Overview of the antimicrobial sensitivity pattern of the clinical isolates collected from patients at intensive care unit of a private hospital in Dhaka

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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July, 2018

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Declaration

I hereby declare that the thesis project „***'Overview of the antimicrobial sensitivity pattern of the clinical isolates collected from patients at intensive care unit of a private hospital in Dhaka'',***

has been written and submitted by me, Prapti Chakraborty and has been carried out under the supervision of Dr M. Mahboob Hossain, Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

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

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Dedicated

TO MY

Parents & sister Dr. Chhowa Chakraborty

Acknowledgment

I acknowledge my esteem to Professor **A F M Yusuf Haider**, Chairperson of MNS Department of BRAC University for allowing me and encouraging me to complete my undergraduate thesis. I am much beholden to Professor Shah M. Faruque, Coordinator of Microbiology Program of Department of Mathematics and Natural Sciences, BRAC University

My regards, gratitude, indebtedness, and appreciation goes to my respected Supervisor Professor **Dr. Mahboob Hossain** for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and never-ending inspiration throughout the entire period of my research work. I would like to thank and express my most profound gratitude for guiding me in my report writing and providing time to time suggestions regarding the setting of experimental designs, interpretation of results and following directions for the whole work without being a bit of impatient.

I would like to extend my appreciation to the respective Lab officer **Shamim Akhter Chowdhury** and Teaching assistant **Akash Ahmed** for their suggestions and moral support during my work.

I will be grateful to **Dr. Abu SyeedMosaddek**, professor and head of Pharmacology, Uttara Adhunik Medical College & Hospital, **Dr. Most. Fhamida Begum,** professor & head of Microbiology, Uttara Adhunik Medical College & Hospital for allowing the collection of samples.

Finally, I would also like to thank my parents, my sister Dr. Chhowa Chakraborty. They have always supported through thick and thin. And I will still be indebted to them. To add more, I would like to show gratitude to my best friends FayruzMaysha (who happened to be my thesis partner as well), FariaTasnimAlam and Tashdik Ahmed Ashik; without them, I would not be able to muster the courage and hold my motivation to complete my thesis.

Prapti Chakraborty

July 2018

Abstract

Antibiotic resistance is one of the significant threat to public health. Every time a person takes an antibiotic, sensitive bacteria gets killed, resistant ones survive and then multiply. This may happen even more if the antibiotic dosage is unregulated and unnecessary. The purpose of this study is to give a clear picture of the position of antibiotic resistance in Dhaka, using 40 clinical samples which were collected from a private hospital, located in Dhaka.

A collection of 40 samples from the intensive care unit of Uttara Adhunik Medical College and Hospital was done. Twenty-three isolates of *Pseudomonas spp.,* nine isolates of *Klebsiellasp*and eight isolates of *E. coli sp* were found. To have an idea about the identities of the organisms, the organisms were cultured in different suitable selective media. In addition to this, eight biochemical tests were done to confirm their identities.

Next, by the Kirby-Bauer disk diffusion method, their antibiotic susceptibilities were tested, to observe how over the years the organisms‟ susceptibility pattern has changed. For the antibiogram test, the most common and reliable antibiotics such as amoxicillin, amoxyclav, aztreonam, imipenem, colistin, gentamycin, cefepime, sulfamethoazole, levofloxacin and a combination of third and fourth generation cephalosporins were selected. In total 11 different antibiotics were used for this study.

All the antibiotics mentioned above were tested, considering the antibiotic susceptibility profile of each of the organisms‟ in the past. All the isolates of *Pseudomonas sp* were resistant to cefotazime, cefexime, amoxicillin, imipenem, cefatrizone, and levofloxacin. Furthermore, all the *Pseudomonas sp.* isolates were sensitive to aztreonam, gentamycin, colistin, amoxyclav, and cefepime.

On the contrary, all the *Klebsiella sp.* isolates showed resistance to cefepime, aztreonam, amoxyclav, amoxicillin, levofloxacin, cefexime, cefotazime, ceftriazone, and imipenem. However, all of the *Klebsiella sp.* isolates showed sensitivity to gentamycin and colistin.

All the *E. coli sp* isolates showed sensitive results to gentamycin, cefepime, colistin, amoxyclav, aztreonam, sulfamethoazole, and cefexime. All the eight strains of *E. coli sp.* were resistant to amoxicillin, imipenem, cefotazime, and levofloxacin.

Contents

Chapter 1

Introduction & literature Review

1.0 Introduction

1.1 Background

Intensive care unit, or in short I.C.U patients, throughout their prognosis period undergo a considerable amount of treatments. Amongst all the therapy, the most quintessential one is said to be antibiotics. In developing countries like Bangladesh, it is seen that a massive bulk of antibiotics are produced, in fact a considerable number of antibiotics are unnecessarily being prescribed daily. This careless action leads to the emergence of the fatal condition called antibiotic resistance.

Patients admitted in the hospital are prone to more infections than any other individual, in fact 5-10 times more than any regular individual. The frequency of infections at anatomic sites of the patients may vary from ICU to ICU, however the likelihood is supremely high (Weber et al,. 1999). Fueling the severity of nosocomial infections, especially in the ICU is the increasing incidence of infections caused by antibiotic-resistant bacteria. It is witnessed that infections caused by antibiotic-resistant bacteria have fatal consequences than patients infected with natural bacteria. It is seen patients with the antibiotic-resistant bacterial infection have been treated with more unnecessary antimicrobials than regular infected patients.

The worldwide use of antimicrobial drugs has continued to rise; and in the year 2005, these chemotherapeutic therapy agents caused an expense of nearly 26 billion pounds. In the United Kingdom, 90% of agents were prescribed and most of them being oral or topical, however, in case of hospitals, they used the remaining 10% of antibiotic prescribing, with a stronger usage of injectable agents.

Drug resistance, can be naturally or can be considered as an acquired characteristic of a microorganism. The reasons behind these can be impaired cell wall or cell wall penetration, enzymatic inactivation, altered binding sites or active extrusion from the cell, which leads to efflux mechanisms. The latter way i.e., acquired characteristic may have taken birth from mutation caused by adaptation or transfer of gene.

