, it is evident from this study that the veterinary undergraduates areusing modern technology for educational purposes and they are aware of the adverse effects of mobile-phone addiction.It appears the contamination of smart phones with Gram positive and Gram negative bacteria owned by veterinary undergraduate students is widely prevalent and regular cleaning of mobile phones and frequent hand washing might reduce the microbial load on the touch pads of the smart phones.

Sweta *et al.*, (2015) stated that the presence of microorganisms on mobile surface is well recognized but literatures, regarding it are still pouring in. This study was conducted 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 surface of phones were swabbed with sterile sticks and immediately streaked on three plates each of Nutrient agar, Potato Dextrose Agar and Glucose yeast agar. The plates were incubated at 35degree Celsius for bacterial growth and 25 degree Celsius for fungal growth. Following incubation the microbial colonies were isolated and identified and tabulated .This study highlights the need and importance of following proper hygiene habits of

using phones as they prove to be potential transmission vehicles for microbial populations responsible for several diseases.

Suganya *et al*., (2012) assessed the Mobile phones are a reservoir of bacteria and may cause nosocomial infections. Because of the achievements and benefits of the mobile phone, it is easy to overlook its hazard to health; this is against the background that many users may have no 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 a good carrier for microbes, especially those associated with the skin resulting in the spread of different microorganisms from user to user. Microbiologists say that the combination of constant handling with the heat generated by the phones creates a prime breeding ground for many microorganisms that are normally found on the skin. Thus the purpose of this study was to investigate microbiological colonization on mobile phones which are not covered and to compare it with those of covered mobiles and thus suggest a cost effective solution for this emerging nosocomial infection.

Meadow *et al*., (2014) viewed Most people on the planet own mobile phones, and these devices are increasingly being utilized to gather data relevant to our personal health, behavior, and environment. During an educational workshop, we investigated the utility of mobile phones to gather data about the personal microbiome — the collection of microorganisms associated with the personal effects of an individual. We characterized microbial communities on smartphone touchscreens to determine whether there was significant overlap with the skin microbiome sampled directly from their owners. We found that about 22% of the bacterial taxa on participants' fingers were also present on their own phones, as compared to 17% they shared on average with other people's phones. When considered as a group, bacterial communities on men's phones were significantly different from those on their fingers, while women's were not. Yet when considered on an individual level, men and women both shared significantly more of their bacterial communities with their own phones than with anyone else's. In fact, 82% of the OTUs were shared between a person's index and phone when considering the dominant taxa (OTUs with more than 0.1% of the sequences in an individual's dataset). Our results suggest that mobile phones hold untapped potential as personal microbiome sensors.

Anuradha *et al*., (2016) reported that Mobile phones carry microorganisms because count of bacteria increases in high temperature and our phones are ideal breeding sites for these microbes as they are kept warm and snug in our pockets and handbags. The important factors of contamination are the personal hygiene level. Though mobile phone use are restricted in hospitals, the probability of the mobile phones of the patients, visitors and healthcare professionals being contaminated by bacteria and microbes in the hospital surrounding is high and may serve as a vehicle for the spread of nosocomial pathogens. The aim of the study is to conduct a survey to identify the level of awareness and to make people aware of transmission of diseases. The method used in this survey is a set of questionnaire were prepared and given to 100 mobile phone users and were asked to answer them without any ambiguity. The data is then collected. Based on the data it was observed that the awareness among people on the presence of microbes on their gadgets were very less. The survey results was shared with the participants, explained them the causes of contamination and its adverse impact, and instigated them to awareness and suggested few preventive measures to avoid the habit of using mobile phones while being with infants, kids, dining and in washrooms. Use of an antimicrobial cleaning agent to clean the mobile phones whenever required is recommended.

CHAPTER 3

MATERIALS AND METHODS

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3.1 Materials

3.1.1 Study area and period

This research was designed during the period from January to June 2018 at the campus of Hajee Mohammad Danesh Science and Technology University Dinajpur, under the bacteriology lab of the Department of Microbiology, Faculty of Veterinary and Animal science. The main aim of this work was to isolate and identify bacteria fromed mobile phones in students and employees of Hajee Mohammad Danesh Science and Technology University (HSTU) Dinajpur, Bangladesh.

3.1.2 Collection of samples

Total numbers of 32 mobile phones were collected from academic staff, nonacademic staff, students and cleaners of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh to isolate bacteria.

3.1.3 Transportation and preparation of samples

Samples from the mobile phones of all participants from the campus were collected randomly during routine daily work. A sterile cotton swab was rolled over all exposed outer surfaces of the cell phones. Care was taken to make sure that the keypad and all buttons were swabbed, since these areas are most frequently in contact with the tips of fingers. Mobile phones were decontaminated with 70% isopropylalchohol and then sampled swabs after decontamination were streaked over Nutrient agar and sub cultured on MacConkey agar, mannitol salt agar, eosin methylene blue agar (EMB), salmonella shigella (SS) agar, blood agar ,Cetrimide agar and also doing plate count agar. Plates were incubated aerobically at 37°C, for 24 hrs.

Plate 1: Sample collection from mobile phones

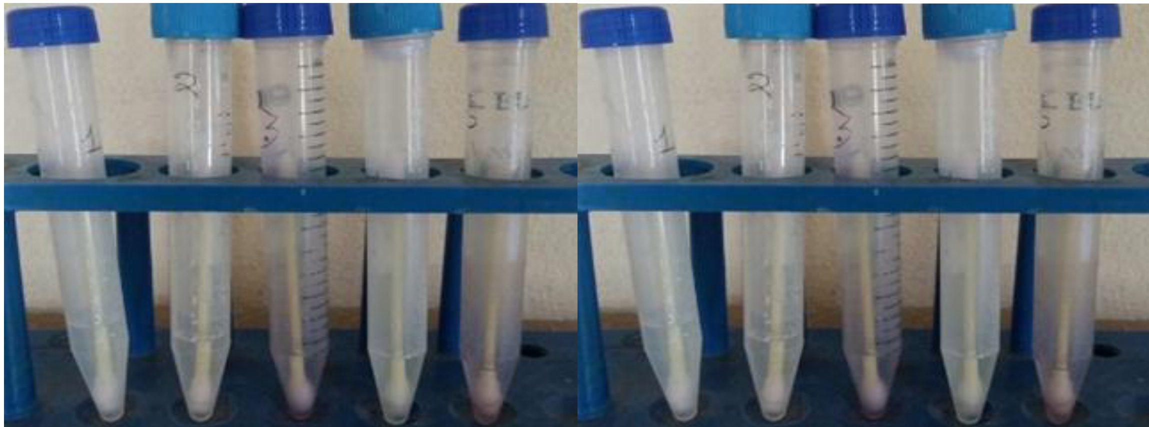

Plate 2: Collected samples from different mobile phones.

Table 1. Microbial growth in different samples

Categories	Number of samples	Microbial growth	No growth
Academic staff	6 samples	4	2
Nonacademic staff	8 samples	6	2
Students	11 samples	9	3
Cleaners	7 samples	6	1

3.1.4 Media for culture

The media and reagents that have been used for the isolation and identification of bacteria are mentioned below:

3.1.5 Solid media

- Nutrient Agar Medium (HI-MEDIA, India)
- MacConkey Agar Medium (HI-MEDIA, India)
- Salmonella and Shigella Agar (HI-MEDIA, India)
- Eosin Methylene Blue Agar (HI-MEDIA, India)
- Mannitol Salt Agar (HI-MEDIA, India)
- Blood Agar Medium (HI-MEDIA, India)
- Cetrimide agar medium (HI-MEDIA, India)
- Plate count Agar

3.1.6 Liquid media

- Methyl Red (MR) media (HI-MEDIA, India)
- Voges Proskauer medium base (VP) media.
- Indole broth, (HI-MEDIA, India)
- Citrate Utilization Test, (HI-MEDIA, India)
- Triple sugar iron (TSI) broth, (HI-MEDIA, India)
- Catalase broth , (HI-MEDIA, India)

3.1.7 Reagents

The reagents used during the experiments were-

- Crystal violet dye
- Grams iodine
- Alcohol
- Safranin
- Saline
- Iodine solution
- Kovac's reagent
- Methyl- red solution
- 3% H_2O_2
- Phenol red
- Phosphate buffered saline (PBS) solution

3.1.8 Instruments and Appliances

The different appliances used during the course of the experiment were as follows:

- Mobile phones
- Cotton swap sticks
- Conical flask
- Petridishes
- Inoculation loop
- Bacteriological incubator
- Test tube
- Slides
- Compound microscope
- Refrigerator
- Hot air oven
- Autoclave

3.1.9 Antibiotic Sensitivity Test

Materials that used drug sensitivity during experiment were as follows:

- Antibiotic disks and Test tube rack
- Inoculation loop or needle
- Sterile swaps and Forceps
- Mueller-Hinton agar plate
- Stock broth culture of experimental bacteria
- Bunsen burner
- Incubator and Ruler

3.1.9.1 Antimicrobial agents with their disc concentration

To determine the drug sensitivity and resistance patterns of isolated organisms with different types of commercially available antimicrobial discs (Oxoid Ltd., UK) were used. The method allowed for the determination of the efficacy of drug by measuring the diameter of inhibition zone that resulted from diffusion of the different agent into medium surrounding the disc. The list of commercially available antimicrobial disc used in this study with their concentration is given below:

Table 2. Antimicrobial agents with their disc concentration

SI.NO	Antibiotic Name	Disc concentration (µg/disc)	Zone diameter		
			Resistant (mm)	Intermediate (mm)	Sensitive (mm)
1	Chloramphenicol(C)	30µg/disc	≤12	13-17	≥18
2	Penicillin (P)	10µg/disc	≤28	-	≥29
3	Methicillin (MET)	5µg/disc	≤9	-	≥10
4	Amoxicillin (AMX)	30µg/disc	≤19	-	≥20
5	Erythromycin (E)	15µg/disc	≤13	14-22	≥23
6	Azithromycin(AZM)	30µg/disc	≤13	14-17	≥18
7	Norfloxacin (NX)	10µg/disc	≤15	-	≥16
8	Cloxacillin (COX)	1µg/disc	≤10	11-12	≥13
9	Ciprofloxacin (CP)	5µg/disc	≤15	16-20	≥20
10	Cefaclor (CEC)	30µg/disc	≤14	15-17	≥18
12	Amikacin (AK)	30µg/disc	≤14	15-16	≥17
13	Cephalexin (CN)	30µg/disc	≤14	15-17	≥18
14	Gentamycin (GEN)	10 µg/disc	≤20	21-28	≥29
15	Ofloxacin (OFX)	5µg/disc	≤12	13-15	≥16

Note SI.NO= Serial number, µg = Microgram.

Clinical and Laboratory Standards Institute (CLSI,2007).

3.2 Methods

The following methods were used for the isolation and identification of bacteria.

3.2.1 Sampling and processing of samples.

The experimental work was divided into two steps: the first step was performed for the isolation and identification of the organisms from the collected sample using cultural, staining and biochemical techniques. The second step was conducted for the determination of antibiotic sensitivity and resistance pattern of isolated organisms of various samples by using different antibiotic discs available in the market. The layout of illustrated the present study is below:

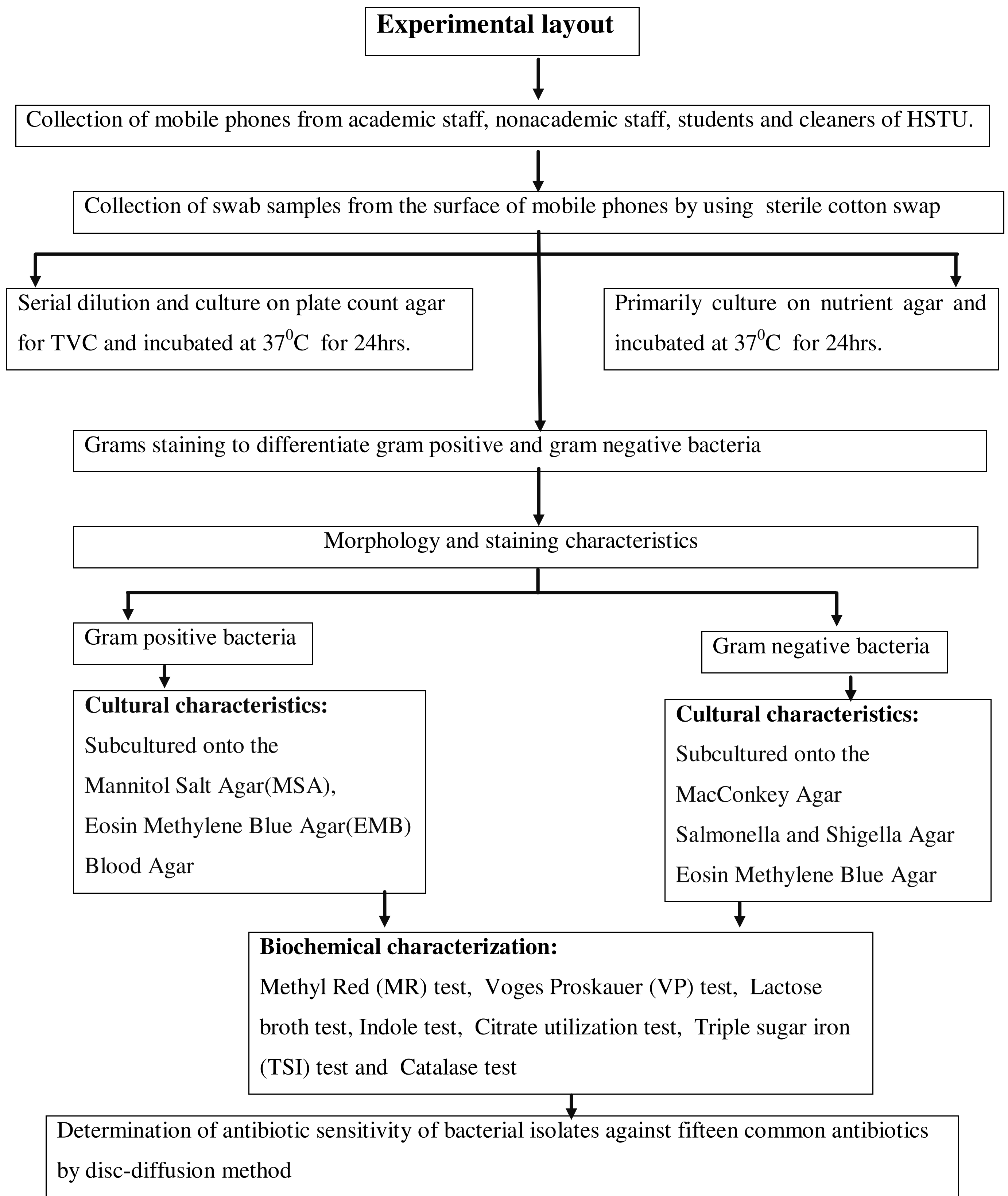

Fig: 1 Schematic illustration of experimental layout

3.2.2 Laboratory preparation

All items of required glassware including test tubes, slides, cylinder flasks, conical flasks, petri plates, glass and vials soaked in a house holding dishwashing detergent solution (Trix, Recket and Colman Bangladesh ltd) for during glassware's were disinfected with 2% sodium solution hydrochloride solution cleaning. The glassware were then clean by brushing washed thoroughly and finally sterilized either by dry heat at 37C for 2 hours or by autoclaving for 15minites at 121C under 15 Ibs pressure per square inch. Autoclaved items were dried in hot air oven over at 50C disposable plastic were. All the glassware was kept over at 50C for future use.

3.2.3 Preparation of culture media

All media broth and reagent use in this experiment were prepared according to instruction.

3.2.3.1 Plate Count Agar (PCA)

Add 17.5g to 1 liter of distilled water dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, the medium was poured into each sterile petri dish and allowed to solidify. After solidification of the medium in the petri dishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use (Cater 1979).

3.2.3.2 Nutrient Agar

28.0 gram of Nutrient agar power (Hi-media India) was suspended in 100 ml of cold distilled water in flask and heated to boiling for dissolving the medium completely. The medium was the sterilized by autoclaving. After autoclave the medium was poured into each sterile Petridish and allowed to solidify. After solidification the medium were incubated at 37C for overnight to check their sterility and use for culture characterization (Carter, 1979).

3.2.3.3 MacConkey Agar

51.50 grams of dehydrated Bacto-MacConkey agar (Difco) was suspended in 1000ml of cold distilled water in conical flask and was heated up to boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in sterile glass pertridishes. The medium was allowed to solidify about 2hours with the cover of the pertridishes partially removed. The sterility of the medium was judged and use for culture characterization or stored at 4C in refrigerator future use (Carter, 1979).

3.2.3.4 Salmonella Shigella Agar

Suspend 50g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by boiling for 5 minutes. After boiling the medium was put into water bath at 45o- 50oC to decrease the temperature. Then medium was poured in 10 ml quantities in sterile glass petri dishes (medium sized) and in 15 ml quantities in sterile glass petri dishes (large sized) to form thick layer there in. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petri dishes partially removed. The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use. Petri dishes, these were incubated at 37° C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use (Cater 1979).

3.2.3.5 Eosin methylene blue agar

EMB contains dyes that are toxic for Gram positive bacteria and bile salt which is toxic for Gram negative bacteria other than coliforms. EMB is the selective and differential medium for coliforms. *Escherichia coli*: Blue-black bulls eye; may have green metallic sheen. *Escherichia coli* colonies grow with a metallic sheen with a dark center, *Aerobacter aerogenes* colonies have a brown center, and nonlactose-fermenting gram-negative and *Klebshella spp* bacteria appear pink (Cheesbrough, 1985).

