PREVALENCE OF CARBAPENEM RESISTANCE IN *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES OBTAINED FROM VARIOUS CLINICAL SAMPLES.

Internship Training Report

Submitted to

Lovely Professional University, Punjab

In partial fulfilment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
Jaspreet Kaur
(Registration No. 11411262)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

PREVALENCE OF CARBAPENEM RESISTANCE IN *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES OBTAINED FROM VARIOUS CLINICAL SAMPLES.

Training Report

Internship Training Report

Submitted to

Lovely Professional University, Punjab

In partial fulfilment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
Jaspreet Kaur
(Registration No. 11411262)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

DECLARATION

I hereby declare that the work embodied in this internship report was carried by Mrs. Jaspreet Kaur bearing Registration Number-11411262 under the supervision of Dr. Anania Arjuna, Assistant professor, Lovely Professional University, Punjab and Dr. Jaspal Kaur, Associate professor, Punjab Institute of Medical Sciences, Jalandhar. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Jaspreet Kaur
Date: May 2016
Place: Lovely Professional Universit

PREVALENCE OF CARBAPENEM RESISTANCE IN *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES OBTAINED FROM VARIOUS CLINICAL SAMPLES.

CERTIFICATE

This is to certify that **Mrs. Jaspreet Kaur** bearing **Registration Number11411262** has completed her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Jaspal Kaur

Dr. Jaspal Kaur
Associate Professor

Department of Microbiology
Punjab Institute of Medical Sciences, Jalandhar

Anania Arjuna 12520

Dr. Anania Arjuna
Assistant Professor
Lovely School of Physiotherapy and Paramedical Sciences
Lovely Professional University, Punjab

ABSTRACT

Carbapenems are drugs which are employed in severe infections not treatable by other classes of drugs due to development of resistance. But nowadays, the bacteria especially members of family *Enterobacteriaceae* for example *K. pneumoniae* and *E. coli* are also rising resistance against Carbapenems. The current study was conducted to check the prevalence of Carbapenems resistant strains among *K. pneumoniae* and *E. coli*. The study deals with *K. pneumoniae* and *E.coli* obtained by standard techniques from different clinical samples like urine, pus, sputum, high vaginal swab, wound swab, tissue, blood from different wards of the hospital. Antibiotic susceptibility testing was done on Mueller Hinton agar following CLSI guidelines. The organisms confirmed as Meropenem resistant and Imipenem resistant were further tested for Amp C production and Carbapenemase production by Amp C test ad Modified Hodge Test. A total of 213 samples were surveyed out of which 142 samples yielded *K. pneumoniae* and *E.coli* were most common in pus (26%) and urine (65.3%) respectively. Likewise, they were most common in ICU (24%) and obstetric gynae (30.5%) wards respectively. 49.4% of *E. coli* came out to be resistant to Meropenem and 12.63% were resistant to imipenem. Similarly, 53.1% of *K. pneumoniae* were found to resistant to Meropenem and 25.5% to imipenem. 61.53% of total isolates were Modified Hodge Test positive and 38.46% were Amp C test positive. *E. coli* ad *K. pneumoniae* are most commonly found bacteria in the hospitals. They show a marked resistance to carbapenems and many are Carbapenemase producing (61.53%) which pose a serious threat to the society. This predicament should be noticed and solved as soon as possible.

ACKNOWLEDGMENT

I would like to offer my special thanks to **Dr. Jaspal Kaur** (Associate professor, Department of Microbiology PIMS, Jalandhar) for her valuable time, constructive suggestions, guidance and continuous encouragement. I am privileged to have my project completed under her supervision. Secondly, I would like to express my great appreciation to my internal guide **Dr. Anania Arjuna** for her constant support, critiques, and precious ideas to improve my report.