Antibiotic resistance can be categorized into three main types: multi-drug resistant (MDR), extensive drug resistant (XDR) and Pan drug-resistant (PDR). Within a brief time, multidrug resistance has increased significantly among various pathogens. Furthermore, Gram-negative bacteria with the ability to produce extended-spectrum beta-lactamase(ESBL) are seen to be more prevalent in hospitals and sometimes they are also the cause of urinary tract infections in

primary care. Currently, multi-drug resistant *E.coli sp*. is to be found abundant in Dhaka using household water (Talukdar et al,. 2003). ESBLs can be transmitted through chromosomes (*Pseudomonas spp., Citrobacter spp*.), as a matter of fact, *Klebsiellaspp* is seldom responsible for hospital outbreaks due to its plasmid-mediated resistance. During the year 1990 till 1993, it was discovered that *Klebsiella pneumonia* became resistant to cefotazime (Neuhauser et al,. 2003). To add more, it has been witnessed that species of *Pseudomonas* and *Acinetobacter*which are carbapenem-resistant are considered to be outbreak strains and often, colistin is used to combat them. (Finch,2001)

Antimicrobial agents not only target the invading organisms but also create an impact on the healthy bacterial flora of our body, mainly of the skin and mucous membranes. As a result, the emergence of microbial overgrowth of resistant bacteria may lead to superinfection. For instance, *C.difficile*, the causative agent of candidiasis is often treated with broad spectrum microbial agents, namely ampicillin or tetracycline. A better example could be the advancement of pseudomembranous colitis that occurs due to the overgrowth of toxin-producing strains of *C.difficile*.With such instances being provided, researches have gauged the problem to be knotting and the consequences to be exceedingly terrible. It has been estimated that over the rolling fifty years, the multidrug resistance will turn into XDR and PDR. The majority of the bacteria that were previously eradicated with antibiotics may re-emerge as antibiotic-resistant bacteria, and cause infections, often referred as “superinfection” and therefore, the organisms to be named “superbug”.

The selection of therapeutic agent highly depends on the clinical diagnosis. To add more, in many situations, the clinical diagnosis might require the microbiological diagnosis to give better and specific treatment. For instance, *Salmonella enterica*, the pathogen responsible for serovarTyphi, can easily be treated by co-trimethoazole, ceftriaxone and ciprofloxacin. But, invariant cases that are not the solution, in fact sometimes clinical diagnosis leads to many possible causes regarding microbiology. To solve situations like these, confirmations from the microbiology is sought and samples are collected, prior, even better, if collected before the antibiotic therapy was initiated. The next steps of laboratory isolation and susceptibility tests will give us the bright idea of what to do next to treat the microorganism. Moreover, in the majority of the cases, these are not done to avoid time lag so the doctors use their knowledge of the

standard microorganism and its susceptibility and therefore, prescribe the drugs. (Greenwood, D.,2007).

In a statement given by the US government committee in June 10, 2010, the Infectious Diseases Society of America states that most frequently used antibiotics cost only a small fraction of dollars for a standard type of treatment, as a matter of fact, a single dose of antibiotic may help to prevent and protect from few diseases for a lifetime. No such alternative can proclaim such achievement that too in such a price. This was compiled as part of the statement given to promote both antibiotic research and appropriate use.

Unfortunately, antibiotics in a way, have become sufferers of their success, the more operative the agent is, it is more likely to have a shorter span of treatment, so the lesser the pay-back to the company that advanced it. The various courses of antibiotic usage last for a week or less than that, as a result the trades growing from them are far lower than those from drugs treating chronic conditions. In combination with the rise in pressure of using antibiotics extravagantly would lead to the less effective drug due toresistance and finally will create the atmosphere for antibiotics less tempting as a therapeutic agent.

1.2 Aims and Objectives:

I. Display the notable difference in the antibiotic susceptibility profile of the most common organisms found in intensive care unit

II. Provide a clear and transparent understanding about the chances of “re-emergence” of certain bacteria and its consequences.

III. Overview of the on-going condition of the intensive care unit in Dhaka.

In this study, we have notably come across to three main organisms and they are *Pseudomonas sp*, *Klebsiellasp* and *E. coli sp*. These three main organisms are the most abundant organisms when it comes to hospitals.

1.3 *Pseudomonas spp.:*

Pseudomonas sp. tops the chart of organisms that can be found in hospitals, generally.

1.3.1 Classification: (Migula, 1894)

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Pseudomonadales
- Family: Pseudomonadaceae
- Genus: Pseudomonas

1.3.2 Characteristics and morphology:

- gram- negative
- aerobe
- Rod-shaped and motile bacteria with one or more flagellum.

1.3.3Role in Disease:

Serious *Pseudomonas sp.* infection mainly occur in hospitals since the patients have weakened immune systems. This infection may include bacteremia, pneumonia, in some severe cases even death. Moreover, these infections are normally not seen in healthy individuals but there is a chance of mild infections when exposed to water, which is inadequately chlorinated. (CDC AR, 2004)

1.3.4 Current setup:

An estimated of 51,000 healthcare associated *P.aeroginosa* infections occur throughout USA. Of which, 6,000 are caused by multi resistant with roughly 400 deaths per year attributed to these infections (CDC AR, 2000)

1.4 *Klebsiellaspp*:

Though, this organism falls under the enteric category and is also a member of the normal flora, however these organisms are often behind the reason for upper respiratory tract infection.

1.4.1 Classification: (Migula, 1894)

- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: *Klebsiella*

1.4.2 Characteristics and Morphology:

- Non-motile
- Gram-negative
- oxidase-negative,
- rod-shaped bacteria with a prominent polysaccharide-based capsule

1.4.3 Role in Disease:

Klebsiellasp organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections. *Klebsiella*species (Patricia et al., 1984) have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies (Sieper, 2011) the majority of human *Klebsiellasp* infections are caused by *K. pneumonia*, followed by *K.oxytoca.* Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer, and most infections involve contamination of an invasive medical device.

1.4.4 Current setup:

The outbreaks of KPC-producing *K. pneumonia* have been recounted in the USA (Woodford et al, 2004) and Israel (Leavitt et al, 2007) currently, similar outbreaks related with patients traveling to endemic areas have also been reported in European countries.

1.5 ***Escherichia Coli spp.:***

One of the core member of the Enterobactericae family, normally it resides in warm blooded organisms part of their normal flora. However, they may also lead to fatal consequences, in few situations. Mostly, this occurs to patients from Intensive Care Unit.

1.5.1 Classification: (Castellani and Chalmers, 1919)

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: *E. coli*

1.5.2 Characteristics and Morphology:

- Gram-negative
- facultative anaerobic
- nonsporulating bacterium
- rod-shaped,
- about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm

1.5.3 Role in Disease:

Most *E. coli sp* strains do not cause disease (Ji Yoon et al., 2017) but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's

disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome, however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli* (Todar, 2007).

1.5.4 Current setup:

Extended-spectrum beta lactamase is an enzyme that allows bacteria to become resistant to a wide variety of penicillins and cephalosporins. Bacteria that contain this enzyme are known as ESBLs or ESBL-producing Enterobacteriacease are resistant to strong antibiotics. This has caused 17,000 deaths, excess of $40,000 worth of medical treatment and 26,000 drug resistant infections are emerging, annually.