3.2.3.6 Mannitol Salt Agar

111 grams Mannitol Salt Agar base powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely (necessary calculation was done for required number of plates). The medium was then sterilized by autoclaving at 1.2 kg/cm2 pressure and 121° C for 15 minutes. After autoclaving the medium was put into water bath at 450- 500C to decrease the temperature. Then medium was poured in 10 ml quantities in sterile glass petri dishes (medium sized) and in 15 ml quantities in sterile glass Petri dishes (large sized) to form thick layer there in. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the Petri dishes partially removed. The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use. Petri dishes, these were incubated at 37° C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use (Cater 1979).

3.2.3.7 Blood agar media

Forty grams of Blood agar base (HI-MEDIA, India) powder was dissolved in 1000 ml of distilled water (necessary calculation was done for required number of plates). The medium was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121° C for 15 minutes and 45° C. Then 5-10 % sterile defibrinated blood was added to the medium and distributed to sterile petri dishes and allowed to solidify. After solidification of the medium in the petri dishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use. (Cater 1979).

3.2.3.8 Cetrimide Agar media

Cetrimide agar base promotes the production of pyocyanin a water-soluble pigment as well as fluorescence, under ultraviolet light, of *Pseudomonas* spp which constitutes a presumptive identification. Cetrimide is the selective agent as it inhibits the growth of the accompanying microbial flora. Typical *Pseudomonas* spp colonies are greenish or yellowish green in color (Cheesbrough, 1985).

3.2.4 Preparation of Gram's staining solution

The four solutions;

- Crystal violet
- Gram's iodine
- 95% alcohol
- Safranins needed for the Gram staining procedure.

3.2.4.1 Gram staining procedure

- Clean glass slides were taken.
- A sterile technique was used; a smear of each of the organisms was prepared. Smear made of a drop of water on the slide was placed then each organism separately to the drop water with a sterile was transferred. A circular motion of the inoculating loop of organism was mixed and spreads.
- Air–dry the smears were allowed then heat-fixed in the usual manner.
- Smears were flooded with crystal violet and let stood for 1 min then slides were washed with tap water.
- Smears were flooded with grams iodine mordant and let stood for 1 min. Slides were washed with tap water.
- Smears were decolorized with 95% ethyl alcohol. Slides were washed with tap water.
- Smears were counter stain with safranin for 30 sec. Slides were washed with tap water.
- Then the slide was blotted with blot paper and was allowed to dry. The slide was
  examined under microscope with high power.

Gram positive (violate colour) organisms are discarded and gram negative (pink coloured), small rod shaped, single or paired arranged organisms were selected.

3.2.5 Biochemical preparation

3.2.5.1 MR solution

The test was conducted by inoculating a colony of the test organism in 0.5ml sterile glucose phosphate peptone broth. After overnight incubation at 37oC a drop of methyl red solution was added. A red coloration is positive and indicates an acid PH of 4.5 or

less resulting from the fermentation of glucose. A yellow coloration is negative (Cheesbruogh, 1985)

3.2.5.2 Voges-proskauer solution

Alpha-naphthol solution

Alpha- naphthol solution was prepared by dissolving 5 gm of Alpha- naphthol in 100 ml of 95% ethyl alcohol.

3.2.5.3 Indole Test

Kovac's Reagent

The solution was prepared by mixing 25 ml of concentrate of Hydrochloric acid in 5ml of amyl alcohol and to this mixture 5 grams of paradimethyl-amino-benzyldehyde crystals were added. This was taken then kept in flask in a flask equipped with rubber cork for future use (Merchant and Packer, 1967).

3.2.5.4 Citrate Utilization Test

Suspected colony was inoculated onto Citrate Utilization Test. Then the medium was incubated at 37oC for 48 hours.

3.2.5.5 Triple sugar Iron (TSI) agar slant

65 grams TSI agar base powder was mixed in 1000 ml of cold distilled water in a flask and mixed thoroughly, then heated to boiling for dissolving the medium completely (necessary calculation was done for required number of test tubes).The medium was then sterilized by autoclaving for 15 minutes at 121°c maintaining a pressure of 1.2 kg/.Then 20/10 ml of medium was poured into each sterilized test tubes and allowed to cool and to solidify (kept in horizontal position). After solidification test tube were used for biochemical characterization and incubated at 37°c for 24 hours.

Procedure

(a) A loop of bacteria was spread across the surface of the agar.
(b) A needle of bacteria was inserted (stabbed) into the bottom (butt) of the tube.
(c) Kept the tubes at 37^{0}C for 24 hours for incubation.
(d) Examined the tubes for the result.

Table 3. Triple sugar Iron (TSI) agar slant

Result(Slant/butt	Symbol	Interpretation
Red/yellow	K/A	Glucose fermentation only
Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/Red	K/K	No fermentation
Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation; Gas produced
Red/Yellow with bubbles	K/A,G	Glucose fermentation only; Gas produced
Red/Yellow with bubbles and black precipitation	K/A,G H_2S	Glucose fermentation only; Gas produced H2S Produced
Red/Yellow with black precipitation	K/A, H_2S	Glucose fermentation only; H_2S Produced
Yellow/Yellow with black precipitation	A/A, H_2S	Glucose and lactose and/or sucrose fermentation; H_2S Produced
No Change/ No Change	NC/NC	No fermentation

Note: A=acid production: K= alkaline reaction: G=gas production: H_2S= Hydrogen sulfide production.

3.2.5.6 Catalase test

This test was used to differentiate bacteria which produce the enzyme catalase. To perform this test, a small colony of good growth pure culture of test organism was smeared on a slide. Then one drop of catalase reagent (3% H_2O_2) was added on the smear. The slide was observed for bubbles formation. Formation of bubble within few seconds was the indication of positive test while the absence of bubble formation indicated negative result (Cheesbrough, 1985).

3.2.5.7 Procedure of Catalase test

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H_2O_2 on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.

4. A negative result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container.

3.2.6 Isolation and identification of bacteria

The cultural examination of mobile phone samples for bacteriological study was done according to the standard method (ICMSF, 1986). Identification of bacteria was performed on the basis of colony morphology; Gram's staining reaction and biochemical test.

3.2.6.1 Culture of organisms

Sterile cotton swaps sticks that rubbed over the surface of mobile phones and immediately steaked Petri-plates of nutrient agar. The plates were incubated 37C for 24hrs and observed for growth.

3.2.6.2 Isolation of bacteria in pure culture

for isolation of bacteria in pure culture, the mixed to obtain isolated colonies as per:

Step1: An inoculum was picked up with a sterile loop and spread on an area of the medium in the Petri dish.

Step 2: the loop was sterilized by being heated as red hot in a flame.

Step 3: The inoculum was spread over the reminder of the plate by drawing the cooled parallel line. This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more time to ensure purity.

3.2.7 Morphological Characteristics of organism by grams staining method

The gram staining was followed to study the morphological and staining characterization of bacteria and provide information about the presumptive bacterial identification as per recommendation of (Cowan and Steel 1979).

Procedure:

- A loopful sterile distilled water was placed in the center of a clean sterile slide.
- A small colony was picked up with a bacteriological loop and was mixed with distilled water of a slide.
- The colony was made to thin smear on a slide.
- The smears were fixed by air driving.

- 0.5% of crystal violet solution was then applied on the for two minutes
- Then washout with clean water
- Grams iodine was then added to act as mordant for one minute 24
- Then washed out with clean water
- Acetone alcohol was then added to decolorize for 1-2 seconds.
- washed out with clean water
- Safranine was as counter a stain and allowed for one minute.
- Washed out with water.
- Then the slide was blotted with blot paper and was allowed to dry. The slide was examined under microscope with high power objective (100X) using with immersion oil.

3.2.8 Subculture into different media

3.2.8.1 MacConkey agar

Samples were subculture on MacConkey agar media and incubated at 37 C for overnight. After that both lactose fermenter bacteria (rose pink color colony) and lactose non fermenter bacteria (pale color colony) were selected.

3.2.8.2 Culture on selective media

3.2.8.3 Eosin methylene Blue (EMB) agar

Sample of positive lactose fermenter were taken and sub-culture on Eosin methylene Blue Agar media and incubate at 37 C for overnight. Same EMB agar plate showed slightly circular colony with dark center metallic sheet. Also in same EMB agar, the growth was indicated by smooth, characteristics mucoid and pink colored colonies which are a consequence of the organism’s abundant polysaccharide capsule.

3.2.8.4 Manitol Salt Agar (MSA)

Gram positive cultures were streaked separated MSA agar plate and incubated at 37°C for overnight. Next day demonstrated morphological characteristics of the bacterial colonies.

3.2.8.5 Blood agar

Yellow colony from Mannitol salt agar was strike on blood agar.

3.2.8.6 Cetrimide Agar

Cetrimide Agar is the selective agent as it inhibits the growth of the accompanying microbial flora. Typical *Pseudomonas* spp colonies are greenish or yellowish green in color (Cheesbrough, 1985).

3.2.9 Antibiotic susceptibility test

Bacterial susceptibility to anti-microbial agent was determined in vitro by using the standardized agar disc-diffusion method known as the *disk diffusion test*; Labeled the covers
of each of the agar plates with name of the test organisms was inoculated.

Using sterile technique, inoculated all agar plates with their respective test organisms as follow:

- o A sterile cotton swab was dipped into a well-mixed saline test culture and removed excess inoculums by pressing the saturated swab against the inner wall of the culture tube.
- o Using the swab streaked the entire agar surface horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface.
  - Allowed all culture plates to dry for about 5 minutes.
  - Distributed the individual antibiotic discs at equal distance with forceps dipped in alcohol and flamed.
  - Gently pressed each disc down with the wooden end of the cotton swab or sterile forceps to ensure that the discs adhered to the surface of the agar.
  - The plates were then inverted and incubated at 37^0C for 24 hours.
  - After incubation, the plates were examined and the diameter of the zones of complete inhibition was measured in mm. The zones of growth inhibition were provided by clinical and Laboratory Standards Institute (CLSI, 2007).

3.2.10 Maintenance of stock culture

Stock culture is a culture of a microorganism maintained solely for the purpose of keeping the microorganism in a viable condition by subculture, as necessary, into fresh medium.

3.2.10.1 Agar slant method

The stock culture was maintained following the procedures, Isolated and identified bacteria were inoculated into nutrient agar slants and incubated at 37°C for 24 hours and then examine for growth. One slant was used for an individual isolate. Then the sterile mineral oil was poured into the tube until the colonies were covered completely. The tube were sealed off with paraffin and kept at room temperature for future use as seed. By this method bacteria can be preserved with no deviation of their original characters for few months (Buxton and Fraser, 1977)

3.2.10.2 Sterile buffered glycerin method

Sterile buffered glycerin (20%) was prepared by mixing 20 parts of pure glycerin and 80 parts of PBS. Then a loopful of thick bacterial culture was mixed with 20%, sterile buffered glycerin in smore vials and was preserved at -20°C This method is more appropriate for preserving bacteria with no deviation of their original characters for several years (Buxton and Fraser, 1977).

RESULTS

CHAPTER 4

RESULTS

The present study was conducted to isolate and identify bacteria on mobile phones from academic staff, nonacademic staff, students and cleaners of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh. A total of 32 samples of mobile phones were processed for isolate and identification of bacteria through culture test, staining, biochemical test and antibiotic sensitivity test. The result presented in different tables and described below under the following heading:-

4.1 Result of Total Viable Count (TVC)

Table 4. Result of Total Viable Count

The TVC of different samples (academic staff, nonacademic staff, students and cleaners) are given below:

Categories	Dilution	Number of colony	Total viable count (TVC)
Academic staff	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	100	1.00 CFU/ml
	10^{-4}	94	9.4 CFU/ml
	10^{-5}	85	8.5 CFU/ml
	10^{-6}	73	7.3 CFU/ml
Nonacademic staff	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	Over 300	TNTC
	10^{-4}	210	2.10 CFU/ml
	10^{-5}	185	1.85 CFU/ml
	10^{-6}	130	1.30 CFU/ml
	10^{-7}	93	9.3 CFU/ml
	10^{-8}	88	8.8 CFU/ml
Cleaners	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	Over 300	TNTC
	10^{-4}	Over 300	TNTC
	10^{-5}	255	2.55 CFU/ml
	10^{-6}	198	1.98 CFU/ml
	10^{-7}	152	1.52 CFU/ml
Students	10^{-1}	Over 300	TNTC

	10^{-2}	Over 300	TNTC
	10^{-3}	Over 300	TNTC
	10^{-4}	Over 300	TNTC
	10^{-5}	Over 300	TNTC
	10^{-6}	260	2.60 CFU/ml
	10^{-7}	200	2.00 CFU/ml
	10^{-8}	197	1.97 CFU/ml
	10^{-9}	154	1.54 CFU/ml
	10^{-10}	100	1.00 CFU/ml
	10^{-11}	91	9.1 CFU/ml

Legend: TNTC= Too numerous to count

4.1.1 Result of Plate Count Agar

Plate count agar plates spread with the samples revealed the growth of bacteria after 24 hours of incubation at 37°C aerobically and were indicated by the growth of circular, small smooth, convex, greenies and gray white or yellowish colonies.

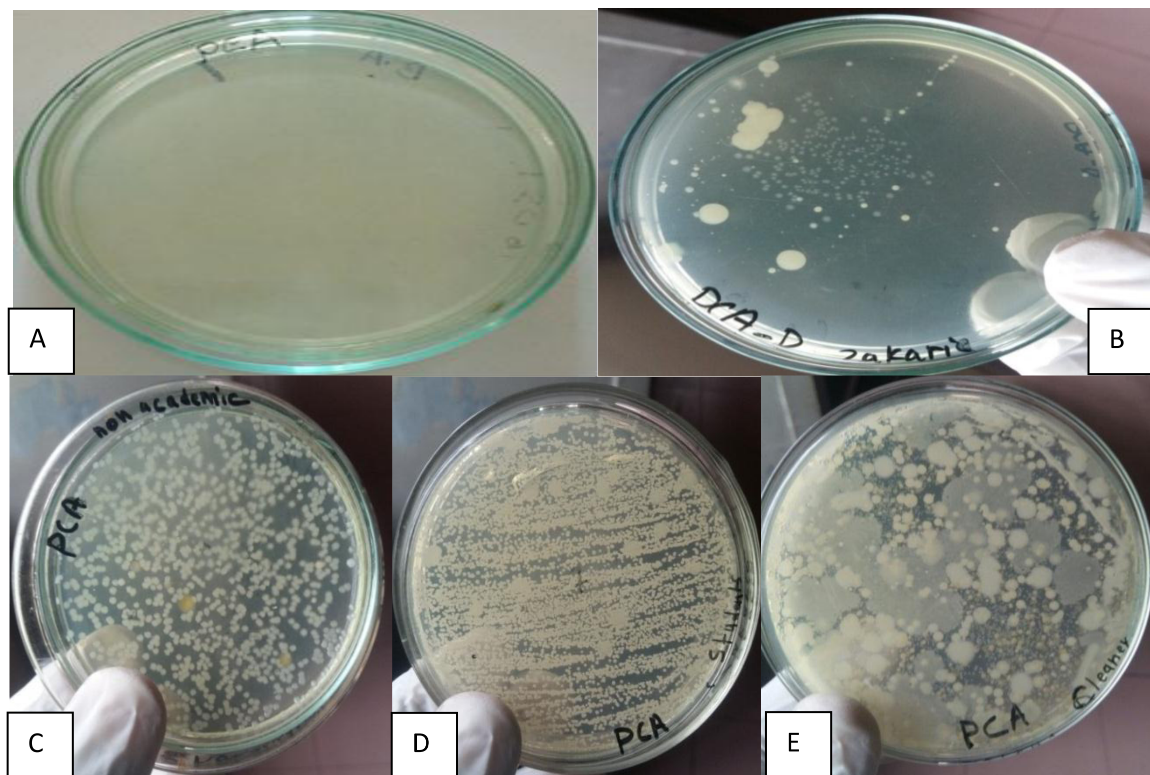

Plate 3: A= Control of Plate Count Agar. B= Microbial growth of samples collected from academic staff. C= Microbial growth of samples collected from nonacademic staff. D= Microbial growth of samples collected from students. E= Microbial growth of samples collected from cleaners.