Thirdly, my grateful thanks are extended to **Dr. Kailash Chand** (Head of the Microbiology Department), **Dr. Shashi, Dr. Sheevani, Dr. Gomty Mahajan** and **Dr. Shivya Thakur** for their consistent support throughout the training for accomplishment of this report.

Moreover, my sincere gratitude to the technical staff of PIMS hospital for their assistance in every aspect.

Lastly, I would like to appreciate the unsustainable reinforcement provided by my parents and my husband, Mr. Opinderdeep Singh, in every part of this study.

JASPREET KAUR

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1 - INTRODUCTION

Both *Klebsiella pneumoniae* and *Escherichia coli* are two pathogens fitting in with family *Enterobacteriaceae* (70). Although *K. pneumoniae* is typical verdure of mouth, skin and intestine (8) *E. coli* are ordinary flora of lower intestine (3). The Carbapenems are antimicrobial specialists having β - lactam rings. They work like other β-lactam antimicrobial operators, i.e. by hindering cell wall formation. This hindrance is accomplished by tying proteins and inactivating them. Carbapenems are the 'final resort medications' which are steady to a large portion of the β – lactamases (58). Contamination with Carbapenems resistant creatures like *K. pneumoniae* and *E. coli* implies a greater morbidity and mortality. Carbapenems are remarkable on the grounds that they are nearly impervious to hydrolysis by the catalysts β - lactamases which corrupt these medications. They have an extraordinary property to restrain β - lactamases alongside their antibacterial ability. The main Carbapenem was Thienamycin which indicated most noteworthy antimicrobial action of early assessed Carbapenems (1, 6). Carbapenems have a positive part in treating multi drug resistant Gram negative bacilli. However, these days, situation has been changed. The microscopic organisms, particularly Gram negative microbes having a place with *Enterobacteriaceae* family, are creating resistance against these medications. This resistance is gained by any of the accompanying techniques; by the creation of β - lactamases, by changes in penicillin binding proteins, by changing the basic arrangement of porins (71), and by expanding the outflow of efflux pumps (58) situated in cell wall. Resistance can be extremely increased in certain bacterial species in case of combination of these mechanisms for example, *K. pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii* (5, 12, 46). Genes for beta-lactamases are normally plasmid encoded. Carbapenemase goes under three classes of b-lactamases catalysts; A, B, or D on the premise of their atomic structure (46). The most widely recognized wellspring of resistance found in *Enterobacteriaceae* family is generation of *K. pneumoniae* Carbapenemase (KPC). Among carbapenemase delivering *Enterobacteriaceae* individuals, KPC is generally found in *K. pneumoniae* isolates (17).

Carbapenems which are clinically utilized by physicians as of now incorporate Imipenem, Doripenem, Meropenem, Imipenem-cilastatin, Ertapenem, Panipenem-Betamapiron and Biapenem (58). For youngsters, Imipenem-cilastatin, Meropenem and Ertapenem are 'food and Drug Administation' affirmed. Meropenem is the main medication among Carbapenems, which is endorsed from FDA (Food and Drug Association) for patients of meningitis including children (23). The colonization of the Carbapenem resistant microscopic organisms or comprehensively, multidrug resistant Gram-negative microorganisms (*K. pneumoniae, E. coli*) have enormously expanded in most recent ten years. The main choices which left behind after advancement of imperviousness Carbapenems are Polymyxine-B and Colistin, which are harmful for human body and are less compelling than Carbapenems (2)

CHAPTER 2 - REVIEW OF LITERATURE

Development of Carbapenem Resistant *Enterobacteriaceae* (CRE) and increasing the rates of resistance have been reported in a number of Asian countries (41, 72, 73).

According to many research papers β-lactamases more specifically, Carbapenemasees in most of the cases are plasmid encoded and also can be incorporated in various mobile genetic elements, as illustration, transposon and integrons (14).

The prevalence of CRE has increased since last decade because of spread of KPC (*K. pneumoniae* Carbapenemase) (63).