Chapter 2
Materials and methodology

2.1 Place of study:

This study was conducted in the laboratory of BRAC University designated for the students of the Department of Mathematical and Natural Science.

2.2 Specimen Collection:

The prime step of this experiment was the collection of the samples. This was conducted under independent scrutiny, starting from the preparation of sterile and autoclaved physiological saline and cotton swabs.

For the collection, we prepared fresh and sterile physiological saline, along with it, we also arranged for sterile autoclaved cotton swabs.

We had selected the ICU of UttaraAdhunik Medical College and Hospital for the collection of our samples. The action of accumulating the mouth swabs was done by the nurses of the hospital, to prevent any cause of sepsis. After the mouth swab was taken, we immediately transferred it to the saline in the test tubes, by swirling the cotton swab quite a while in the saline, followed by discard of the used cotton swabs.

2.3 Transfer of the mouth swabs to culture media:

As soon as we completed our collection, we headed towards our lab and used the spread plate method on nutrient agar, to transfer the sample on a culture media and then the samples were sent for the incubation period at 37 degrees Celsius.

The next day, subsequently we studied the numerous colonies that had grown on the nutrient agar culture plate. Then, we tried our best to select the most distinct and random colonies for the next step, that is the isolation of pure colony. The purpose of pure colonies has a significant role in the aspect of identification.

2.4 Identification of the bacteria:

All the steps taken under this category were repeatedly done, to avoid any erroneous results for each of the samples.

2.4.1 Selective culture media:

For this category of identification, we chose to work with MaConkey agar, eosin methylene blue agar, cetrimide agar and hi-chrome agar. All the media were prepared for each sample, inferring, each of the samples‟ pure colony was cultured on all the mentioned media.

2.4.1.1 The purpose of using the following media are:

•Maconkey agar has crystal violet the enables the inhibition of any gram-positive bacteria along with the presence of lactose that helps us to distinguish between the existence of any lactose or non-lactose fermenter exhibited by the change of color of the media. Organisms like E. coli sp. will give pink colonies, with a matte finish.

•Eosin methylene blue (EMB), this media has the inhibiting ingredient to prevent any growth if gram-positive bacteria. In addition to it, it is designed to seek the significant appearance of nucleated colonies representing lactose fermentation. For instance, E. coli sp.will show green metallic sheen in this media.

•Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent when comes in contact with the bacterial cell, causes the release of nitrogen and phosphorous which in turn has denaturing effects on membrane proteins of the bacterial cell. It exhibits inhibitory actions on a wide variety of microorganisms including Pseudomonas species other than Pseudomonas aeruginosa.

•Hi-chrome UTI agar, the purpose behind using this agar was too confirm the organisms since the media has chromogenic substrates that the organism uses to give a discrete colony color.

2.4.2 Biochemical Tests:

A series of biochemical tests were done such as Gram staining, catalase, oxidase, methyl-red (MR), Vogues Proskauer (VP), indole, citrate, triple sugar iron (TSI), motility indole urease (MIU). All of the mentioned tests were followed as described by Buxton and Fraser (1977), Merchant and Packer (1967) and OIE (2004).

2.4.2.1 Methyl- Red (MR) test:

•Five ml of dextrose phosphate (MR-VP) broth were prepared for the forty-one test tubes, representing the individual forty isolates.

•Next, the test tubes were labeled according to the isolate being tested.

•All the forty isolates that were to be tested were inoculated into the broth and then incubated at 37 degrees Celsius for 24 hours, along with a control.

•After the incubation period was over, five drops of methyl red indicator were added to the forty test tubes and also to the monitor, for comparison.

•The appearance of red color confirmed the test to be positive, and the presence of orange meant negative result.

2.4.2.2 Vogues-Proskauer (VP) test:

•Five ml of dextrose phosphate was freshly prepared for forty-one test tubes, representing the forty isolates and one control

•Next, the test tubes were labeled according to the forty strains and also, a command was labeled too.

•All the forty isolates that were to be tested were inoculated into the broth and then incubated at 37 degrees Celsius, alongside the control.

•After the incubation period was over, we added ten drops of Barritt‟s reagent A, immediately followed by Barritt‟s reagent B. After the addition of the two reagents, the test tubes are agitated.

•The test tubes are shaken within regular intervals of 3-4 minutes for the time span of 15 minutes.

•Development of rose pink color will confirm a positive result, whereas, no color change will indicate the result to be negative.

2.4.2.3 Triple sugar iron (TSI) test:

•Seven ml of TSI media was freshly prepared for forty –one test tubes, including a control. During the solidification of the media, the test tubes were kept in a slant position.

•Next, all the test tubes were labeled according to the isolates.

•A small inoculum was taken from a pure colony of nutrient agar and then stabbed to the butt of the media and slowly streaking on the slant. With all these being done, the inoculated test tubes were sent for incubation at 37 degrees Celsius for 24-48 hours.

•With the incubation period being over, the test tubes were taken out of the incubator, and the color change of the media was observed and compared with the control.

•If the media turned yellow (butt and slant), this would indicate that all the three sugars (dextrose, sucrose, and lactose) were fermented by the organism and proved to become acidic. In addition to it, if we witness any cracks in the media, then that would mean the production of gas like carbon dioxide. However, any black colored precipitation would mean the production of hydrogen sulfide.

•On the contrary, if the organism fails to ferment any of the three sugars, then the media remains red and alkaline (butt and slant).

2.4.2.4 Citrate test:

•3ml of fresh citrate media were prepared for forty-one vials, including a control. After the media was taken out of the autoclave, it was positioned in a slanted way.

•All the vials were labeled according to the forty-one isolates.

•A small inoculum was stabbed to the butt of the citrate media for forty vials. All the inoculated forty vials and the control was sent for incubation of 24 hours at 37 degrees Celsius.

•Over these 24 hours, if the color of the media turned from green to blue, it will indicate a positive result. If no certain change in color is to be observed, then that means a negative result.

2.4.2.5 Indole test:

•5 ml solution of sodium chloride and peptone was freshly prepared for forty-one test tubes, including a control.

•All the test tubes were labeled correctly in accordance to the forty isolates and control.

•A small inoculum of bacteria was inoculated into the broth in forty test tubes, and control was kept. Followed by a period of incubation of 24- 48 hours at 37 degrees Celsius.

•At the end of the incubation period, five drops of Kovac‟s reagent must be added. After the addition, we wait for the emergence of a ring.

•If there is the appearance of pink color then it means, the result is positive. If no such color emerges, the result is said to be negative.

3.4.2.6 Catalase test:

•Some autoclaved slides were taken and were labeled according to the forty isolates.