4.2 Result of identification of bacteria by different bacteriological methods

Table 5. Result of identification of bacteria by different bacteriological methods

Name of media	Colony characteristics	Staining characteristic	Isolated bacteria
Nutrient Agar	Circular small yellowish colonies.	Gram positive cluster liked violet colour.	***Staphylococcus spp.***
Manitol Salt Agar	Yellowish color colonies.		
Blood agar	β-hemolytic colonies of *Staphylococcus spp* on Blood		
Nutrient agar	Thick grayish – white, or cream coloured colonies.	Gram positive rod shaped purple color	***Bacillus spp.***
Blood agar	Large cream colonies.		
Nutrient agar	Smooth. Opaque, translucent colonies.	Gram negative small rod shaped pink colour.	***Salmonella spp.***
Salmonella-Shigella Agar	Pale colour colony.		
Nutrient Agar	Large colony.	Gram negative rod shaped pink colour.	***Klebsiella spp.***
Mac-Conkey's Agar	Large, red, mucoid lactose fermented colony.		
Eosin Methylene Blue	produce pink color		
Nutrient Agar	Large, smooth, low convex and greenish pigment with fruity odor.	Gram negative small rod shaped pink colour	***Pseudomonas spp.***
Cetrimide agar	Slant yellowish in color colonies		

4.2.1 Result in Nutrient Agar

Nutrient Agar plates streaked with the organisms separately revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the growth
of circular small yellowish colonies

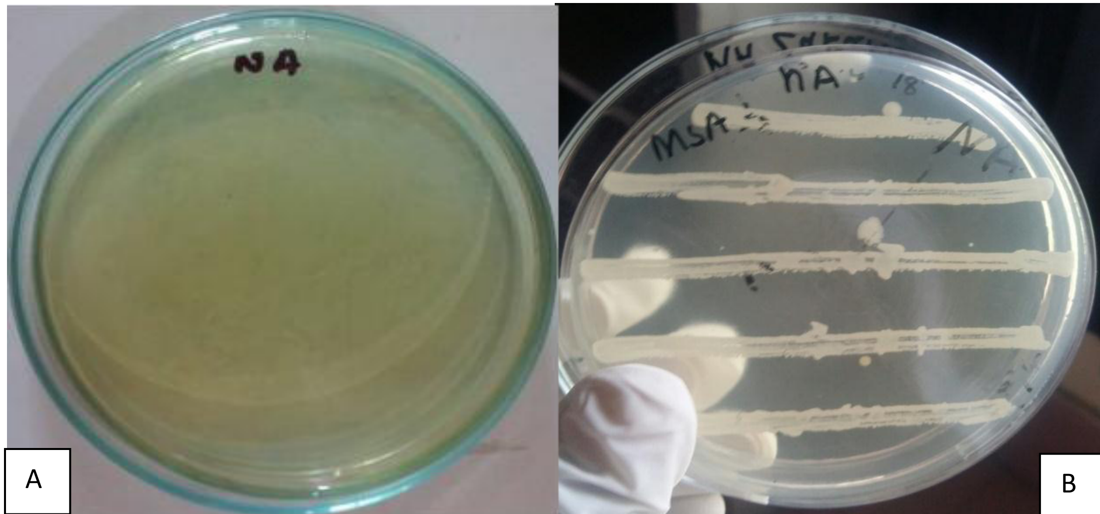

Plate 4: A= Control Nutrient Agar. B= Culture of organism on Nutrient's agar.

4.2.2 Result in Mac-Conkey Agar

Mac-conkey agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the colorless colonies after prolonged incubation pink color and colorless colonies.

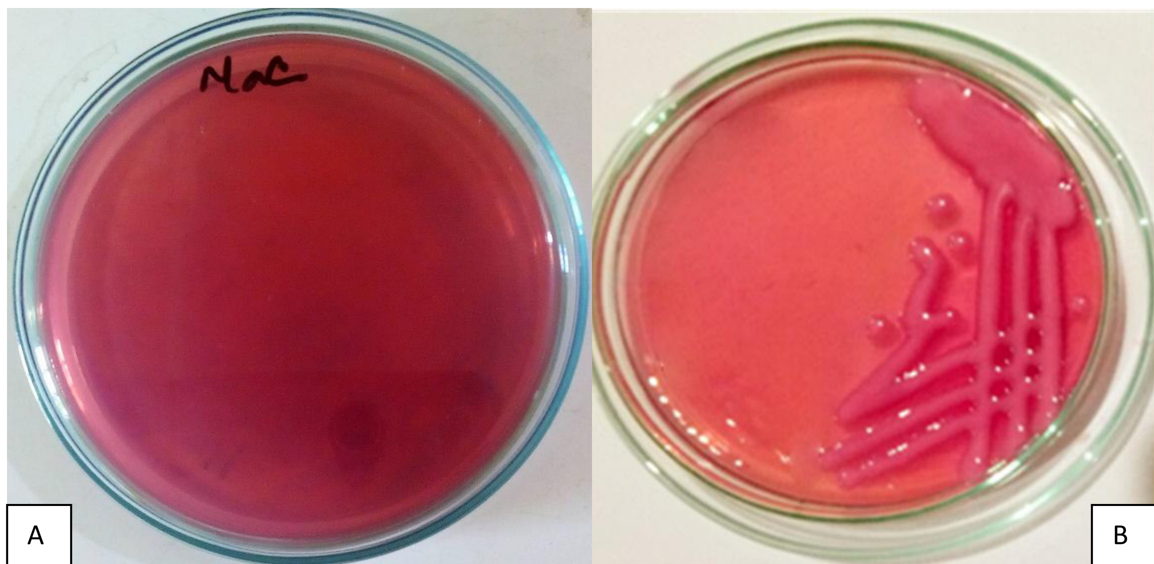

Plate 5: A Control Mac-Conkey Agar   B= *Klebsiella* spp. on Mac-Conkey Agar

4.2.3 Result in Eosin Methylene Blue agar

Eosin methylene blue agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the growth of smooth, circular , black center with blue-green metallic sheen and gray-brown center with pink color colonies.

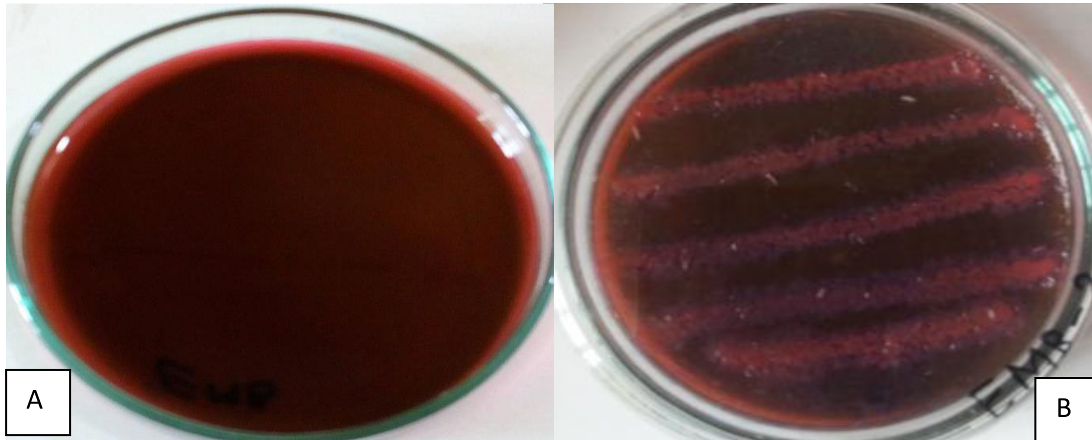

Plate 6: A Control Eosin Methylene Blue Agar B=*klebsiella spp* on Eosin Methylene Blue agar.

4.2.4 Result in Salmonella-Shigella Agar

Salmonella-shigella agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the clear, black center colony, transparent colonies.

Plate 7: A Control Salmonella-Shigella Agar B= *Salmonella spp* on Salmonella-Shigella Agar

4.2.5 Result in Manitol Salt Agar

Manitol salt agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the pink and yellow color colonies.

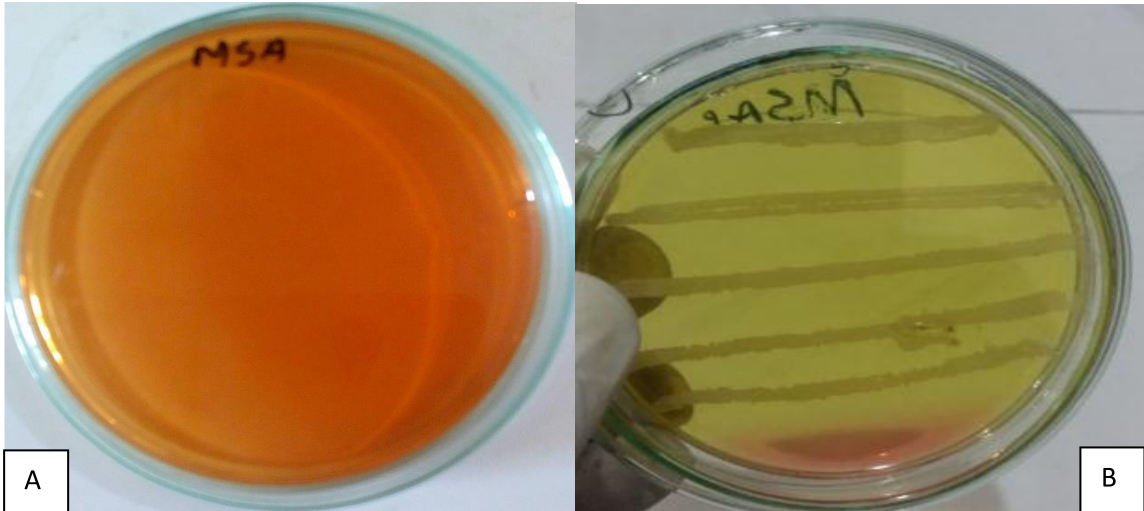

Plate 8: A Control Manitol SaltAgar.   B= *Staphylococcus spp* on Manitol Salt Agar.

4.2.6 Result in Blood Agar

Blood agar plates streaked separately with the organism and incubated at 37^0C aerobically for 24 hours and observed β-hemolytic colonies.

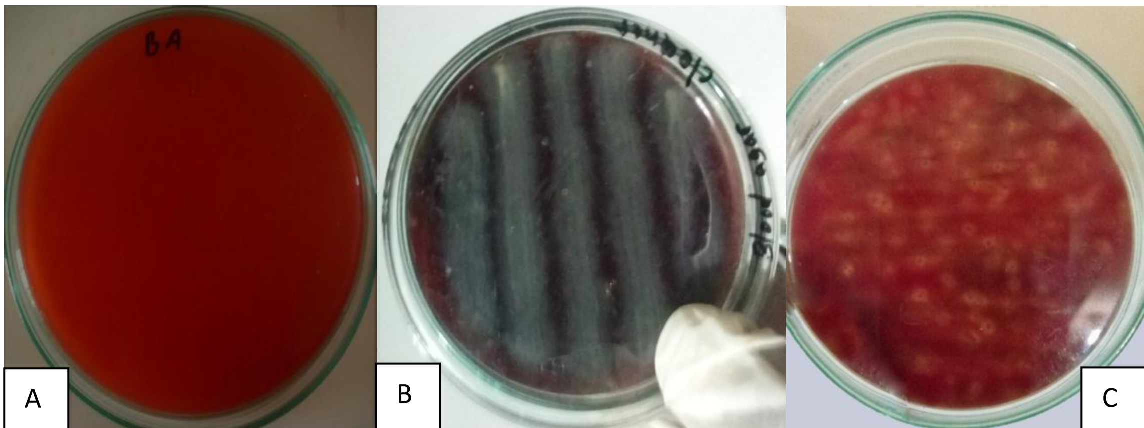

Plate 9: A Control blood Agar. B= *Bacillus* on blood agar. C= β-hemolytic colonies of *Staphylococcus spp* on Blood

4.2.7 Result in Cetrimide Agar

Cetrimide agar streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^{0}C aerobically and observed greenish color colonies.

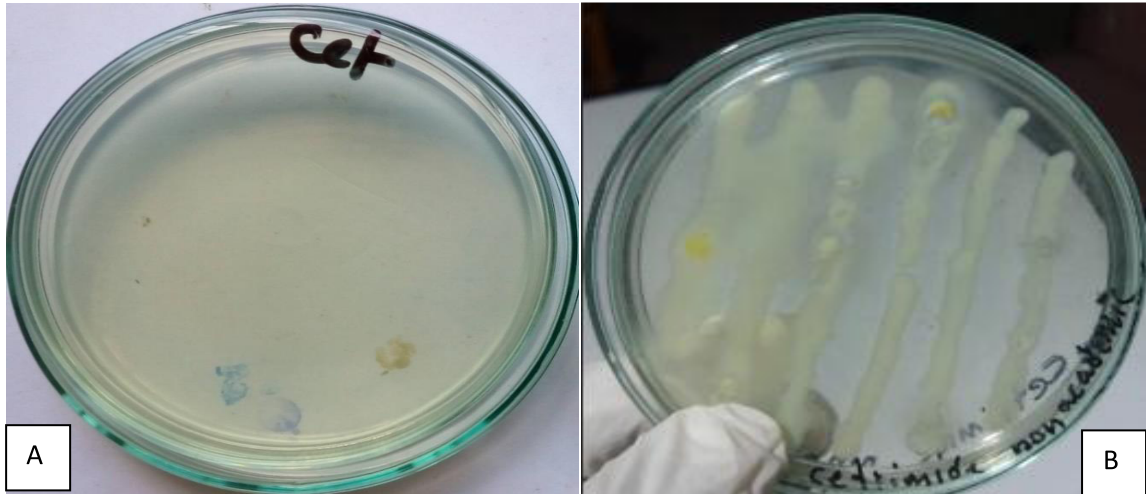

Plate 10: A Control Cetrimide Agar. B= *Pseudomonas spp* on Cetrimide Agar.

Table 6. Result of cultural examination of different isolated organisms.

Organisms	Nutrient agar	Manitol Salt Agar	Mac Conkey agar	Eosin Methylene Blue agar	Salmonella-Shigella Agar	Blood agar	Simon Citrate Agar
Staphylococcus spp.	Growth	+	-	-	-	+	-
Bacillus spp.	Growth	-	-	-	-	+	-
Salmonella spp.	Growth	-	+	+	+	-	-
Klebsiella spp.	Growth	-	+	+	-	-	-
Pseudomonas spp.	Growth	-	-	-	-	-	+

Table 7. Result of frequency distribution of isolated organisms in samples of different academic staffs.

Result of frequency distribution of isolated organisms in samples of different academic staffs.					
SI. No	**Staphylococcus**	**Bacillus**	**Salmonella**	**Klebsiella**	**Pseudomonas**
AC.1	+	+	-	-	-
AC.2	-	-	-	+	-
AC.3	+	-	+	-	-
AC.4	-	+	-	-	+
AC.5	+	-	+	+	-
AC.6	+	+	-	-	-

Legend: AC = Academic

Table 8. Result of frequency distribution of isolated organisms in samples of different nonacademic staffs.

Result of frequency distribution of isolated organisms in samples of different nonacademic staffs.					
SI. No	**Staphylococcus**	**Bacillus**	**Salmonella**	**Klebsiella**	**Pseudomonas**
NA.1	+	+	+	-	-
NA.2	-	-	+	+	-
NA.3	+	-	+	-	+
NA.4	-	+	-	+	+
NA.5	+	-	-	+	-
NA.6	+	+	-	+	-
NA.7	-	-	+	-	+
NA.8	+	+	+	-	+

Legend: NA = Non Academic

Table 9. Result of frequency distribution of isolated organisms isolated in samples of different students.

Result of frequency distribution of isolated organisms isolated in samples of different students.					
SI. No	**Staphylococcus**	**Bacillus**	**Salmonella**	**Klebsiella**	**Pseudomonas**
S.1	+	+	-	-	-
S.2	+	-	-	-	-
S.3	+	+	+	-	+
S.4	-	+	-	+	+
S.5	+	-	-	+	-
S.6	+	+	-	+	-
S.7	-	-	-	-	+
S.8	+	-	-	+	-
S.9	+	+	-	-	+
S.10	-	+	+	+	-
S.11	+	-	+	+	+

Legend: S = Student

Table 10. Result of frequency distribution of isolated organisms in samples of different cleaners.

Result of frequency distribution of isolated organisms in samples of different cleaners.					
SI. No	**Staphylococcus**	**Bacillus**	**Salmonella**	**Klebsiella**	**Pseudomonas**
C.1	+	-	-	-	+
C.2	+	+	-	+	+
C.3	+	-	+	+	-
C.4	-	+	+	-	+
C.5	-	-	+	+	+
C.6	+	+	-	-	-
C.7	-	+	-	+	+

Legend: C = Cleaners

4.3 Types of bacteria isolated from different samples

Academic staff was *staphylococcus spp* is 4 and *Pseudomonas spp* 1, *bacillus spp, klebsiella spp and salmonella spp.* From nonacademic staff were *staphylococcus spp and salmonella spp was* 5, *bacillus spp* is 4, *klebsiella spp and Pseudomonas spp.* From the isolated organism students were *Staphylococcus spp* was 8 and *salmonella spp* 3, *bacillus spp*, *klebsiella spp and Pseudomonas spp.* From the organism were cleaners *Pseudomonas spp* 5 and *Salmonella spp* 3, *Staphylococcus spp, Bacillus spp and Klebsiella spp.*

Table 11. Types of bacteria isolated from different samples

Categories	Number of collected samples	Isolated bacteria				
		Staphylococcus spp. (%)	***Bacillus spp. (%)***	***Klebsiella spp. (%)***	***Pseudomonas spp. (%)***	***Salmonella spp. (%)***
Academic staff	6 samples	4	3	2	1	2
Nonacademic staff	8 samples	5	4	4	4	5
Students	11 samples	8	6	6	5	3
Cleaners	7 samples	4	4	4	5	3
Total		21 (25.6%)	17 (20.7%)	16 (19.5%)	15 (18%)	13 (15.85%)

Table 12. Result of Characterization of isolated bacteria by gram staining technique.