In the beginning reports regarding *Klebsiella pneumoniae* Carbapenemase, Carbapenem resistance was considered due to over production of Amp C mediated β-lactamases or ESBLs in the organisms having mutations in porin proteins (10, 16, 45).

But presently, Carbapenemases have become another source of Carbapenem resistance among CRE in US. The commonest Carbapenemase found in United States is KPC. KPC is an ambler molecular class A enzyme, that hydrolyze many β- lactams by using serine at the active site (18).

KPC- producing *Enterobacteriaceae* was first found in a sample from a patient in North Carolina in 2001.[4] Moreover, KPC producing *Enterobacteriaceae* are reported from other parts of the world. Some of them are concerned with the receipt of medical care in the United States, representing the emergence of these organisms worldwide (42).

The Metallo β- lactamases (MBL) belong to Ambler class B. the difference between MBLs and other Carbapenems is the usage of zinc for hydrolysiation at active site (43). the most commonly found MBLs in *Enterobacteriaceae* worldwide until recently were Verona integrin-encoded MBLs and IMPs(active to imipenem). A new MBL was described in 2009, namely New Delhi MBL (NDM).

NDM was first found in a patient from Sweden who had received the treatment in India. The organism in which this Matello-β-lactamase was found was *K. pneumoniae*and in UK it was immediately found to be a rising mechanism of resistance in many species of *Enterobacteriaceae* (44).

Besides Carbapenem resistance due to the production of ESBLs, plasmid encoded Amp C β-lactamases, porin loss (26). The main cause of Carbapenem resistance is Carbapenmases especially KPC and MBLs, for example VIM and IPM (55).

Production and spread of NDM-producing *Enterobacteriaceae* has been reported in India, Pakistan, the UK and other countries (54, 57).

Among Carbapenem resistant *Enterobacteriaceae, E. coli* and *K. pneumoniae* account for the largest proportion. Percentage of Carbapenem resistant *K. Pneumoniae* among *Enterobacteriaceae* is 39.3% and *E. coli* is 22.0% (76).The highest resistance for Carbapenem in *Klebsiella spp*. is not surprising, because of the fact that *K. pneumoniae* is an ill-famed "collector" of multidrug resistance plasmids. *K. pneumoniae* Carbapenemase is one of the most

important Carbapenemase which spread mainly in *Klebsiella spp.* and decrease the sensitivity towards Carbapenems (67).

In addition to common trend and resistance pattern, variation according to geographic regions is also seen. In the time period of 2000-2012 in UAE the Carbapenem resistance in epidemic area varied from 35.7% to 29.8%. This is higher than the normal rates of resistance (27, 74, 38).

In the samples reported to the national healthcare safety network (NHSN), from the year 2006-2007, the percentage of Carbapenem resistance in *E. coli* was up to 4.0% and 10.8% in *K. pneumoniae*. These results were associated certain device related infections (26).

According to Korean nationwide surveillance of antimicrobial resistance (KONSAR), all the strains isolated were sensitive to Carbapenems in 2000 (7), but in 2009 resistance to Carbapenems in *E. coli* was 0.1% and in *K. Pneumoniae* it was 0.5%.

According to Meropenem yearly susceptibility test information collection program, Meropenem resistance in *K. pneumoniae* increased in various clinical isolates from 0.6% to 5.6% in the period of 4 years i.e. 2004 to 2005 (39).

From 2000 to 2004, Meropenem resistance in *K. pneumoniae* was 0.3% and Imipenem resistance was 0.5%. Further, in years 2005 to 2008 Imipenem resistance in *K. pneumoniae* was found to be 0.4% and Meropenem resistance was found to be 0.3%. In addition to this, the resistance against Meropenem and Imipenem was greatly increased in years 2009 to 2012. In *K. Pneumoniae* it raised to 1.9% against Imipenem and 2.4% in case of Meropenem.