•A drop of catalase reagent, 3% hydrogen peroxide was added to the slide. Followed by a small inoculum taken from nutrient agar is smeared on the slide with the reagent added, beforehand.

•Presence of bubbles, within a few seconds, indicates the positive result.

•The same procedure was repeated for the rest of the isolates.

2.4.2.7 Motility Urease indole test (MIU):

•3ml of the MIU media base was freshly prepared, followed by the addition of urease solution to it in forty- one vial, inclusive of a control.

•All the vials were correctly labeled in accordance to the forty isolates and control.

•With stern concentration, using an inoculating needle, an inoculum is taken and very carefully stabbed into the media, to avoid any misplaced stabbing. Afterward, the vials are sent for incubation for 24- 48 hours.

•Followed by the incubation period, the vials are sought for any color changes. Since it is a test for three components, we first observe the color change, if the media turns pink, it indicates a positive result for urease; any sign of turbidity indicates motility test being positive and lastly, with all these being done we again add five drops of Kovac‟s reagent in search of any pink colored ring.

2.4.2.8 Gram staining:

•Five ml of saline were freshly prepared with the intention of formulating the smear.

•We took a loopful of saline, smeared it on the slides and then again, we took small inoculum of the isolate to the saline and then heat fixed it.

•Afterward, we added crystal violet and after minutes washed it.

•Followed by the addition of mordant which also stayed on the smear for a minute.

•Next, we added ethanol or acetone and kept it for 15 seconds.

•Lastly, we add safranin.

•And observe the slides under the microscope with lens 10x, 40x and 100x lens.

•Under the microscope, any signs of purple colored cells will be considered gram-positive bacteria and pink or red colored cells will be considered to be gram-negative bacteria.

2.5 Methods for the detection of antibacterial activity:

•Forty test tubes of 5ml saline were freshly prepared by each of the sample, with the purpose of standardizing the sample.

•Followed by the inoculating of the sample with each of the samples. This was done by using inoculating loops and then picking one or two colonies from the subculture plate.

•The test tubes were later vortexed to create a smooth suspension.

2..5.1 Comparison with the McFarland solution:

This solution is one the quintessential part of any test concerning the antibacterial sensitivity. This solution is anticipated to serve as the reference to adjust the turbidity of any given bacterial suspension. The purpose behind it is to ensure the uniformity of the number of bacteria within a range to standardize the microbial testing.

This action will also enable us to prevent any errors in results since any chance of too much dilution or the suspension being too heavy would cause a significant error in the test.

Afterward, the bacterial suspension is compared to the commercially produced McFarland solution 2(for detection of inhibition rate) and McFarland solution 0.5 (for detection of the zone of inhibition by agar disc/well diffusion method)

It is usually gauged that if a bacterial suspension matches with McFarland solution 2, it is supposed to contain 6×10^8 colonies per ml. Moreover, a bacterial suspension matching with McFarland solution 0.5 supposedly has 1.5×10^8 colonies per ml.

2.5.2 Mueller- Hinton agar (MHA):

For any antimicrobial activity test, Mueller-Hinton agar has been used for ages. Mainly because it is a non-selective, non-differential medium. This means that almost all organisms plated on here will grow. It contains starch. Starch is known to absorb toxins released from bacteria so that they cannot interfere with the antibiotics. It also mediates the rate of diffusion of the antibiotics through the agar. It is a loose agar. This allows for better diffusion of the antibiotics than most other plates. A better diffusion leads to a more right zone of inhibition. MHA shows acceptable batch-to-batch reproducibility for susceptibility testing. MHA is low in sulfonamide, trimethoprim, and tetracycline inhibitors (i.e., the concentration of inhibitors thymidine and thymine is low in MHA). Both the para-aminobenzoic acid (PABA) and thymine/thymidine content in Mueller Hinton Agar is reduced to a minimum, thus markedly reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antibiotics.

2.5.3 Selection of antibiotics:

For designing the antibiogram test to observe the antibiotic resistance and susceptibility, 11 antibiotics were chosen. They are:

- Amoxicillin
- Amoxyclav
- Aztreonam
- Colistin
- Cefepime
- Ceftriazone
- Cefixime
- Cefotaxime
- Gentamycin
- Imipenem
- Levofloxacin
- Sulfamethoazole

2.5.4 Preparation of the lawn culture of bacterial suspension on MHA media:

•Autoclaved cotton swabs were considered and then dipped into the bacterial suspensions, and the test organisms were taken from a 24 hours‟ nutrient media culture plate.

•After the dip, the swab was pressed against the wall of the test tube, to discard any excess fluid.

•The swab was then swiped multiple times, in different angles to achieve uniform distribution of the bacteria.

•The plate was rimmed with the swab to pick up any excess liquids.

•Leaving the lid slightly ajar, the plate was allowed to soak and the suspension for 3-4 minutes before placing the antibiotic.

2.5.5 Placement of antibiotic disc on the lawn:

•The discs were placed very carefully, using tweezers. The tweezers were at first dipped in alcohol and the burnt.

•The discs were gently placed on the lawn and then pressed against it, ensuring complete contact with the lawn.

•A total of forty lawns were made and labeled accordingly.

•Once all the discs were placed, the plate was covered by the lid and was not inverted. The plates were then sent for incubation for 24 hours at 37 degrees Celsius.

2..5.6 Measurement of the zone of inhibition:

•After the incubation period, the zones were observed and measured cautiously.

•The measurements were taken by a centimeter graduated ruler. The ruler was placed against the back of the plate, where the zone was visible enough, and the diameter was measured.

•After the measurements were taken, the results were recorded and converted into millimeters.