Grams staining				
Clour	**shape**	**Arrangement**	**Grams stain reaction(+/-)**	**Identification.**
Purple color	Cocci-shape	Spherical	Gram positive	*Staphyloccocus spp.*
Purple color	Rod-shaped	Arranged in single	Gram positive	*Bacillus spp.*
Pinkish color	Rod-shape	Single or pair.	Gram negative	*Salmonella spp.*
Pinkish color	Rod-shape	Single,pairs or short chain.	Gram negative	*Klebsiella spp.*
Pinkish color	Rod-shaped	Single or pairs.	Gram negative	*Pseudomonas spp.*

4.4 Result of microscopic examination of isolated bacteria.

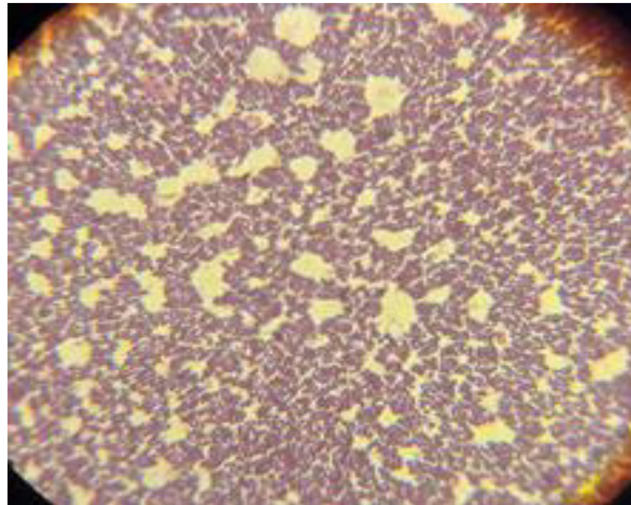

Plate 11: Gram positive spherical shaped purple color *Staphylococcus spp* 100x microscopy.

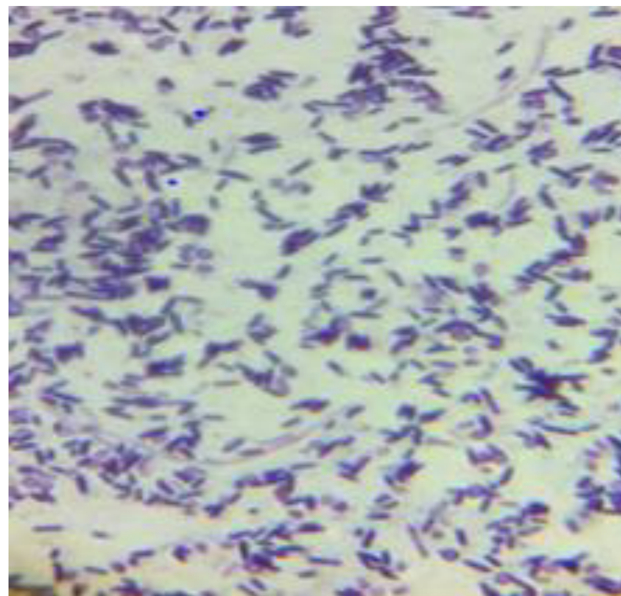

Plate 12: Gram positive rod shaped purple color *Bacillus spp* 100x microscopy.

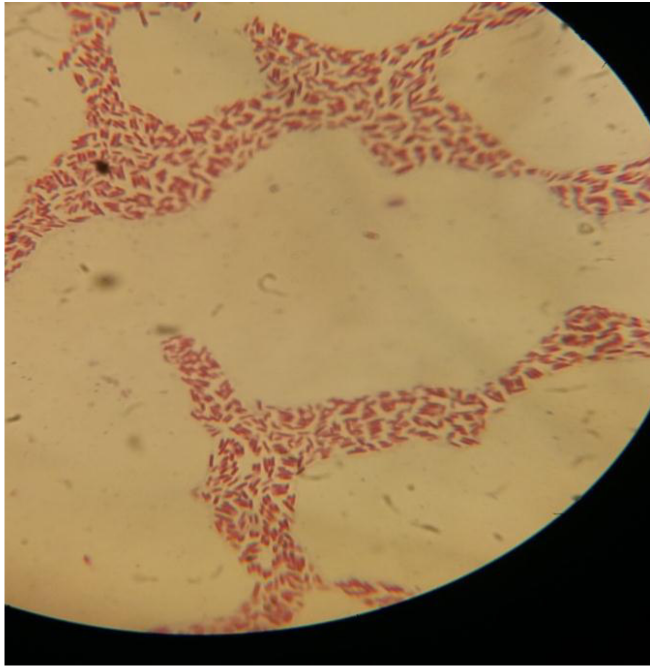

Plate 13: Gram negative rod shaped *Klebsiella* spp under 100x microscopy.

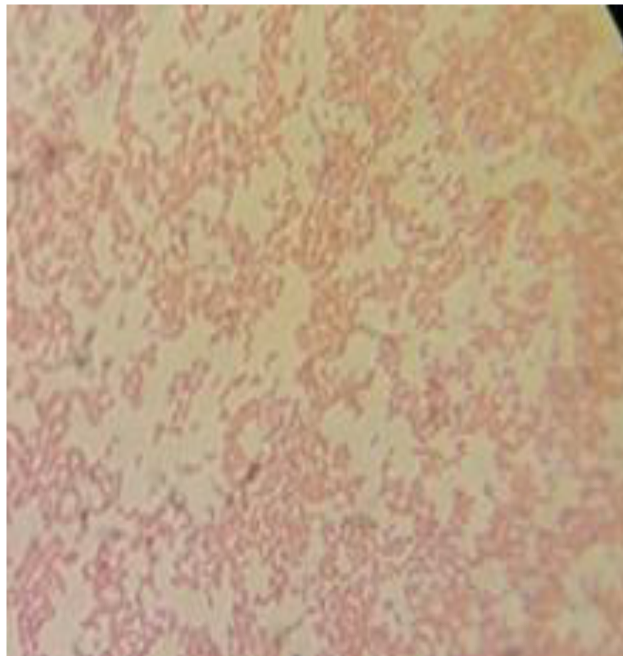

Plate 14: Gram negative small rod shaped pink colour *Salmonella spp* 100x microscopy.

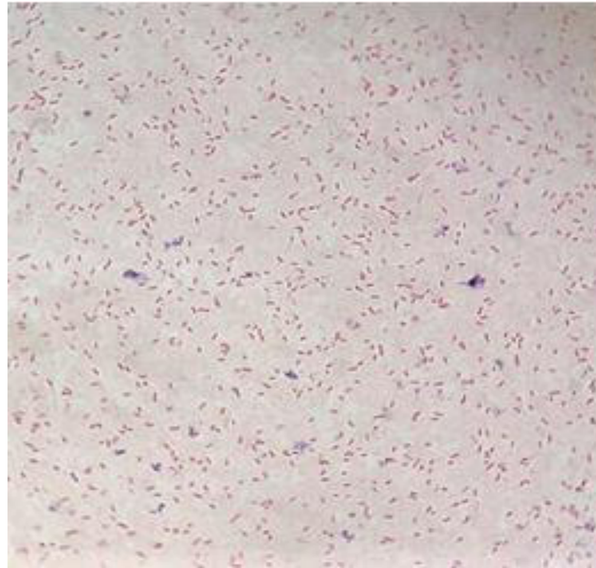

Plate 15: Gram negative small rod shaped *Pseudomonas spp* under 100x microscopy.

4.5 Result of Biochemical test

Table13. Result in biochemical test of isolated bacteria.

Organisms	MR	VP	TSI	CIRTARE	CATALASE	INDOLE
Staphylococcus spp.	-	-	+	+	+	-
Bacillus spp.	-	-	-	+	+	-
Salmonella spp.	+	+	+	+	+	+
Klebsiella spp.	+	+	-	+	+	-
Pseudomonas spp.	-	-	-	+	+	-

Biochemical test result of *Staphylococcus spp*

Catalase Test

Plate 16: *Staphylococcus spp* showing positive result for catalase test.

Methyl Red Test

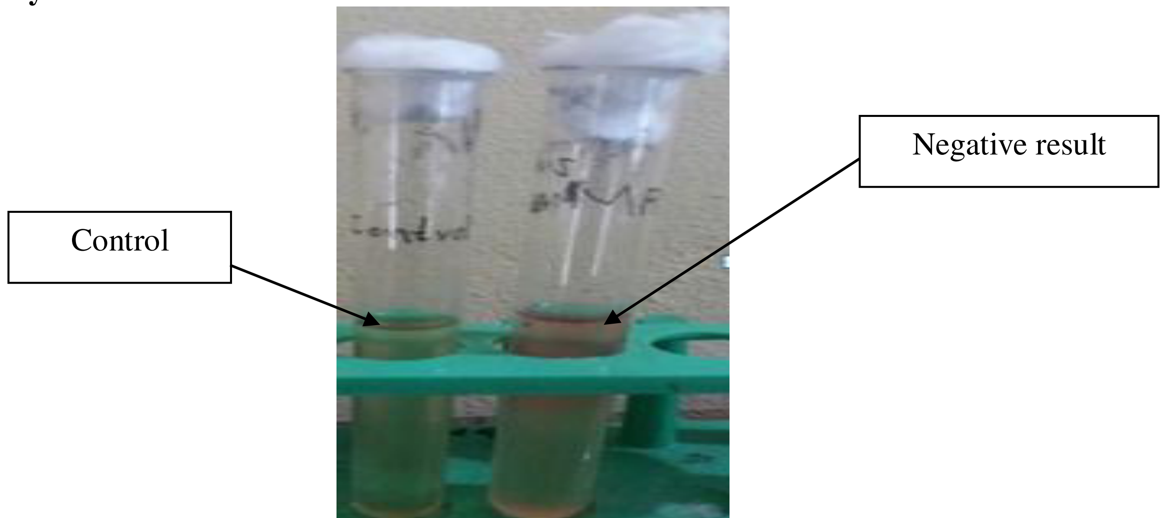

Plate 17: *Staphylococcus spp* showing negative result (Right) on Methyl red test with control (Left).

Voges-Proskauer Test

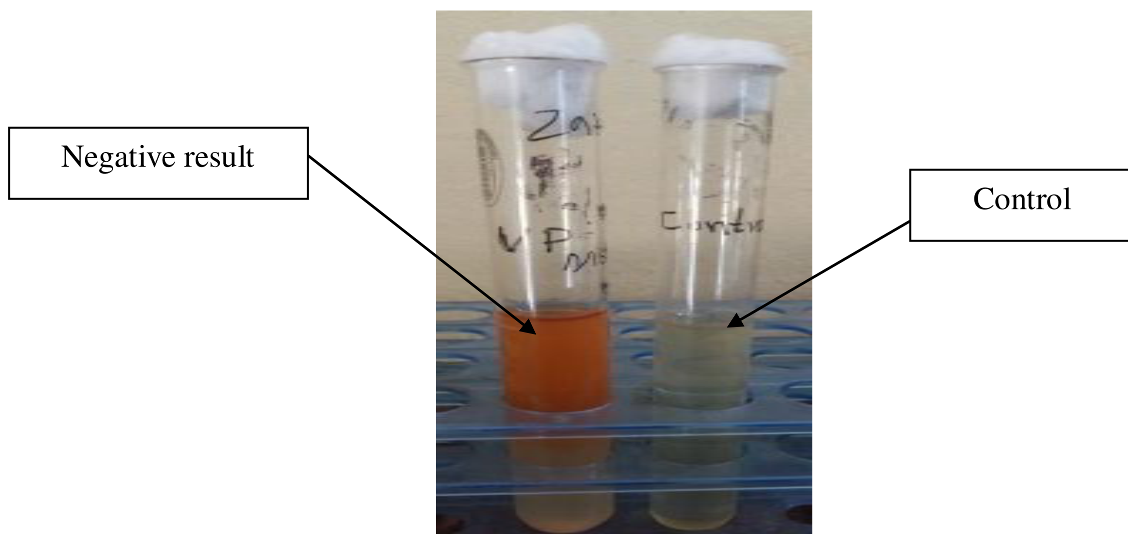

Plate18: *Staphylococcus spp* showing negative result (Left) on Voges-Proskauer test with control (Right).

Triple Sugar Iron Test.

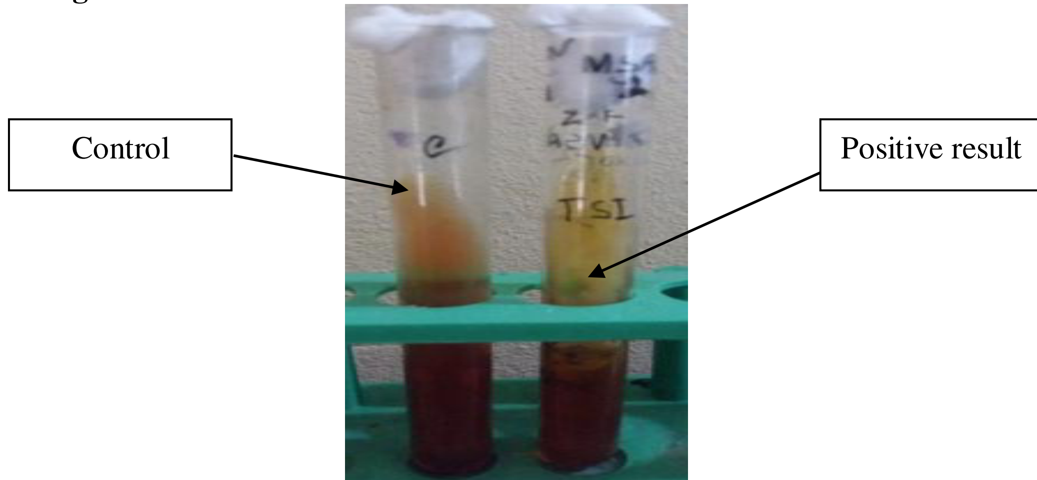

Plate 19: *Staphylococcus spp* showing positive result (Right) on Triple sugar Iron test with control (Left).

Citrate Utilization Test

Plate 20: *Staphylococcus spp* showing positive result (Right) on citrate utilization test with control (Left).

Indole Test

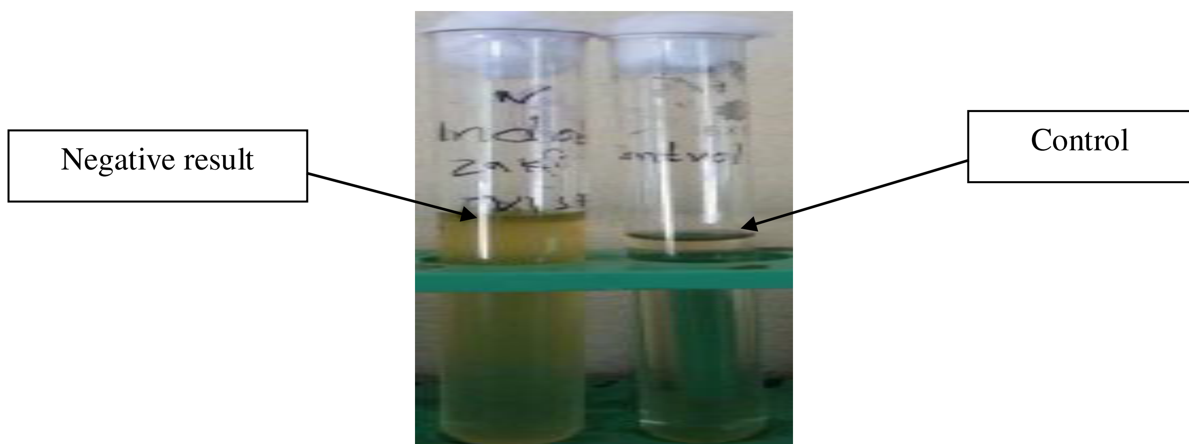

Plate 21: *Staphylococcus spp* showing negative result (Left) on Indole test with control (Right).

Biocehmic tests result of *Bacillus spp.*

Catalase Test

Plate 22: *Bacillus spp* showing positive result for catalase test.