Meropenem resistance in *E.coli* in year 2000-2004 was found to be 0.1 and imipenem resistance in the same time period was 0.2. In the later years from 2005-2008 Meropenem resistance was 0.1 and imipenem resistance was 0.3. Furthermore, in years 2009 to 2012 the resistance in *E.coli* was reported as 0.2 to imipenem and 0.5 to Meropenem (6, 13, 15, 19, 20, 24, 25, 27, 28, 32, 37, 40, 47, 51, 56, 61, 62, 64, 68, 74, 75).

In china, imipenem resistance in *E.coli* was reported as zero in years 2000-2004 which climbed to 0.5% in year 2010. *K. pneumoniae* showed higher rates of resistance. It was 0.7% in 2004-2005, but boomed up to 2.7 % in year 2010 (29, 60).

Among Gram negative bacilli *K. Pneumoniae* is the second most frequently isolated pathogen in Shanghai bacterial resistance surveillance program (21, 22).

In Shanghai, China the prevalence of Carbapenem resistant *K. pneumoniae* was 0.91% in 2005, which increased greatly in 2009 to 12.87% (69).

Outbreaks of *K. pneumoniae* resistant to Carbapenems have been reported worldwide (65, 66). In North America, Carbapenem resistant *K. pneumoniae* is the most common type of CRE (48, 50). According to a study in US, the Carbapenem resistant *K. pneumoniae* raised from mere 0.1% to 4.5% in the time period of 2002 to 2010.

According to a report from CDC in 2001, the analysis of hospital surveillance data shows that 8% of *K. pneumoniae* isolates are Carbapenem resistant but the recent surveys show, that this percentage has increased to 5-24% in hospitalized patients (11, 35, 36). Moreover the Carbapenem resistant *K. pneumoniae* causes more deaths than the *K. pneumoniae* sensitive to Carbapenems having mortality between 47% and 57% (9, 33, 34).

CHAPTER 3 - AIMS AND OBJECTIVES

3.1 AIM

To check the prevalence of Carbapenem resistance strains among *K. pneumoniae* and *E. coli.*

3.2 OBJECTIVES

3.2.1 GENERAL OBJECTIVES

- To spot the occurrence of *K. pneumoniae* and *E. coli* in Punjab Institute of Medical Sciences, Jalandhar.
- To check the Antibiogram of all the *K. pneumoniae* and *E. coli* isolates.

3.2.2 SPECIFIC OBJECTIVES

- To isolate strains of *K. pneumoniae* and *E. coli* resistant to carbapenems.
- To determine the prevalence of Amp C producers and Carbapenemase producers among Carbapenem resistant isolates.

CHAPTER 4 - MATERIAL AND METHODS

4.1 MATERIALS

A list of materials used to conduct the study is provided in Appendix – II

4.2 METHODS

This study was carried out in Department of Microbiology in Punjab Institute of Medical Sciences (PIMS) at Jalandhar.

4.2.1 WARDS FROM WHICH SAMPLES WERE RECEIVED

The study was conducted using samples from Out Patient Department (OPD) and In Patient Departments (IPD) including:-

- Gynaecology
- Surgery
- Medicine
- Emergency
- Paediatrics
- ICU
- NICU wards.
- HDU
- Orthopaedics
- OBG
- Chest and TB
- Special

4.2.2 TYPES OF SPECIMEN RECEIVED

Number of samples received for culture and sensitivity from 1st January 2016 to 31st April 2016. The various clinical samples consisted of the following:-

- Swab
- Pus
- Discharge
- Urine
- Body fluids
- Sputum
- Throat swab
- Endo tracheal secretions
- Catheter tip
- HVS
- Wound swab

- Tracheal secretions
- Necrotic fascia or soft tissue
- Blood
- Stool
- Bile
- Tissue

4.2.3 PROCESSING OF SAMPLES

Culture of specimen - Inoculation of all the samples was done on two solid agar medium:-

- MacConkey Agar.
- Blood Agar.