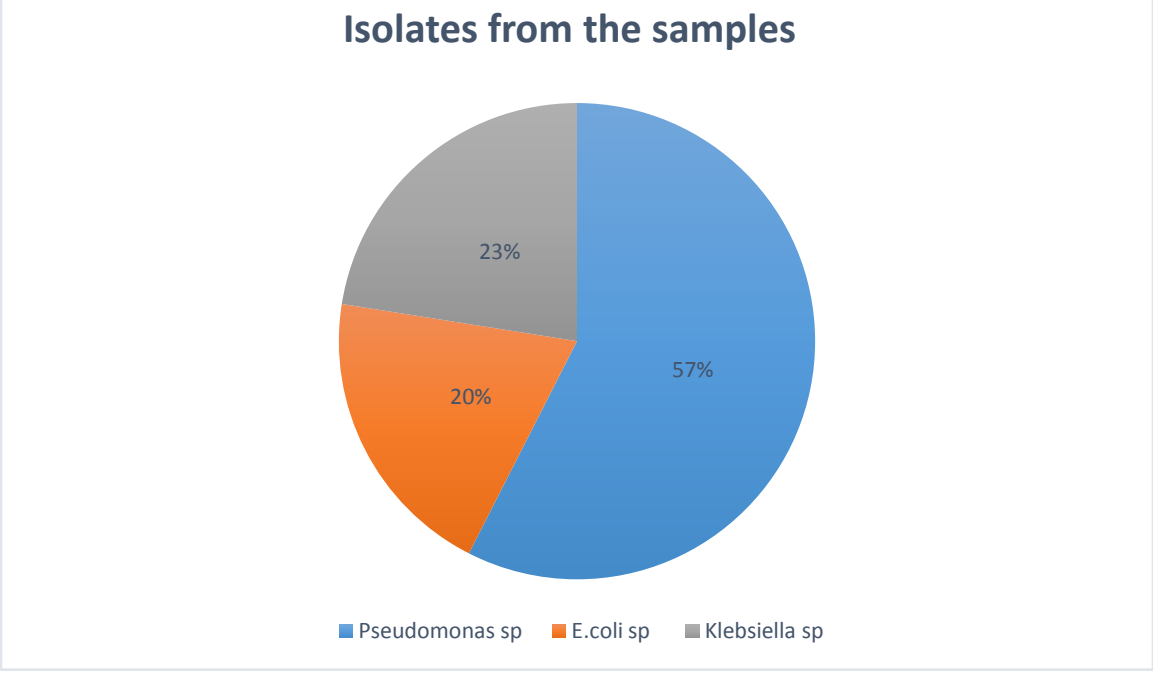
•Followed by the comparison with antibiotic susceptibility chart.

Chapter 3
Results

3.1 Growth on Nutrient Agar

In the beginning, all the samples were transferred on a media, in order to achieve pure colonies for the next coming tests like: selective culture media, biochemical tests and lastly antimicrobial susceptibility test.

After the collection from the patients of intensive care unit, we had spread plated the 61 samples on a nutrient agar. Furthermore, random colonies were selected based on their colony characteristics and then again, cultured separately on nutrient agar, with the purpose of achieving pure colony isolates. Finally, we were able to separate forty pure colony isolates from the 61 clinical specimens



3.2 Growth on selective Media:

3.2.1 Maconkey agar:

For the first trial, we selected Maconkey agar to conduct this test for the forty pure colony isolates. The results seemed to be mixture of growth. 23 of the isolates produced mucoid surfaced, green colonies as a result, indicating the presence of *Pseudomonas sp*. Moreover, eight isolates gave pink colored colonies with darker pink shade on the periphery, sort of confirming the presence of *E. coli sp*. Lastly, 9 of the isolates produced pink mucoid colonies, specifying the culture to be of *Klebsiella sp.*

3.2.2 Eosin methylene Blue (EMB):

After getting over with Maconkey agar, we shifted to EMB media. The forty pure colonies were cultured on this media. The results, to a great extent sustains with the result given by Maconkey agar. A characteristic green metallic sheen was witnessed in eight of the samples, thoroughly confirming the growth to be of *E. coli's*. Next, there were nine isolates giving purple mucoid colonies on EMB, though this not confirmed yet to be *Klebsiellasp*, however gives us an idea of it. At last, there were a major number of results, to be precise 23 of them giving absolutely transparent colonies on the media, giving an inkling of the organism being *Pseudomonassp*, however more tests were yet to be performed for more solid confirmation.

3.2.3 Cetrimide :

All the previous selective media were prepared for the search of gram- negative lactose fermenters. Next, we selected the selective media cetrimide. As the incubation period was over for the forty samples, it was witnessed that 23 of the pure isolates gave bright green growth on the cetrimide agar, these were exactly the 23 isolates that previously were thought to be

Pseudomonas sp. With this test, we get a confirmation of them being *Pseudomonassp*. Unlike the other selective media, the rest 17 did not produce any growth on this media.

3.2.4 Hi-Chrome Agar:

In order to confirm the identities in the culture media manner, we decided to culture all of the forty samples on Hi-Chrome Agar, since hi-chrome will give colony colors that specify organisms. Followed by the end of the incubation period, we got to observe nine isolates gave blue- green mucoid colonies, eight of the isolated produced small circular pink colonies and 23 of the isolates gave deep green colonies; more likely to be a colorless colony with green pigment, the following result once again validated our previous conclusions of the organisms to be *Klebsiellasp*, *E. colisp*and *Pseudomonasp.*

Table 1 results on selective media

Isolates	Appearance on maconkey	Appearance on EMB	Appearance on Cetrimide	Appearance on Hi-Chrome
P01	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P02	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P03	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P04	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P05	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P06	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P07	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P08	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P09	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P10	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P11	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P12	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P13	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P14	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P15	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P16	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P17	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P18	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P19	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P20	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P21	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P22	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
P23	Colorless with green pigment	Colorless colonies	Bright green colonies	Green colonies
K01	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K02	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K03	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K04	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K05	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K06	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K07	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K08	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies
K09	Pink ; mucoid colonies	Purple ; mucoid colonies	-	Blue-green colonies

E01	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E02	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E03	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E04	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E05	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E06	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E07	Pink ; matt colonies	Green metallic sheen	-	Pink colonies
E08	Pink ; matt colonies	Green metallic sheen	-	Pink colonies

3.3 Biochemical tests:

3.3.1 Catalase Test:

To begin with the biochemical tests, we at first performed the catalase test. This catalase test was done for the forty pure isolates. With the addition of 3% hydrogen peroxide, this test effectively produced immediate bubbles. After all the isolates were treated with hydrogen peroxide, it was seen that all of the forty isolates gave positive result for the test.

3.3.2 Triple sugar iron (TSI) test:

For many organisms, they are expected to ferment certain sugars like sucrose, lactose and dextrose in addition to it, they are also expected to produce certain gases such as carbon dioxide and hydrogen sulfide. For this test, all of the forty isolates were stabbed at the bottom of the test tube and streaked along the slant. After the incubation period, 23 of the samples simply changed the colour of the media from orange to red (both the slant and butt); with no production of gases as a result no cracking of the media. Furthermore, 17 of the isolates produced yellow slants and yellow, in fact the 17 isolates cracked the media, confirming the production of carbon dioxide.

3.3 Vogues-Proskauer:

Next, we went for the traditional imVic test, one of the tests comprising the ImVic test, is Vogues Proskauer. The reuslts for this test is considered to be postive if the solution turns the transparent into pink colour solutions; if negative, then the solution will be as it was before. We performed the test for the forty isolates, nine of them gave positive result hence, pink colour was observed. However, 31 of them failed to change the colour, therefore, a negative result was considered.

3.3.4 Methyl-Red test:

The subsequent test, then is methyl red, which is one of the ImVic test. After the indicator is added, the test provides with two results of two different colors and they are: red for positive and

yellow for negative result. 8 of the isolates, out of forty gave positive results followed by, 32 of the isolates giving negative results.