Methyl Red Test

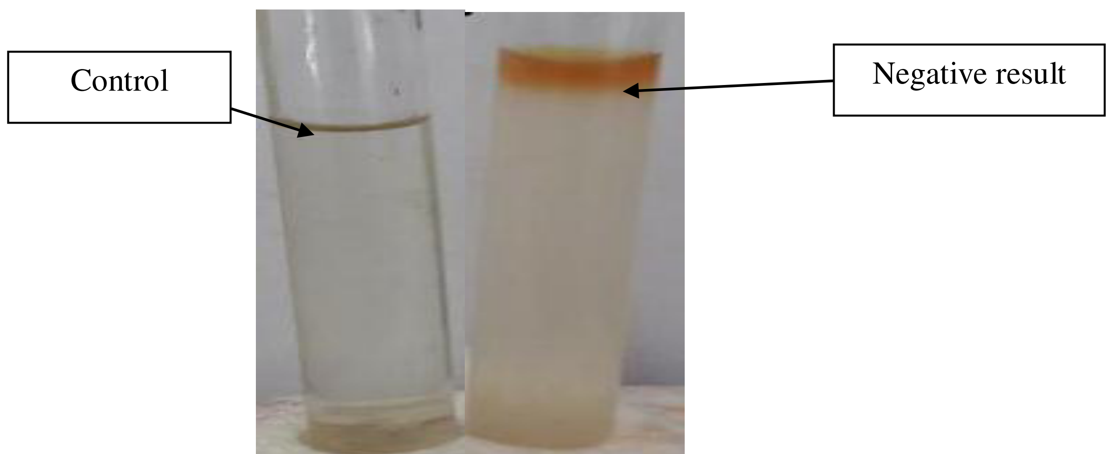

Plate 23: *Bacillus spp* showing negative result (Right) on Methyl Red test with control (Left).

iii) Voges-Proskauer Test

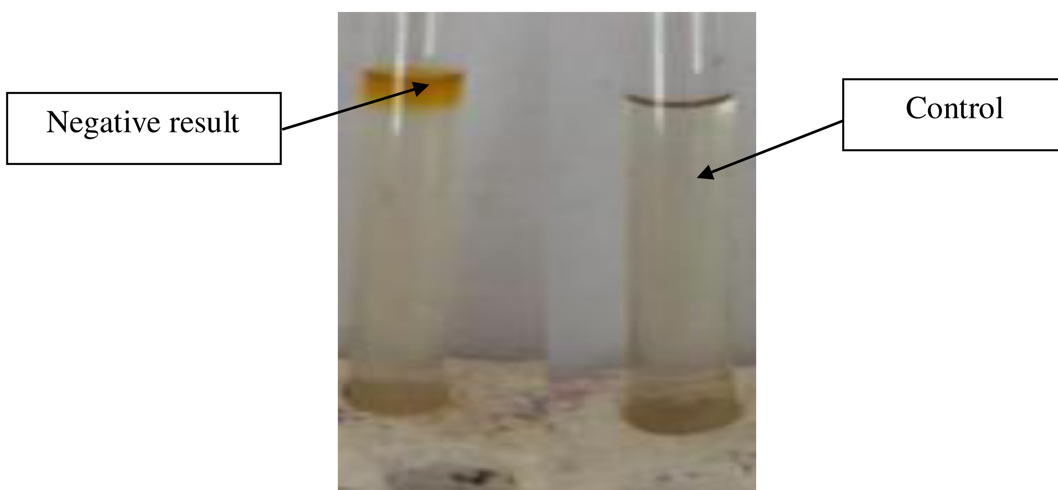

Plate 24: *Bacillus spp* showing negative result (Left) on Voges-Proskauer test with control (Right)

Triple Sugar Iron (TSI) Test

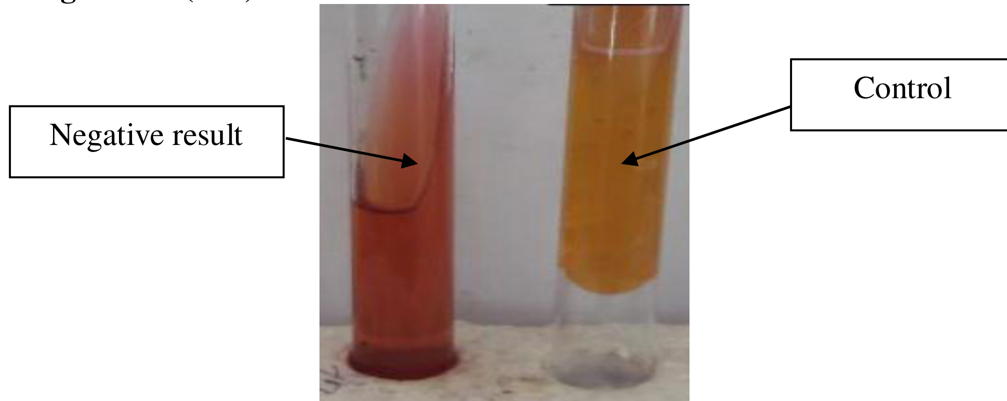

Plate 25: *Bacillus spp* showing negative result (Left) on Triple sugar iron test with control (Right).

Citrate Utilization Test

Plate 26: *Bacillus spp* showing positive result (Right) on citrate utilization test with control (Left).

Indole Test

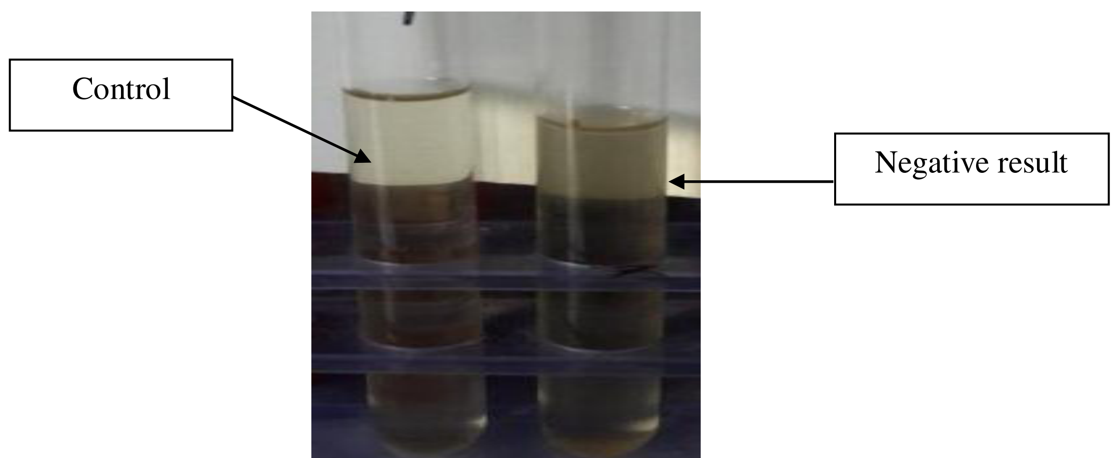

Plate 27: *Bacillus spp* showing negative result (Right) on Indole test with control (Left).

Biocehmic tests result of of *Salmonella spp.*

Catalase Test

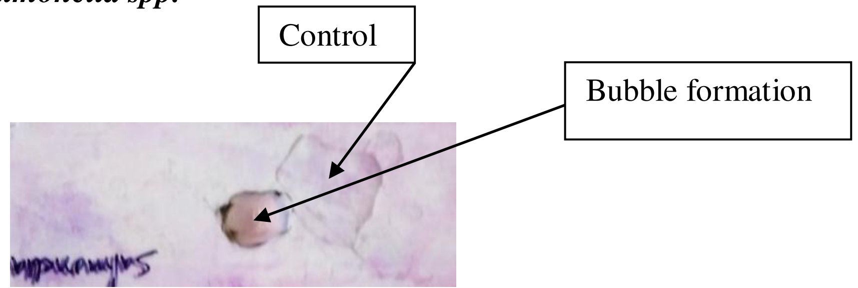

Plate 28: *Salmonella spp* showing positive result on catalase test.

Methyl Red Test

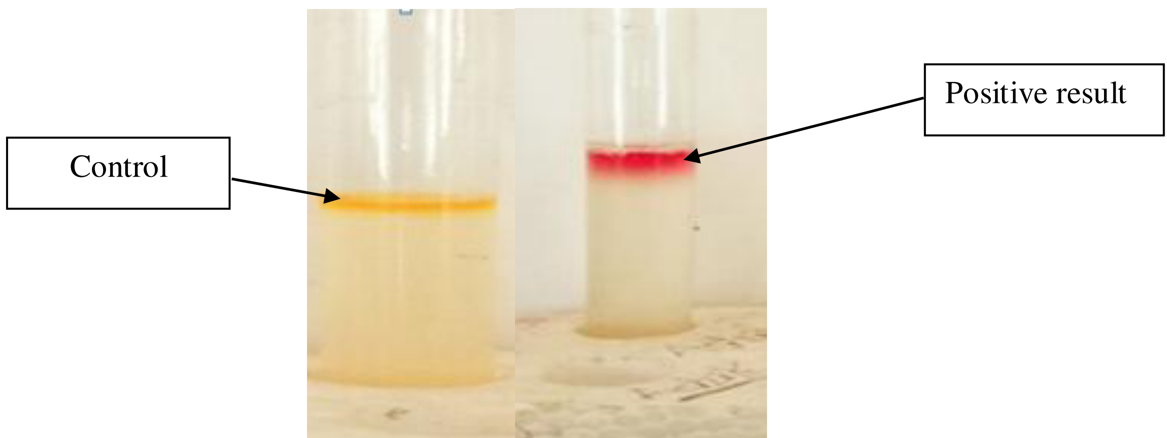

Plate 29: *Salmonella spp* showing positive result (Right) on Methyl Red test with control (Left).

Voges-Proskauer Test

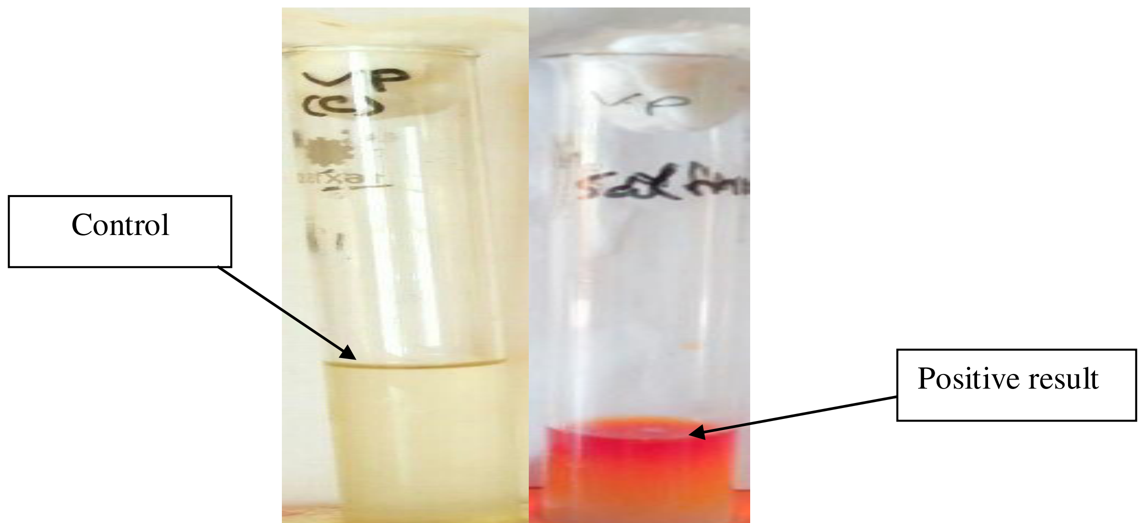

Plate 30: *Salmonella spp* showing positive result (Right) on Voges-Proskauer test with control (Left).

Triple Sugar Iron (TSI) Test

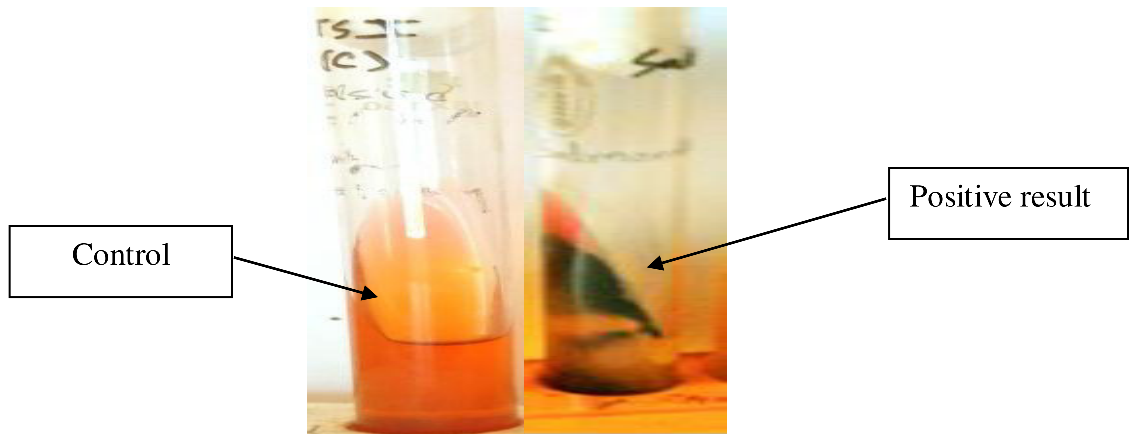

Plate 31: *Salmonella spp* showing positive result (Right) on Triple sugar iron (TSI) test with control (Left).

Citrate Utilization Test

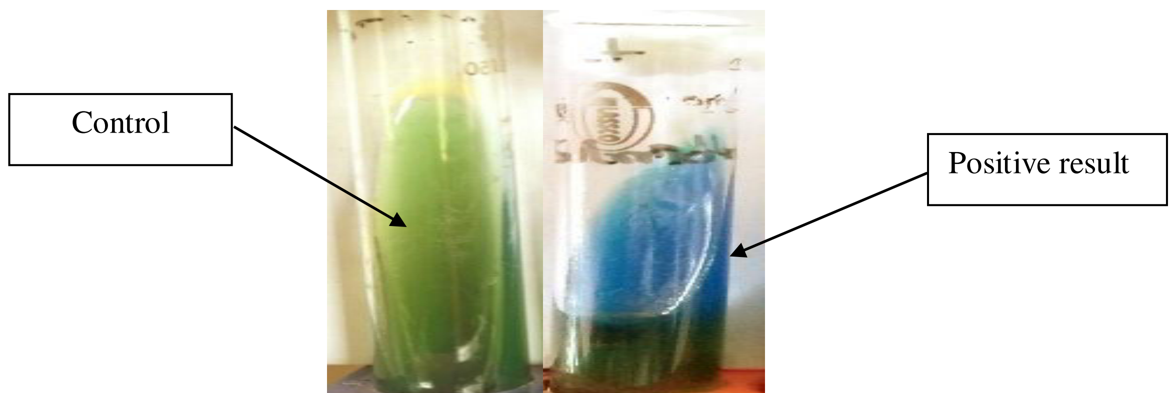

Plate 32: *Salmonella spp* showing positive result (Right) on citrate utilization test with control (Left).

Indole Test

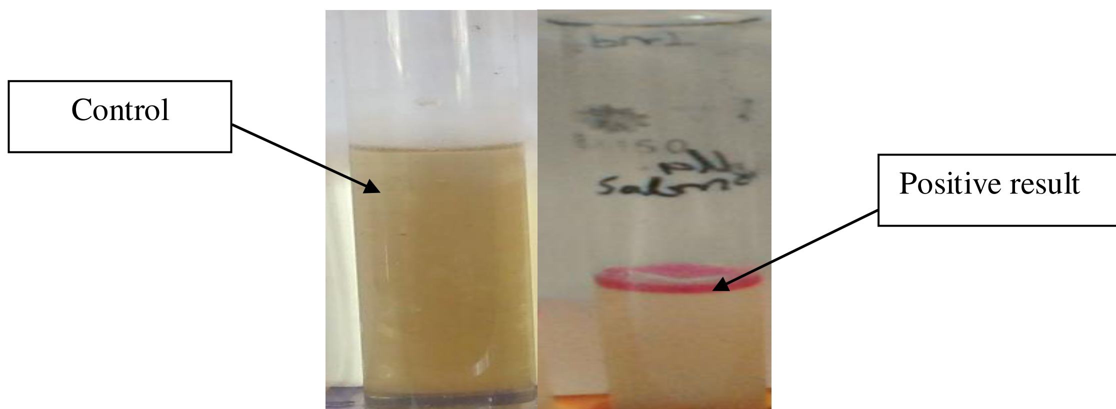

Plate 33: *Salmonella spp* showing positive result (Right) on Indole test with control (Left).

Biocehmic tests result of *Klebsiella spp* in.

Catalase Test

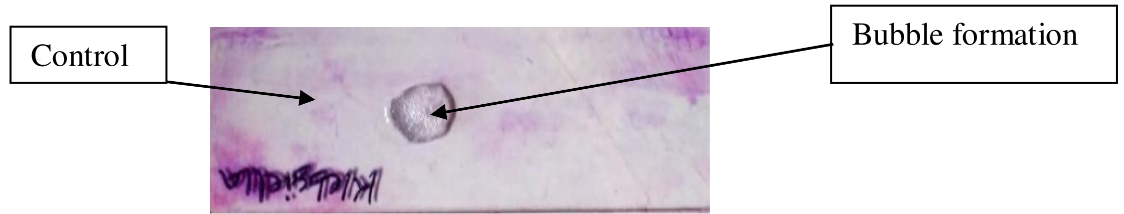

Plate 34: *Klebsiella spp* showing positive result on catalase test.

Methyl Red Test

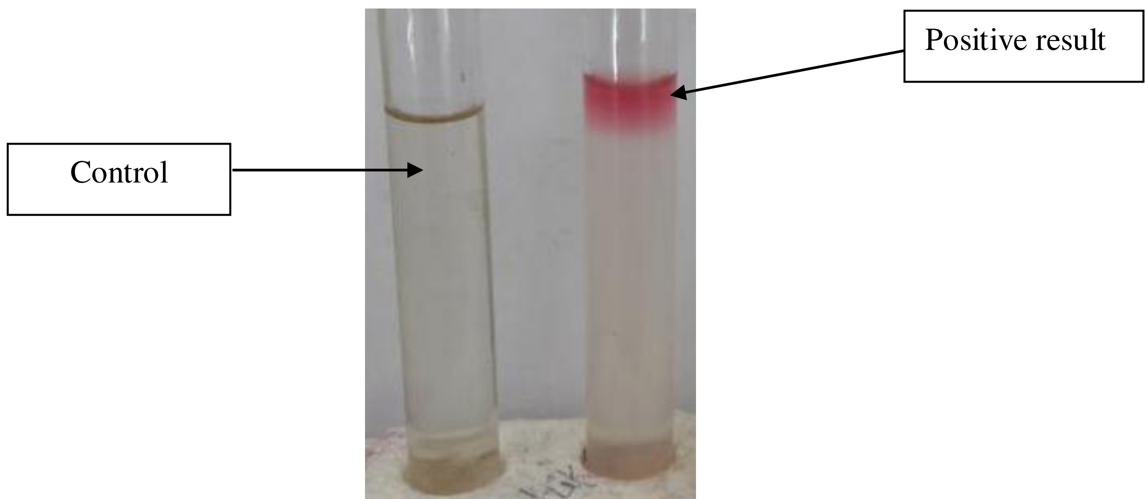

Plate 35: *Klebsiella spp* showing positive result (Right) on Methyl red test with control (Left).