3.3.5 Indole Test:

In the succeeding indole test, a pink ring reflects positive result and yellow ring endorses negative result. While performing these tests, it was seen to give a mixture of both positive and negative results. In fact, 32 of them gave negative results and only eight of them gave pink ring, inferring it to be positive results.

3.3.6 Citrate Test:

The results of the citrate test are said to be positive, if the color of the media turns to blue from green, if there is no change then, the result is said to be negative. In this case, the test was performed for forty isolates. Out of them, 8 of them produced negative results and the rest 32 gave positive results.

3.3.7 Motility Urease Test (MIU):

This test was done to investigate to check for urease, motility and indole. As the indole was done before, this test focuses only on urease and motility. The results for urease is checked by the color change of the media, since the media is orange in it non-inoculated form, so if the organism has the enzyme urease then the media will turn pink and if no such activity occurs, then the media will turn yellow. Since it is a dual test, the other test being motility is judged on the basis of the media becoming turbid. If the media is turbid, the organism is said to be motile, otherwise not. In this case, there were nine isolates which showed turbidity however turned the media to pink color, inferring it to be only urease positive and non-motile. Next, 31 of them showed motility but turned the media to yellow, proving that they were urease negative but motile organisms.

Table 2 Results from the biochemical tests:

Isolates	MR-VP		Catalse	Citrate	MIU			TSI						Gram staining	Organism
	MR	VP	+/-	Blue/green	motility	Urease	Indole	Sucrose	Glucose	Lactose	Gas production	H_2S production	Slant/butt	color	
P01	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P02	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P03	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P04	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P05	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P06	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P07	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P08	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P09	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P10	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P11	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P12	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P13	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P14	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P15	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P16	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P17	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P18	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P19	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P20	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P21	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P22	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
P23	-	-	+	Blue	+	-	-	-	-	-	-	-	R/R	Pink	*Pseudomonas sp.*
K01	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K02	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K03	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K04	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K05	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K06	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K07	-	+	+	Blue	-	+	-	+-	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K08	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*Klebsiella sp.*
K09	-	+	+	Blue	-	+	-	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E01	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E02	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E03	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E04	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E05	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E06	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*

E07	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*
E08	+	-	+	Green	+	-	+	+	+	+	+	-	Y/Y	Pink	*E.coli sp.*

3.4 Antimicrobial Susceptibility Test:

Biochemical tests suggest that the isolates were *Klebsiellasp*, *Pseudomonassp* and *E.colisp*. With these results, we started antimicrobial sensitivity test. As mentioned before, we selected 11 antibiotics for each of the isolates

Table 3 Antimicrobial susceptibility test result for the isolates of *Klebsiella sp.*

	Name of the antibiotics																			
	Amoxicillin		amoxyclav		Aztreonam		Colistin		Cefepime		Cefexime		Imipenem		Gentamycin		Ceftriazone		Levofloxacin		cefotazime	
Isolate	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I
K01	0	R	2	R	11	R	18	S	11	R	3	R	4	R	15	S	7	R	0	R	2	R
K02	0	R	5	R	7	R	15	S	2	R	0	R	1	R	15	S	7	R	0	R	2	R
K03	4	R	6	R	10	R	15	S	4	R	3	R	1	R	15	S	2	R	3	R	3	R
K04	4	R	2	R	8	R	14	S	2	R	2	R	3	R	14	S	3	R	2	R	2	R
K05	6	R	1	R	9	R	20	S	8	R	5	R	5	R	18	S	5	R	0	R	0	R
K06	7	R	4	R	10	R	18	S	0	R	0	R	4	R	20	S	0	R	0	R	0	R
K07	3	R	7	R	13	R	17	S	9	R	0	R	2	R	16	S	0	R	1	R	3	R
K08	4	R	1	R	8	R	13	S	1	R	0	R	1	R	18	S	0	R	5	R	2	R
K09	4	R	1	R	13	R	18	S	13	R	8	R	5	R	18	S	0	R	2	R	1	R

4.4.1 All of the isolates of *Klebsiellasp* showed resistance to nine antibiotics; amoxicillin, amoxyclav, aztreonam, cefepime, cefexime, imipenem, ceftriazone, levofloxacin and cefotazime. Here, 9 out of 11 antibiotics were ineffective against all the isolates of *Klebsiella sp*. And, all the isolates of *Klebsiellasp* showed sensitivity to two antibiotics; gentamycin and colistin. Therefore, 2out of 11 antibiotics were effective against all the isolates of *Klebsiella sp*.

Table 4 Antimicrobial susceptibility test for the isolates of *Pseudomonas sp.*

	Name of the antibiotics																					
	Amoxicillin		amoxyclav		Aztreonam		Colistin		Cefepime		Cefexime		Imipenem		Gentamycin		Ceftriazone		Levofloxacin		cefotazime	
Isolate	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I
P01	0	R	5	R	21	S	23	S	18	S	9	R	3	R	15	S	13	R	1	R	10	R
P02	0	R	0	R	31	S	30	S	23	S	3	R	4	R	16	S	11	R	12	R	13	R
P03	1	R	10	R	30	S	21	S	22	S	1	R	5	R	20	S	8	R	0	R	11	R
P04	5	R	10	R	25	S	19	S	22	S	1	R	5	R	17	S	12	R	9	R	7	R
P05	0	R	1	R	27	S	23	S	19	S	12	R	5	R	19	S	7	R	5	R	9	R
P06	2	R	8	R	21	S	12	S	21	S	2	R	1	R	19	S	10	R	2	R	7	R
P07	0	R	5	R	32	S	20	S	24	S	11	R	2	R	18	S	10	R	2	R	11	R
P08	4	R	2	R	28	S	30	S	21	S	4	R	0	R	15	S	8	R	6	R	12	R
P09	3	R	5	R	28	S	15	S	18	S	10	R	0	R	19	S	9	R	5	R	9	R
P10	2	R	6	R	22	S	12	S	19	S	1	R	5	R	20	S	8	R	1	R	7	R
P11	5	R	8	R	27	S	14	S	20	S	10	R	2	R	18	S	11	R	0	R	8	R
P12	4	R	3	R	25	S	18	S	30	S	9	R	1	R	15	S	8	R	12	R	13	R
P13	4	R	5	R	23	S	18	S	28	S	8	R	3	R	22	S	10	R	5	R	10	R
P14	3	R	0	R	23	S	30	S	17	S	1	R	4	R	17	S	13	R	8	R	8	R
P15	5	R	9	R	28	S	24	S	14	S	1	R	4	R	19	S	4	R	9	R	7	R
P16	5	R	3	R	22	S	26	S	23	S	12	R	0	R	22	S	5	R	5	R	5	R
P17	2	R	4	R	13	S	21	S	20	S	6	R	5	R	15	S	12	R	6	R	2	R
P18	5	R	2	R	16	S	19	S	22	S	7	R	3	R	17	S	8	R	8	R	0	R
P19	4	R	7	R	22	S	31	S	17	S	10	R	1	R	22	S	7	R	15	R	0	R
P20	1	R	8	R	13	S	29	S	21	S	12	R	1	R	18	S	2	R	15	R	1	R
P21	2	R	7	R	16	S	14	S	11	S	1	R	0	R	24	S	0	R	6	R	9	R
P22	4	R	10	R	22	S	22	S	16	S	7	R	0	R	22	S	1	R	13	R	0	R
P23	1	R	1	R	11	S	30	S	20	S	8	R	0	R	22	S	11	R	12	R	0	R

4.4.2 All the isolates of *Pseudomonassp*showed resistance to the antibiotics; amoxicillin, amoxyclav, cefexime, imipenem, ceftriazone, levofloxacin and cefotazime. Here, 4 (64.64%) out of 11 antibiotics were ineffective against all the isolates of *Pseudomonas sp*. In contrast, all the isolates of *Pseudomonassp* showed sensitivity to aztreonam, colistin, cefepime and gentamycin. Here,4 (36.36%) out of 11 antibiotics were effective against all the isolates of *Pseudomonas sp.*

Table 5 Antimicrobial susceptibility test for the isolates of*E.coli sp.*

	Name of the antibiotics																					
	Amoxicillin		Amoxyclav		Aztreonam		Colistin		Cefepime		Cefexime		Imipenem		Gentamycin		Ceftriazone		Sulfamethoazole		cefotazime	
Isolate	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I	ZOI(mm)	I
E01	0	R	26	S	15	S	19	S	23	S	28	S	0	R	1	R	0	R	21	S	8	R
E02	4	R	22	S	23	S	15	S	22	S	28	S	0	R	2	R	0	R	24	S	10	R
E03	2	R	24	S	20	S	13	S	30	S	23	S	5	R	1	R	0	R	19	S	11	R
E04	04	R	22	S	20	S	20	S	23	S	21	S	1	R	4	R	2	R	30	S	4	R
E05	0	R	25	S	30	S	22	S	25	S	22	S	2	R	4	R	1	R	20	S	5	R
E06	1	R	27	S	20	S	27	S	26	S	22	S	2	R	1	R	1	R	23	S	1	R
E07	1	R	25	S	22	S	30	S	21	S	22	S	5	R	1	R	1	R	30	S	0	R
E08	0	R	22	S	30	S	20	S	20	S	24	S	1	R	1	R	2	R	30	S	0	R

4.4.3 All the isolates of *E.coli* showed resistance to five antibiotics; gentamycin, amoxicillin, imipenem, ceftriazone and cefotazime. Therefore, 5 (45.46%) out of 11 antibiotics were ineffective against all the isolates of *E.coli sp*. On the other hand, all the isolates of *E.colisp*showed sensitivity to six antibiotics; aztreonam, colistin, cefepime, cefexime, sulfamethoazole and amoxyclav. Hence, six (54.55%) out 11 antibiotics were effective against all of the isolates of *E.coli sp.*

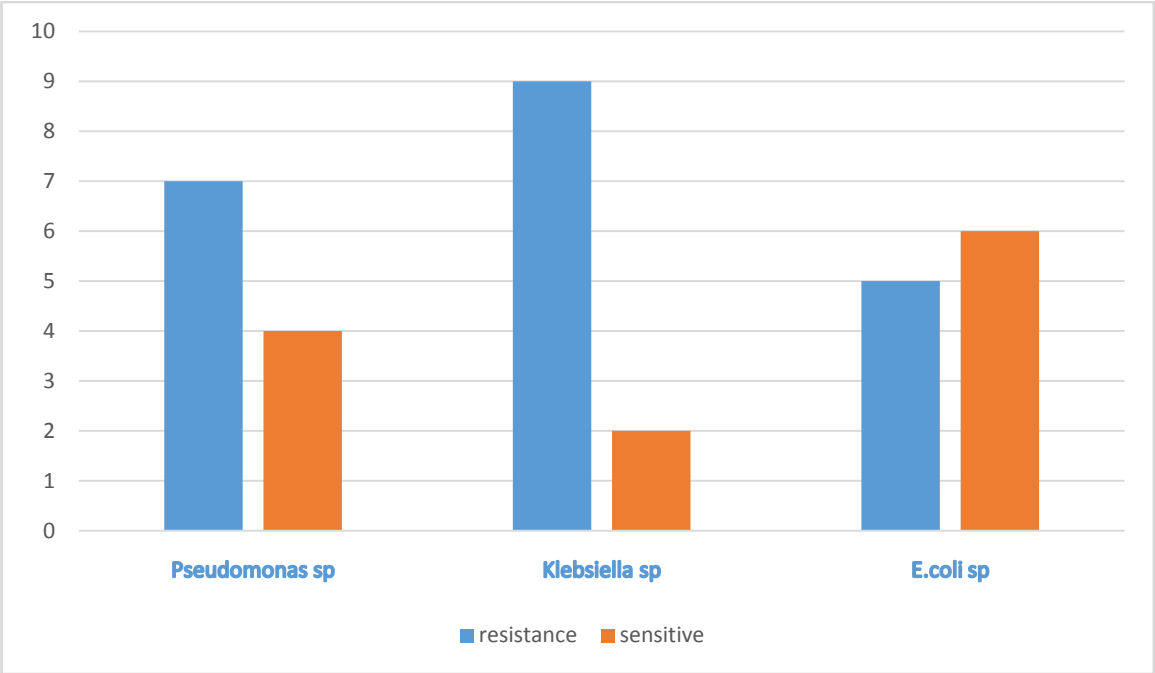


Figure 1 shows the antibiotic susceptibility pattern of the three organisms

Chapter 4
Discussions

Discussions:

In the present study microbiological status and their antimicrobial sensitivity test were performed on the intensive care unit patients of UttaraAdhunikMedical College and Hospital. A total of 40 isolates were grown multiple selective culture media steps followed by various biochemical tests to confirm the identities. The study consists of 23 strains of *Pseudomonas sp*, nine isolates of *Klebsiellasp* and eight isolates of *E. colisp*.

The antimicrobial susceptibility test for the forty isolates as performed by Kirby-Bauer disk diffusion method. For the study, 11 antibiotics were selected for the strains of each of the organisms. The selection of antibiotics was based on the profile of antimicrobial susceptibility.