Voges-Proskauer Test

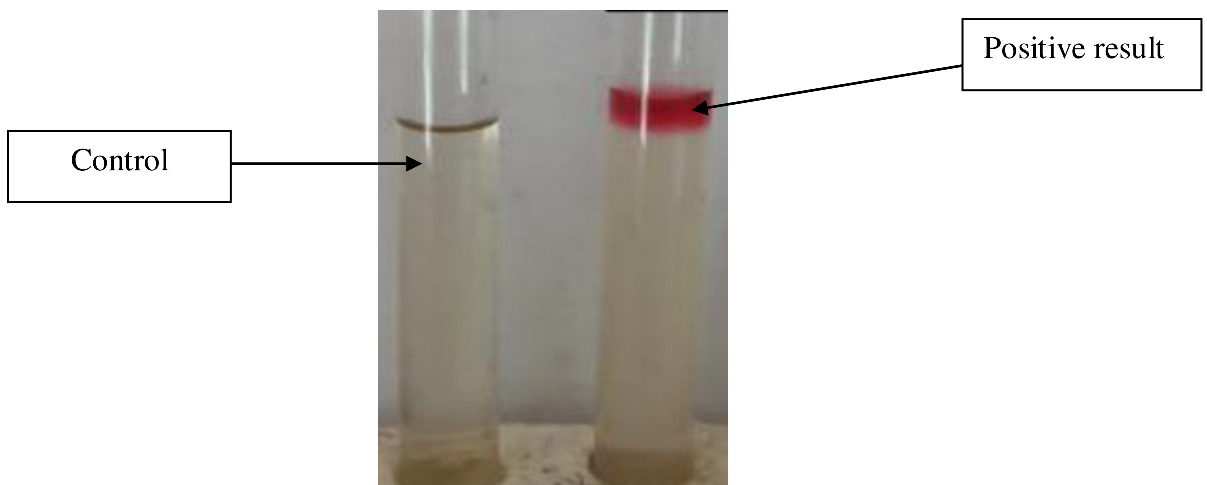

Plate 36: *Klebsiella spp* showing positive result (Right) on Voges-Proskauer test with control (Left).

Triple Sugar Iron (TSI) Test

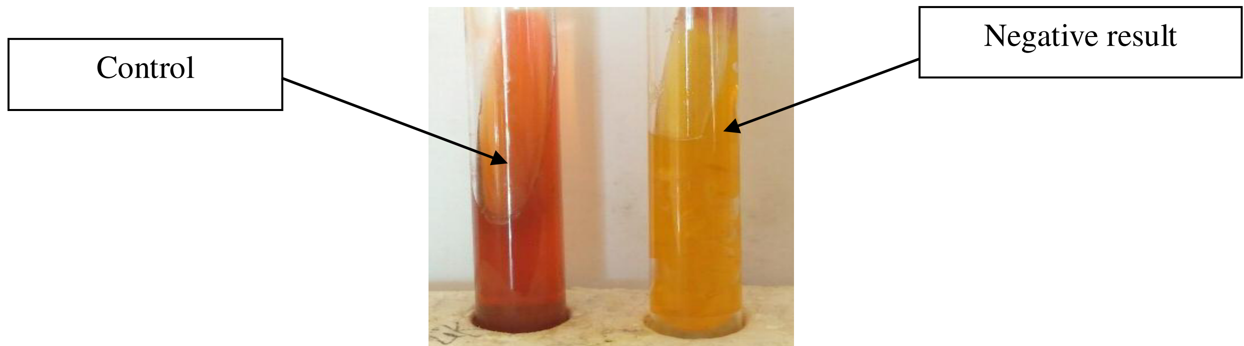

Plate 37: *Klebsiella spp* showing negative result (Right) on Triple sugar iron (TSI) test with control (Left).

Citrate Utilization Test

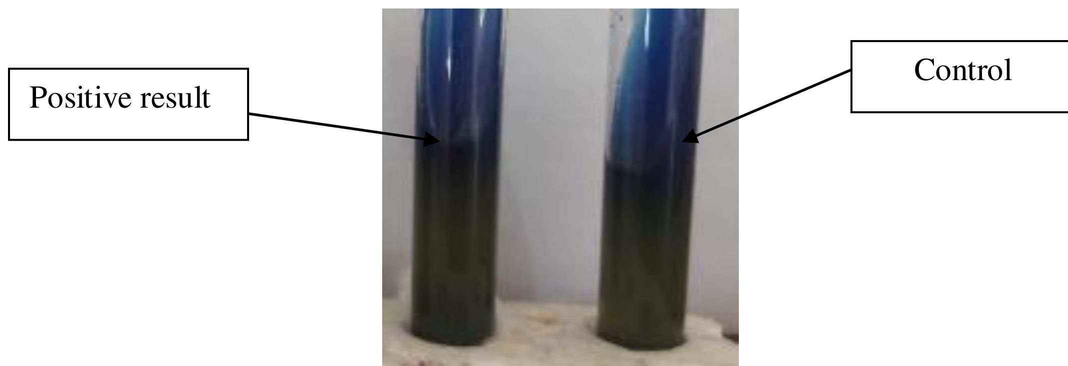

Plate 38: *Klebsiella spp* showing positive result (Left) on citrate utilization test with control (Right).

Indole test

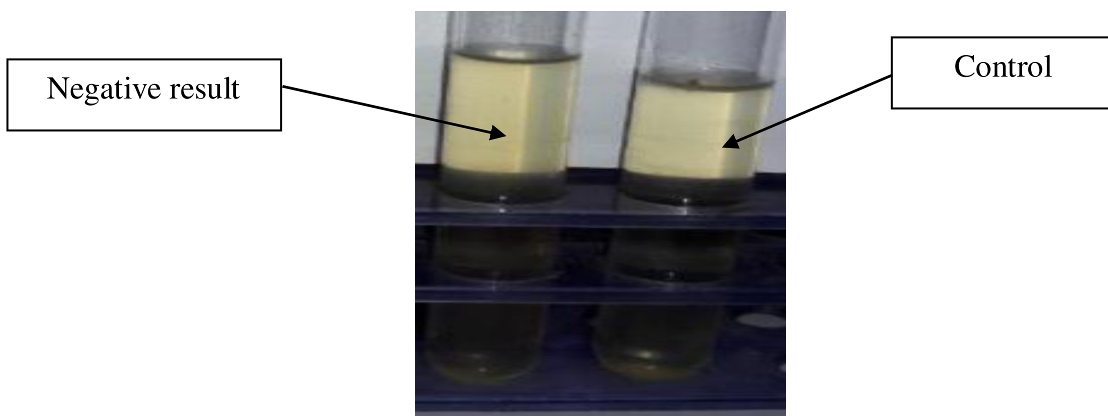

Plate 39: *Klebsiella spp* showing negative result (Left) on Indole test with control(Right).

Biocehmic tests result of *Pseudomonas spp* in.

Catalase Test

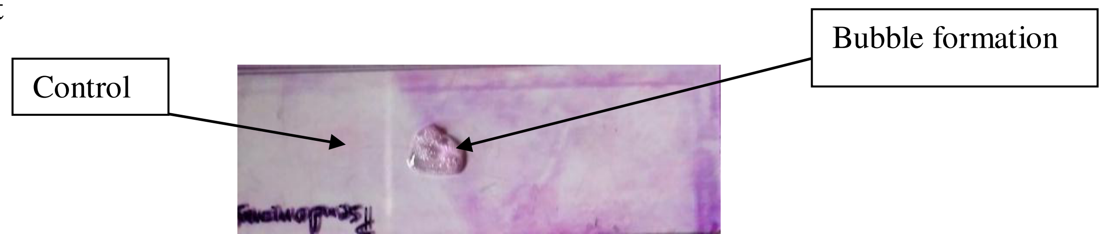

Plate 40: *Pseudomonas spp* showing positive result on catalase test.

Methyl Red Test

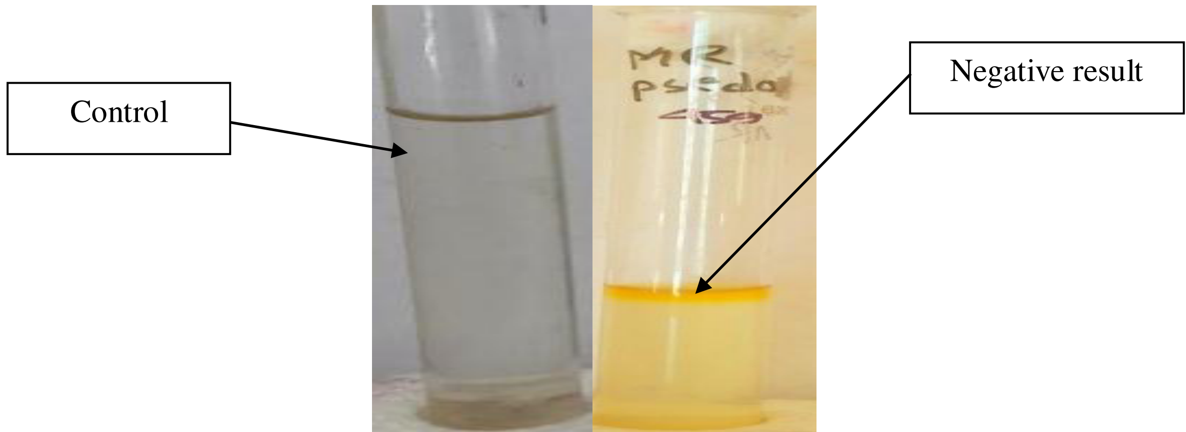

Plate 41: *Pseudomonas spp* showing negative result (Right) on Methyl red test with control (Left).

Voges-Proskauer Test

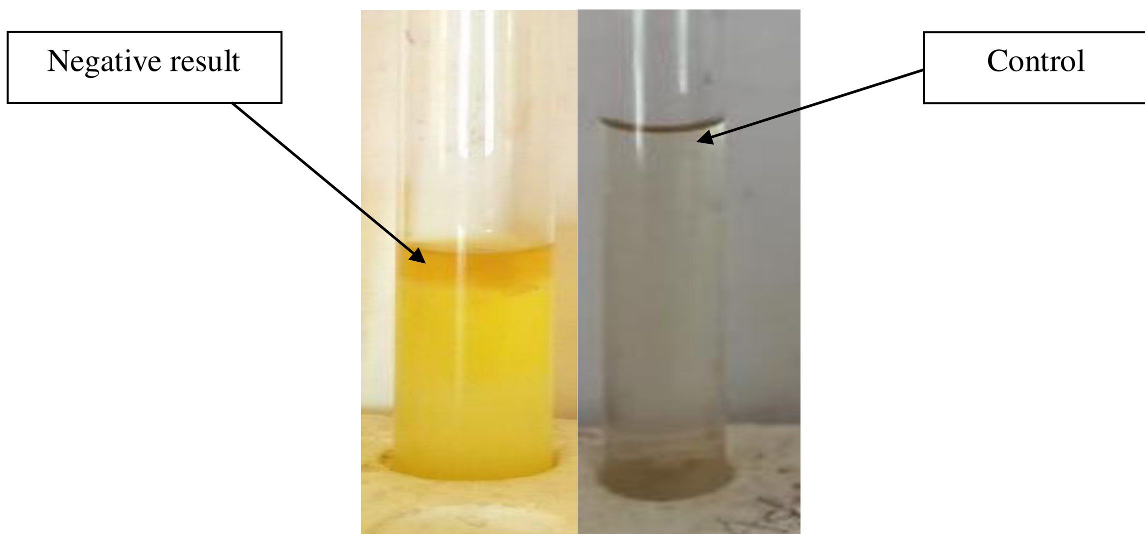

Plate 42: *Pseudomonas spp* showing Negative result (Left) on Voges-Proskauer test with control (Right).

Triple Sugar Iron (TSI) Test

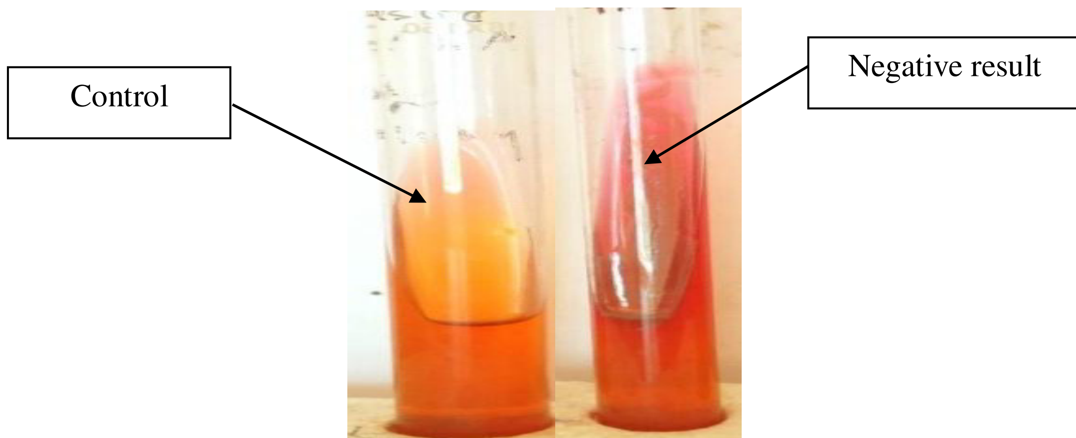

Plate 43: *Pseudomonas spp* showing Negative result (Right) on Triple sugar iron (TSI) test with control (Left).

Citrate Utilization Test

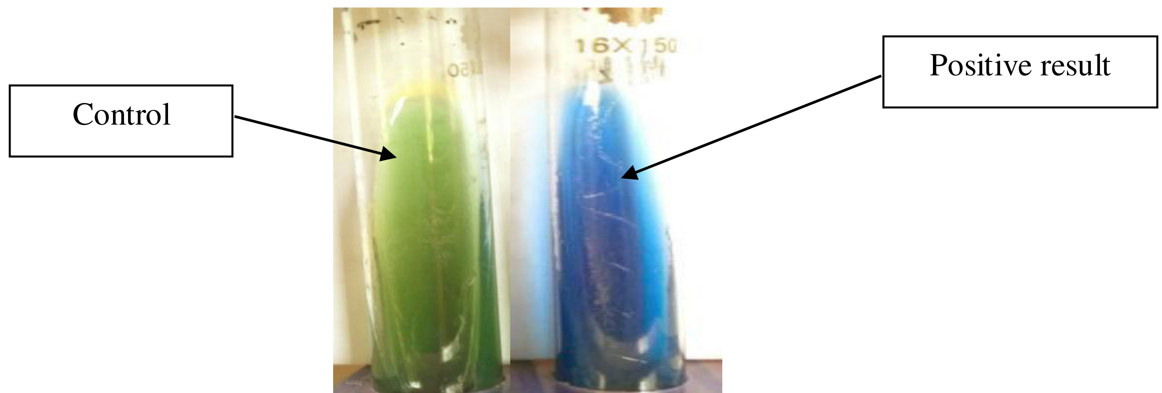

Plate 44: *Pseudomonas spp* showing positive result (Right) on citrate utilization test with control (Left).

Indole test

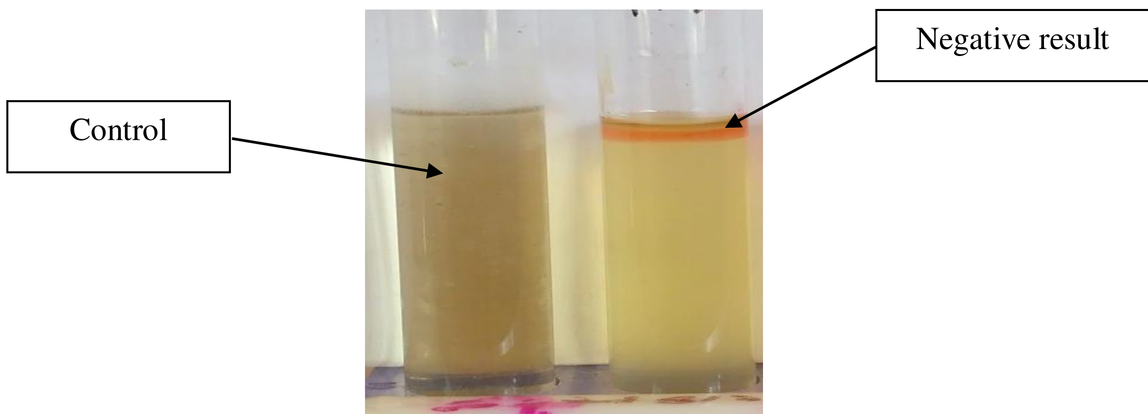

Plate 45: *Pseudomonas spp* showing negative result (Right) on indole test with control (Left).