For the 23 isolates of *Pseudomonassp*, 11 antibiotics were selected and were amoxicillin, amoxyclav, aztreonam, colistin, cefepime, cefexime, ceftriazone, cefotazime, imipenem, levofloxacin and gentamycin. Furthermore, All the isolates of Pseudomonas sp showed resistance to the antibiotics; amoxicillin, amoxyclav, cefexime, imipenem, ceftriazone, levofloxacin and cefotazime. Here, 4 out of 11 antibiotics were ineffective against all the isolates of *Pseudomonassp*. In contrast, all the isolates of *Pseudomonas sp*showed sensitivity to aztreonam, colistin, cefepime, and gentamycin. Here, 7 out of 11 antibiotics were effective against all the isolates of *Pseudomonassp*.

Pseudomonasaeruginosa continues to be a significant cause of infections, in part because of the intrinsic property to antibiotics (Hancock &Speert, 1990). From 1993 to 2002, general increases in antimicrobial resistance were most significant for ciprofloxacin, imipenem, tobramycin, and aztreonam. Rates of multidrug resistance (resistance to ≥3 of the following drugs: ceftazidime ciprofloxacin, tobramycin, and imipenem) increased from 4% in 1993 to 14% in 2002(Obritsch et al., 2004).

In contrast to this, all of the isolates of *Klebsiellasp* showed resistance to nine antibiotics; amoxicillin, amoxyclav, aztreonam, cefepime, cefexime, imipenem, ceftriazone, levofloxacin, and cefotazime. Here, 9 out of 11 antibiotics were ineffective against all the isolates of *Klebsiellasp*. And, all the strains of *Klebsiellasp* showed sensitivity to two antibiotics; gentamycin and colistin. Therefore, 2out of 11 antibiotics were effective against all the isolates of *Klebsiellasp*.

The prevalence of multidrug resistance, defined as resistance to at least one extended-spectrum cephalosporin, one aminoglycoside, and ciprofloxacin, increased substantially among ICU isolates of *Acinetobactersp*, *P.aeroginosa*, *K.pneumonia* and *E.cloacae*. (Lockhart et al., 2007).

On the contrary, All the isolates of *E.colisp* showed resistance to five antibiotics; gentamycin, amoxicillin, imipenem, ceftriazone, and cefotazime. Therefore, 5 out of 11 antibiotics were ineffective against all the isolates of *E.colisp*. On the other hand, all the strains of *E.colisp* showed sensitivity to six antibiotics; aztreonam, colistin, cefepime, cefexime, sulfamethoazole, and amoxyclav. Hence, six out of 11 antibiotics were effective against all of the isolates of *E.coli sp*.
More than 20% of patients admitted to European intensive care units (ICUs) develop an ICU-acquired infection. A high prevalence of decreased antibiotic susceptibility among gram-negative bacilli has been reported from ICU patients in France, Belgium, Germany and the Netherlands during 1990 and 1991 in the United States between 1990 and 1993, and Belgium and Sweden during 1994 and 1995 (Hanberger et al., 1999). The high incidence of reduced antibiotic susceptibility among gram-negative bacteria in these ICUs suggests that more effective strategies are needed to control the selection and spread of resistant organisms.

Pseudomonas sp, which just used to be a causative agent of a burn wound has now become a leading reason behind of nosocomial infection. And of course, over the years of mutation, it has also acquired resistance against valuable antibiotic, which in this study has clearly been proven and validated.

The next soon-to-be antibiotic resistant bacteria is going to *Klebsiellasp* species, undeniably. With its high resistant profile, it is a very unfortunate thing to say that soon people will be infected and even face death, if the proper measures are not taken.

The enteric organism *E.colisp* one of the most quintessential bacteria of the ecosystem might soon revolt against the antibiotic that will be used in the form of treatment. If not in the coming days but soon; as per my study.

In a country like Bangladesh, immediate measures must be taken alongside the treatment facilities. Since we have just begun to grip the economic stability, a sudden blow comprising of these will leave the country shattered.

Conclusion:

The illustrious invention, also known as “antibiotic” during the 20th century has given us the power to eliminate the most concerning organism, bacteria; this organism is threat to the mankind from its discovery and with antibiotic on our side, we could have easily eradicated it. However, over the years, no such thing happened, on the contrary, the exact opposite scenario is taking its shape.

That day is not far when the once eradicated bacteria will re-emerge and the mankind will have nothing to fight against it. The singular reason behind this turmoil is the pristine ignorance of prescribing antibiotic in a futile and unnecessary manner. In the hope of making antibiotic abundant in order to help the ailing people, it has turned into a curse.

References:

- Obritsch, M., MacLaren, R., &jung, R. (2003). National Surveillance of Antimicrobial Resistance in Pseudomonas aeruginosa Isolates Obtained from Intensive Care Unit Patients from 1993 to 2002. doi: DOI: 10.1128/AAC

- Hanberger, H., Garcia-Rodriguez,, J., &Gobernado, M. (1999),Antibiotic Susceptibility Among Aerobic Gram-negative Bacilli in Intensive Care Units in 5 European Countries, 281(1):67-71

- Hanberger, H., Garcia-Rodriguez,, J., &Gobernado, M. (1999). Antibiotic Susceptibility Among Aerobic Gram-negative Bacilli in Intensive Care Units in 5 European Countries,289(7):885-888

- Finch,R.G.,Greenwood,D.,Norrby S.R & Whitley R.(2003) Antibiotic and Chemotherapy, 8th edn.ChurchillLivingstone,London.

- Bruce,J., MacKenzie, F.,Cookson, B.,et al(2009) Antibiotic Stewardship and Consumption:*findings from a pan-European hospital study*. *J Antimicrobchemother,***64**, 853-860.

- Dellit, T.H.,Owens,R.C.,McGowan,J.E.Jr,*et al(*2007)Infectious Diseases Society for Healthcare Epidemiology of America Guidelines for Developing an Instituitional Program to Enhance Antimicrobial Stewardship. *Clin Infect Dis*, 44, 159-177

- Satter S, Mahbub H, ShamsuzzamanSM.,(2018) Antibiotic Resistance Pattern and Prevalence of Aminoglycoside-Modifying Enzymes in *Escherichia Coli* and *Klebsiella*species Isolated from a Tertiary Care Hospital in Bangladesh.,27(3):561-566

- Quinn, J., Shlaes, D., Fine, M., Chow, J., & Hooper, D. (1991). Enterobacter Bacteremia: Clinical Features and Emergence of Antibiotic Resistance during Therapy. Annals Of Internal Medicine Logo.,115(8):585-590.

Appendix

Appendix 1
Media Composition

Nutrient Agar

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

Muller Hilton Agar

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

Physiological saline

Component	Amount (g/L)
Sodium Chloride	9.0