4.6 Result of antibiotic susceptibility pattern of isolated organisms from different mobile phones.

Total numbers of 32 mobile phones were collected from academic staff, nonacademic staff, students and cleaners of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh to isolate bacteria. The table below showing only the average zone of inhibition for those organisms was tested against at least 15 different antibiotics that are available in the market. Mobiles were isolates for antibiotic susceptibility test on the basis of their sampling site importance

Table 14. Result of antibiotic susceptibility pattern of isolated organisms from different mobile phones.

Name of the tested organism	Name of selected antibiotic	Disc concentration (µg/disc)	Zone of Inhibition (unit)	Interpretation
Staphylococcus spp.	Chloramphenicol(C)	30µg/disc	17	Intermediate
	Penicillin (P)	10µg/disc	0	Resistance
	Methicillin (MET)	5µg/disc	0	Resistance
	Amoxicillin (AMX)	30µg/disc	0	Resistance
	Erythromycin (E)	15µg/disc	4	Resistance
Bacillus spp.	Chloramphenicol(C)	30µg/disc	20	Susceptible
	Azithromycin(AZM)	30µg/disc	14	Intermediate
	Amoxicillin (AMX)	30 µg/disc	0	Resistance
	Norfloxacin (NX)	10µg/disc	5	Resistance
	Cloxacillin (COX)	1µg/disc	0	Resistance
Salmonella spp.	Gentamycin (GEN)	10µg/disc	21	Intermediate
	Erythromycin (E)	15µg/disc	0	Resistance
	Cefaclor (CEC)	30µg/disc	0	Resistance
	Amoxicillin (AMX)	30µg/disc	0	Resistance
	Cloxacillin (COX)	1µg/disc	0	Resistance
Klebsiella spp.	Gentamycin (GEN)	10 µg/disc	18	Resistance
	Amoxicillin (AMX)	30 µg/disc	15	Resistance
	Cefaclor (CEC)	30 µg/disc	0	Resistance
	Ofloxacin (OFX)	5µg/disc	17	Intermediate
	Erythromycin (E)	15µg/disc	0	Resistance
Pseudomonas spp.	Cephalexin (CN)	30µg/disc	0	Resistance
	Penicillin (P)	10µg/disc	0	Resistance
	Ciprofloxacin (CP)	5µg/disc	4	Resistance
	Amoxicillin (AMX)	30 µg/disc	0	Resistance
	Amikacin (AK)	30 µg/disc	3	Resistance

The *Staphylococcus spp* was intermediate sensitive to Chloramphenicol and resistant to Erythromycin, Penicillin, Methicillin and Amoxicillin. *Bacillus spp* was susceptible to Chloramphenicol, and intermediate to Azithromycin and resistant to sensitive Norfloxacin through Amoxicillin and Cloxacillin. *Salmonella spp* was intermediate sensitive to Gentamycin, and resistance through Erythromycin, Cefaclor, Amoxicillin and Cloxacillin. *Klebsiella spp* was intermediate sensitive to Ofloxacin and resistant through Gentamycin, Amoxicillin, Cefaclor and Erythromycin. *Pseudomonas spp* was resistance sensitive to Cephalexin, Penicillin, Ciprofloxacin, Amoxicillin and Amikacin.

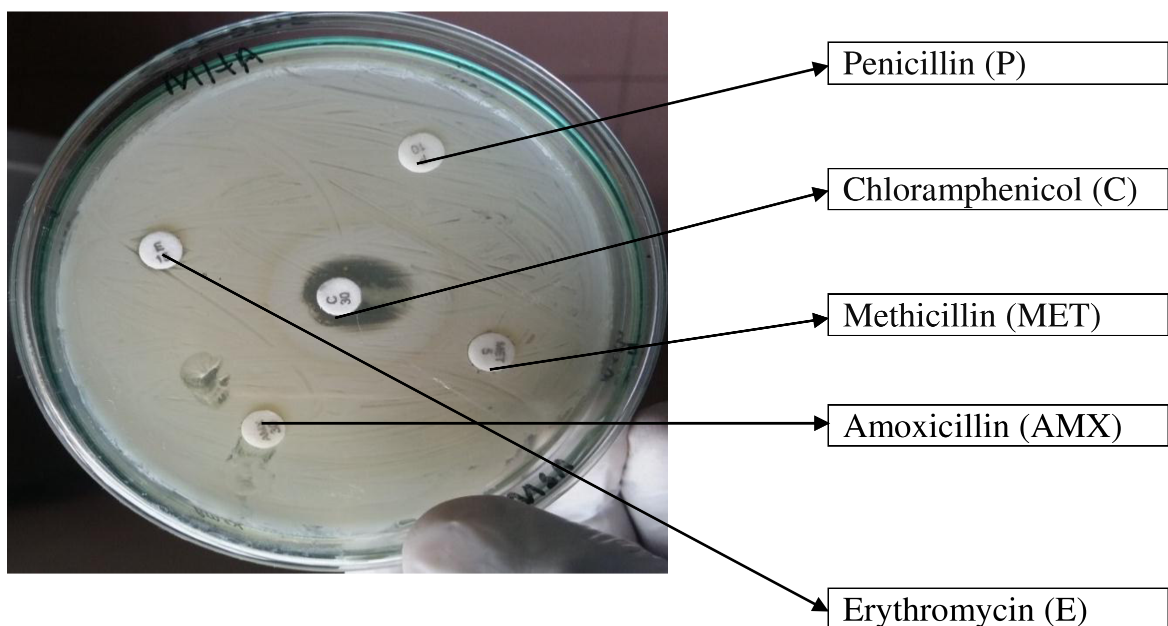

Plate 46: Antibiogram assay of *Staphylococcus spp* against different antibiotics.

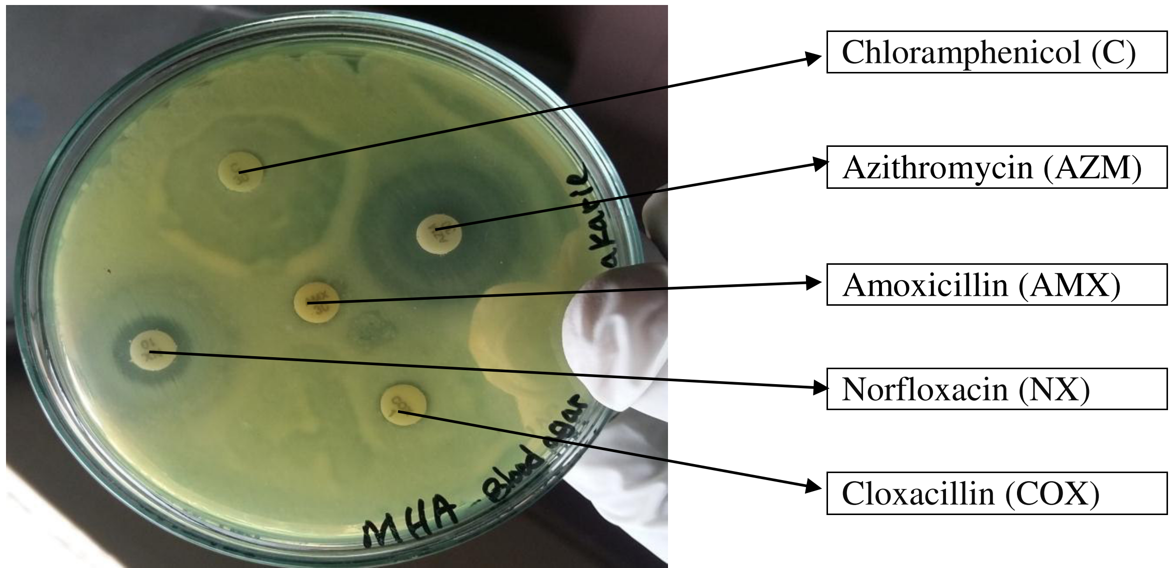

Plate 47: Antibiogram assay of *Bacillus spp* against different antibiotics.

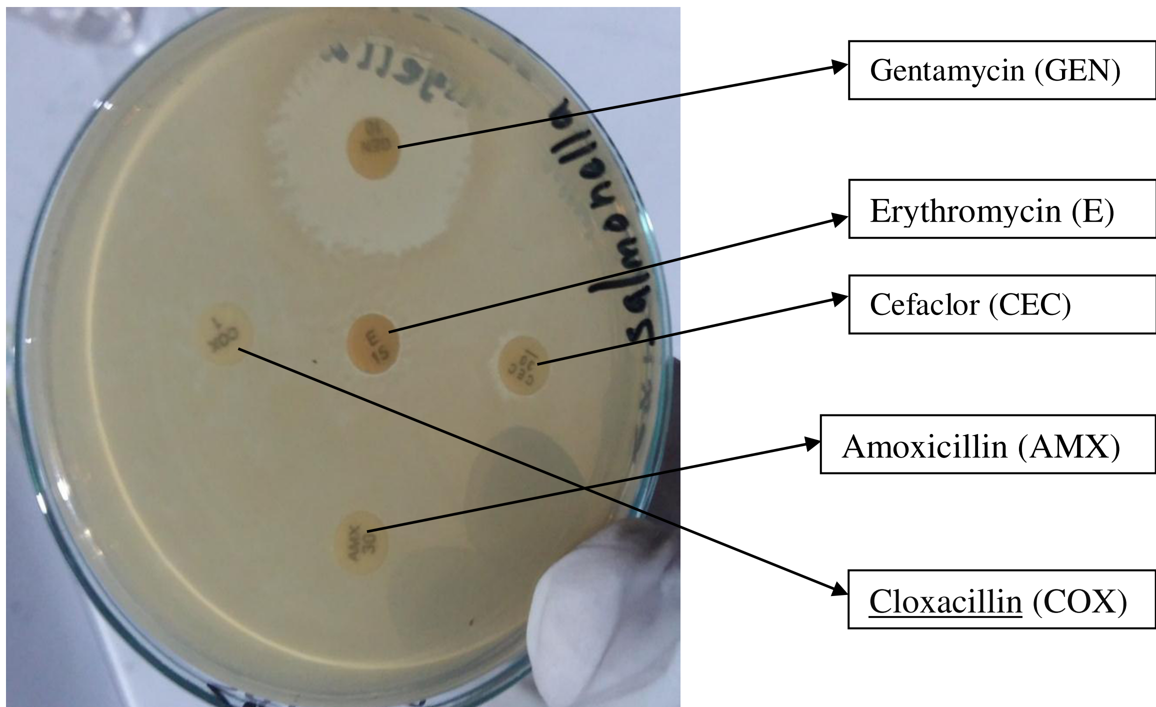

Plate 48: Antibiogram assay of *Salmonella spp* against different antibiotics.

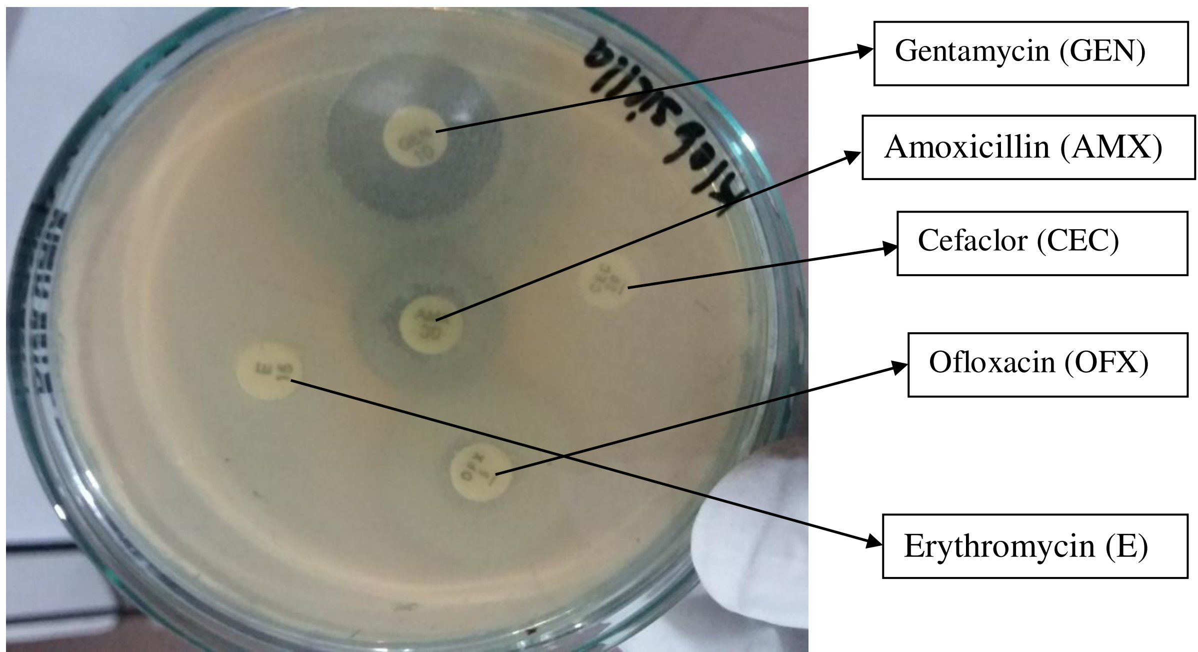

Plate 49: Antibiogram assay of *Klebsiella spp* against different antibiotics.

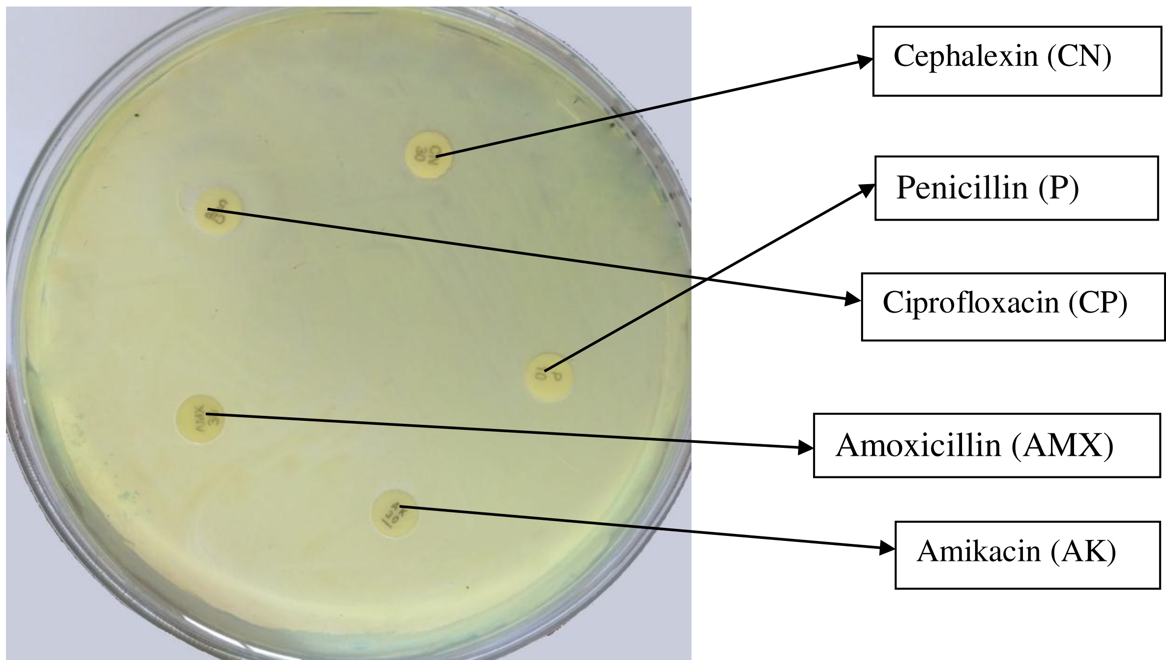

Plate 50: Antibiogram assay of *Pseudomonas spp* against different antibiotics.

DISCUSSION

CHAPTER 5

DISCUSSION

We are living in a world, which is full of microbes, it is not possible to make this world microbe free but microbiological standards and hygiene practices should be adapted by the society for a healthy life. This investigation of this study was aimed to isolate and identify bacteria and create awareness that mobile could also serve as vector for transfer of bacteria from one individual to another, therefore personal hygiene and mobile decontamination is very important. For this study, A total of 32 swap samples of mobile phones were collected from academic staff, nonacademic staff, students and cleaners of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh. This study confirmed as variety of microbes on mobile phones belong to five genera (*Staphylococcus spp, Bacillus spp, Klebsiella spp, Pseudomonas spp, Salmonella spp)* were identified. Depending on environmental conditions, pathogens may remain infectious on surfaces for weeks after being contaminated. In humid conditions, pathogens may actively colonize surfaces, transforming a passive reservoir into an active one. Mobile phones due to their personal nature and proximity to sensitive part of our bodies in usage such as faces, ears, lips and hands of users could become veritable reservoirs of pathogens that could result in infections. The prevalence of *Staphylococcus spp* 21 (25.6%), Bacillus *spp,* 17 (20.7%), *Klebsiella spp* 16 (19.5%), *Pseudomonas spp* 15 (18%) and *Salmonella spp* 13 (15.85%).

Findings of our research work are nearly similar with findings Oguz Karabay *et al.* (2007). The bacteria isolated from the smart phones were *Pseudomonas*, *Bacillus* and *Staphylococci*.

Findings of our research work are nearly similar with findings Bone, (1993). They presence Gram-negative rod. The possibility of the presence of the faecal contamination on the mobile phone by *Klebsiella* spp and *Pseudomonas aeruginosa spp.*

Findings of our research work are nearly similar with findings Ergey and Breed (1957). The isolated bacteria from cell phone of hospital staff and college students were identified with the help of Bergey's Manual of determinative bacteriology. We isolated different type of bacterial pathogens such as *Staphylococcus spp, Bacillus spp, Klebsiella spp, Pseudomonas spp* and *Salmonella spp.*

The organisms were consistently isolated from the environment and humans. The roles of these organisms in both nosocomial and community acquired infections have been stressed (Topley *et al*., 1990; Walther *et al*., 2004). According to my study *Staphylococcus spp* find in most of the phones. Commercial phones had the largest variety of bacteria. This may be as a result of multiple usage and longtime of exposure to the environment. The surface of the patients' phones carries more pathogenic bacteria than the ear piece. Nurses' phones carry the least array of bacteria. This result shows the frequency of the use and exposure of cell phones to environmental microbes on the hand and skin of the users. This result is in agreement with the findings of Rusin *et al*., (2000). This is another mean by which pathogens from the hands of health care workers can be transmitted to the both the sick and healthy individuals. (Ferroni *et al*., 1998).

The vitro antibiotic sensitivity test of isolated bacteria *Staphylococcus spp, Bacillus spp, Klebsiella spp, Pseudomonas spp and Salmonella spp* showed resistant to Penicillin, Amoxicillin, Cefaclor, Ofloxacin and Ciprofloxacin.

CONCLUSION

CHAPTER 6

CONCLUSION

Through experiment different type of bacterial pathogens were collected such as *Staphylococcus spp, Bacillus spp, Salmonella spp, Klebsiella spp* and *Pseudomonas spp.* Isolated microorganisms were identified on the basis of morphological characteristics, cultural characteristics and biochemical characterization. The results of present study are conclusive evidence for prevalence of bacterial pathogenicity, due to the sharing of mobile phones and sensitive parts of our bodies in contact with it such as faces, hands and ears. The personal hygienic sanitation is required for decontamination mobile phones such hands cleaning and washing when mobile phone is used. The use of mobile phones by lectures in the classroom and technologists in the laboratories may have serious hygiene consequences, because unlike fixed phones, mobile phones are often carried about within and outside the classrooms and laboratories. The laboratory environment plays a critical role in the transmission of organisms associated with infections. Micro-organisms can be transferred from person to person from inanimate objects such as (microscopes, fixed telephones, autoclaves, ovens, incubators fridges etc.).

Suggestions

- Control measures are quite simple. Mobile phones cleaning and disinfection of appropriate mobile surface to reduce transmission of pathogenic organisms from these gadgets to persons in addition, people should be informed that these devices may be a source for transmission of infections to and from the community.
- Regular cleaning of mobile phones with a suitable cleaning fluid as well as frequent hand washing should be encouraged as means of curtailing any potential disease transmission from mobile phones.

REFERENCES

REFERENCES

Adetona, A.A., Akinyemi, F.S., Olabisio, O.O. and Audu, B.O. (2011). The potential role of mobile phones in the spread of bacterial infection, Department of Microbiology Lagos State University, 3:628 – 632.

Aiello, A.E., Larson E.L., (2002). What is the evidence for a causal link between Hygiene and Infections.Lancet Infection Dis. 2:103–110.

Amira, H.A., Al-Abdalall. (2010). Isolation and identifcation of microbes associated with mobile phones in Dammam in eastern Saudi Arabia. Journal of Family and Community Medicine. 17(1):11-14

Anuradha, S.N., Arunkumar.S., Mekhanayakee, Wai Yoke Shyen and Arwintharao (2016).Study on awareness of microbial contamination through mobile phones. Asian pacific journal of health sciences.3(4):313-323.

Bergey, D.H., Breed R.S., 1957. Bergey's manual of determinative bacteriology. American Society of Microbiology, New York.

Beveridge, T. J., Martin, S. A., Kadurugamuwa, J. L., & Li, Z.. (1997). Interactions between biofilms and the environment. FEMS Microbiology Reviews, 20(3-4), 291-303.

Bone, R.C., Gram- negative sepsis: a dilemma of modern medicine. *Clin. Microbial. Rev*. 1993; 6(1):57- 68.

Brady, R.R., Fraser S.F., Dunlop M.G., Paterson-Brown, S., and Gibb, A.P. (2007). Bacterial contamination of mobile communication devices in the operative environment. Journal Hospital Infection. 66: 397-8.

Brady, R.R., Wasson A., Sterling I., M.c., Allister C., and Damani N.(2006). Is your phone bugged? The incidence of bacteria known to cause nosocomial infection on health care workers' mobile phones. The Journal Hospital Information. 62 (1):123 – 125.

Brady RR, Verran J, Damani NN, Gibb AP. (2009). Review of mobile communication devices as potential reservoirs of nosocomial pathogens. Jounal Hospital Infection. 71: 295–300.

Carter, G.R. (1979). Diagnosis procedures in veterinary bacteriology and mycology 3rd end. Charles C, Thomas publisher U.S.A.398-417

Chawla K., Mukhopadhayay C., Gurung B., Bhate P. and Bairy I.(2009) Bacterial 'Cell' Phones: Do cell phones carry potential pathogens? Online Journal Health Allied Science.8:8.

Cheesbrough, M., (1985). Medical laboratory manual for tropical countries. 1st end.vol.02 microbiology, English language Book Socity, London, 35: 40-57.

Cowan, S.T. and steel, K.T, (1979) manual for identification of medical bacteria. 2and Education, Cambridge university press, London. 22-122.

Deepak Kumar Verma, Alemu Barasa, Dantse Dara, Habtamu W/Medehen, Hilina Asrat, Natnael Demissie, Kibrnesh Tegenaw, Wagaw Sendeku and Nega Berhane (2015). Conducted Isolation and characterization of bacteria from mobile phones of students and employees at university of Gondra, Ethopia. Bulletin of Pharmaceutical Research. 5(3):96-100.

Famurewa, O. and David, O. M. (2009). Cell phone is a medium of transmission of bacterial pathogens. Marsland Press World Rural Observations. 1(2): 69-72.

Ferroni, A., Nguyan, L., Pron, B. Quseslle, G., Brusset, M. C. and Bercher, P. (1998): Outbraek of nosocomial urinary tract infection due to *Pseudomonas aeruginosa* In a pediatric surgical unit associated with tap water contamination. *J. Hos. Infec*. 38(4): 301-307.

Haider S., Kadhem, Ahmed A. Abed Ali, Osama M. and Hassan (2016). Isolation and identification of bacteria isolated from different parts of cell phones. World journal of experimental Bioscience. 4(1):29 -31.

Hui Y.H., Satar S.A., Murrell K.D., Nip W.K. and Stanfeld P.S.(2001). Food borne disease handbook. 2nd ed., Vol. 2. Viruses, parasites, pathogens and HACCP. New York: McGraw-Hill Professional.

Ibrahim T.A., Akenroye, O.M., Opawale, B.O., and Osabiya OJ (2013). Isolation and Identification of Bacterial Pathogens from Mobile Phones of Volunteered

Technologists in Rufus Giwa Polytechnic, Owo, Ondo State. Journal of microbiology and biotechnology. 3(1):2347-2286.

International Commission on Microbiology Specification for foods (ICMSF), (1986). Microorganisms in food 2-sampling for microbiology analysis: principle and specific.

Isolation and characterization of bacteria from mobile phones of students and employees at university of Gondar, Ethiopia. *Bulletin of Pharmaceutical Research.* 5(3):96-100.

James, F., Meadow, Adam E. Altrichter Jessica L. Green (2014) Mobile phones carry the personal microbiome of their owners. Peer Journal.2:447.

Jayachandra T., Lakshmi Prassana A., Venkateshwara Rao A. (2011). A study on isolation and identification of bacteria causing nasocomial infection on mobile phones of health care worker, Calicut medical journal.5: 1-6.

Jeske H.C., Tiefenthaler W, Hohlrieder M, Hinterberger G, Benzer A (2007). Bacterial contamination of anaesthetists' hands by personal mobile phone and fixed phone use in the operating theatre. Anaesthesia. 62: 904-906.

Khivsara A., Sushma T.V., Dahashree B (2006). Typing of *Staphylococcus aureus* from mobile phones and clinical samples. Curr Science. 90: 910-912.

Kilic, I.H., Ozaslan, M., Karagoz, I.D. and Davatoglu, V. (2009). The microbial colonisation of mobile phones used by health care staffs, Pakistan journals of biological science, 78: 882-884.

Marchat I.A and Packer, R.A. (1967) veterinary bacteriology and virology 07th edition. The lowa state university press USA.

Melnick J.and Edward A. Medical Microbiology.(2004). 23th ed. New York: McGraw-Hill Professional.

Mofolorunsho C.K. and Onwe, C.S. (2013). Isolation and characterization of bacterial pathogens associated with mobile phones in Anyigba, Nigeria. Journal of Science & Multidisciplinary Research. 2:19-21.

Mohamed M., Hassan and Ismail A., Ismail (2014). Isolation and molecular characterization of some pathogenic mobile phone bacteria. International journal of biochemistry and biotechnology 3(3):516-522.

Mrs. Sweta Dave, Dr. Kishor Shende (2015). Isolation and Identification of Microbes Associated With Mobile Phones in Durg District in Chhattisgarh Region, India. Journal of Environmental Science, Toxicology and Food Technology.1(6):71-73.

Ogus Karabay, Esra Kocoglu, Mustafa Tahtaci (2007) the role of mobile phones in the spread of bacteria associated with nosocomial infections.

PURNIMA R. CHITLANGE (2014). Contamination of cell phones by pathogenic microorganisms, Comparison between hospital staff and college students. 6(2):203-206.

Rafferty K.M. and Pancoast S.J. (1984). Bacteriological sampling of telephones and other hospital staff hand-contact objects. Jounal Infection Control 5(11):533-5.

Roberta Basol, Jean Beckel, Judy Gilsdorf-Gracie, Amy Hilleren-Listerud, McCaffrey, Sherri Reischl, Pamela Rickbeil, Mary Schimnich, Kirsten Skillings and Mary A. Struffert. (2014). Bacteria on shared mobile phones can lead to infections. Nursingcriticalcare. 9(4):5-7.

Rusin, P., Maxwell, S, S., & Gerba, C. (2002). Comparative surface- to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, ggram-negative bacteria, and phage. Journal of Applied Microbiology, 93(4), 585-592.

Sadat AM, Al-Omran AK, Azam Q, Bukari H. (2010). Bacterial flora on cell phones of health care providers in a teaching institution. Americal journal Infection Control. 38: 404-405.

Shahaby, A.F., Awad, N.S., El-Tarras, A.E., and Bahobial A.S. (2012). Mobile phone as potential reservoirs of bacterial pathogens African Journal of Biotechnology. 11(92): 15896-15904.

Soto C.B., Albaladejo M.D., Martinez P., Sanchez-Gascon F., TomasBarberan F. and Espin J.C. (2006). Pomegranate juice supplementation in chronic obstructive

pulmonary disease: a 5-week randomized, double-blind, placebo-controlled trial. Europ J Clin Nutr+ 60: 245-253.

Soto R.G., Chu L.F., Goldman J.M., Rampil I.J. and Ruskin K.J. (2006). Comminication in critical care environments: Mobile telephones inmprove patient care. Anesth Analog. 102: 535-541.

Suganya.S, Judia Harriet Sumathy.V (2012). Isolation and identification of bacteria from covered and uncovered mobile phones. 3 (1):0976 – 4402.

Tagoe D.N., Gyande V K, Ansah E O (2011). Bacterial Contamination of Mobile Phones: When Your Mobile Phone Could Transmit More Than Just a Call Webmed Central Microbiology.2(10):2046-1690.

Tagoe D.N., Gyande V.K. and Ansah E.O. (2011). Bacterial contamination of mobile phones. Central microbiology. 65:121-125.

Telecom Regulatory Authority India. Annual report 2009-2010. Available at http://www.trai.gov.in/WriteReadData/UserFiles/Documents/AnuualReports/ ar_09_10.

Topley, W.W. and Wilson, G. S. (1990). *Hospital acquired infections. Principle of Bacteriology, Virology and Immunity*. 8th Edition, Butler and Tanner Ltd, London. Vol. 3, pp. 267-287.

Ulger, F., Esen, S., Dilek, A., Yanik, K, Gunaydin, M., & Leblebicioglu, H. (2009). Are we aware how contaminated our mobile phones with nosocomial pathogens? Annals of Clinical Microbiology and Antimicrobials, 8 (1), 7.

Vivekanandan Annet Viveka (2017). Isolation and Identification of Common Bacterial Contaminants in Mobile Phones Owned by Veterinary Undergraduate Students Journal of Health, Medicine and Nursing.35:2422-8419.

Walther, B. A. and Ewald, P. W. (2004). Pathogens survival in the external environment and the evolution of virulence. *Bio. Rev. Camb. Philo. Soc*. 79: 849-869.

APPENDICES

APPENDICES

APPENDIX-I

Composition of the media used:

Nutrient Agar	**Grams/Liter**
Peptone	5.0 gm
Bacto beef extract	3.0 gm
NaC1	5.0 gm
Agar	15.0 gm
Distilled water	100 ml
PH	7.2

Sterilized at 121°C under 15 lb/in2 presssure for 15 minutes.

MacConkey agar	**Grams/Liter**
Bctoeptone	17.0 gm
Proteas Peptone	3.0 gm
Lactose	10.0 gm
Bile Salt	1.5 gm
Agar	15.0 gm
Neutral red	0.03 gm
Crystal violet	0.100 gm
Distilled water	1000 ml
PH	71

Sterilized at 121°C under 15 lb/in2 pressure for 15 minutes.

Eosine methylene blue(EME) agar	**Gram/Liter**
Peptone	10.0 gm
Lactose	10.0 gm
K_2HpO_4	2.0 gm
Eosin	0.4 gm

Methylene blue	0.065 gm
Agar	20.0 gm
Distilled water	1000 ml
PH	6.8

Sterilized at 121°C under 1 Sib/in^2 pressure for 15 minutes.

Mueller Hinton Agar	**Gram/Liter**
Beef infusion	20.0 gm
Bacto casamino acid (technical)	17.5 gm
Starch	1.5 gm
Bacto agar	17.5 gm
Distilled water	1000 ml
pH	73

Sterilized at 121°C under 151 b/in^{2}pressure for 15 minutes.

Mannitol Salt Agar	**Gram/Liter**
Proteas peptone	10.0 gm
Beef extract	1.0 gm
D-Mannitol	10.0 gm
NaCl	75.0 gm
Phenol red	0.025 gm
Agar	20 gm
Distilled water	1000ml

Sterilized at 121°C under 15 lb/in^{2}pressure for 15 minutes.

Salmonella Shigella Agar	**Gram/Liter**
Lactose	10.0 gm
Bile salt No.3	8.5 gm
Sodium citrate	8.5 gm
Sodium Thiosulfate	8.5 gm
Beef extract	5.0 gm

Proteose peptone	5.0 gm
Ferric citrate	1.0 gm
Brilliant Green	0.33 gm
Nuetral Red	0.025 gm
Agar	13.5 gm
Distilled water	1000ml

Sterilized at 121°C under 15 lb/in^2 pressure for 15 minutes

Blood Agar	**Gram/Liter**
Blood Agar	60 gm
Distilled Water	1000 ml
Bovine blood	5.00 ml
Or Nutrient agar	500 ml
Sterile Defibrinated blood	25 ml

Normal Saline	**Gram/Liter**
NaCl	0.85gm
Distilled water	1000ml

Autoclaved at 121°C for 15 minutes

APPENDIX-II

Composition of the media used in biochemical test

MR-VP broth	Gram/Liter
Peptone	7.0
Dextrose	50
Dipotassiuni phosphate	5.0
Distilled water	1000ml
PH	6.9

Sterilized at 121°C under 151 b/in^{2}pressure for 15 minutes.

Triple Sugar Iron TSI Agar	Gram Liter
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Lactose	10.0
Saccharose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosuiphate	0.3
Phenol Red	0.024
Agar	12.0
PH	7.4

Sterilized at 121°C under 151 b/in^{2}pressure for 15 minutes.

Simmons citrate Agar	**Gram/Liter**
Magnesium sulphate	0.2
Manoammonium phosphate	1.0
Dipotassium phosphate	1.0

Sodium citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Brom-Thymol Blue	0.08
PH	6.8

Sterilized at 121°C under 15 lb/in^{2}pressure for 15 minutes.

Indole tryptopon broth medium	**Gram/Liter**
Tryptone	10.0
Distilled water	1000ml

Sterilized at 121°C under 15 lb/in^{2}pressure for 15 minutes.

APPENDIX III

Composition of chemicals and reagents

Crystal violet

Solution-A

Crystal violet (90% dye content)	2.0 g
Ethyl alcohol (95%)	20.0 ml
Solution-B	
Ammonium oxalate	0.8
Distilled water	80.0 ml

Note-Mix the solution A and B

Gram's iodine

Iodine	1.0g
Potassium iodide	2.0 g
Distilled water	300.0 ml
Ethyl alcohol	(95%)
Ethyl alcohol (100%)	95.5 ml
Distilled water	5.0 ml

Safranin

Safranin O	0.25 ml
Ethyl alcohol (95%)	10.0 ml
Distilled water	100. 0 ml