MacConkey agar

MacConkey Agar is selective, differential and an indicator media.

Blood agar

Blood Agar comes under the category enriched and deferential media. It is mainly employed for isolation of fastidious organisms. Blood Agar is a nutritional medium having whole blood cells which provides nutrients for growth of bacteria. It differentiates the organisms on the basis of their hemolytic properties (breakdown of red blood cells).

Name of the Media	Media preparation
MacConkey agar	55.04 grams of MacConkey agar provided by HiMedia Laboratories Pvt. Ltd. Was dissolved in 1000 ml of distilled water. pH was adjusted to 7.2±0.2. Sterilization was done by autoclaving at 15 lbs pressure and 121˚C for 15 minutes. Then the media was poured into sterilized Petri plates and allowed to solidify.
Blood Agar	40.0 grams of blood agar base provided by HiMedia Laboratories Pvt. Ltd. was added to 1000 ml distilled water. Media was sterilized by moist heat sterilization method i.e. by autoclaving at 15 lbs pressure and 121˚C for 15 minutes. 50 ml of blood was added when the temperature of media became 55ºC. Then pouring was done in aseptic conditions.

Inoculation of specimen – All the samples were inoculated on MacConkey Agar, Blood Agar and Chocolate Agar by streaking method. An inoculation well was made and from it, primary, secondary and tertiary streaking was done and loop was sterilized after each and every step.

Incubation of plates- Incubation was done for 24-48 hours for overnight at 37°C.

4.2.4 DETECTION AND VERIFICATION OF *K. PNEUMONIAE* AND *E. COLI* ACCORDING TO ACCEPETED MICROBIOLOGICAL PROTOCOL.

The isolates identified as *K. pneumoniae* and *E. coli* on the basis of cultural characteristics, gram staining and biochemical reactions were further studied for antimicrobial susceptibility, Carbapenemase and Amp C production. Antibiotic susceptibility test was performed by Kirby Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines. CLSI, Wayne, PA (2011). Carbapenemase detection was done by MHT and Amp C tests. The bacterial isolates were identified according to standard microbiological procedure. .

4.2.4.1 GRAM STAINING

Gram staining was done to categorize the morphology of the organisms obtained on culture media after the incubation. Gram staining is used for differentiation of Gram positive and Gram negative bacteria.

4.2.4.2 MICROSCOPY

Microscopy was done to check the morphology and type of bacteria.

4.2.4.3 BIOCHEMICAL TESTS FOR *K. PNEUMONIAE* AND *E. COLI*

Biochemical tests were performed to differentiate the bacterial species that cannot be differentiated by the morphology and cultural characteristics. Biochemical tests are based on fact that different bacterial species differ in their capacity to metabolize carbohydrates, proteins and fats.

MOST BIOCHEMICAL TESTS ARE BASED ON-

- The presence of specific enzymes such has catalase, oxidase, urease, gelatinase and others.
- The production of metabolic end product of some compounds, like sugar fermentation produces acid by the enzymatic action of some bacteria

4.2.4.4 IMViC TESTS

IMViC tests are four tests done for the detection of various organisms which have specific results for these tests. They include four tests namely, Indole test, Methyl Red test, Voges Proskaeur test and citrate utilization test. These are chiefly done to detect the members of family Enterobacteriaceae.

4.2.4.5 INDOLE TEST

This test checks the ability of an organism to produce indole. This biochemical test is performed to predict the species of bacteria

4.2.4.6 METHYL RED (MR) TEST

This test is done to check the capability of an organism to manufacture and sustain acidic end products from glucose fermentation. Some bacteria are able to produce large amount of acids which can neutralize the buffer action of the system. Methyl Red is a pH indicator of the medium, which remains red in colour at a pH of 4.4 or less**.**

4.2.4.7 CITRATE UTILIZATION TEST

This test detects organism which can utilize citrate as the sole source of carbon and energy. Medium contains sodium citrate and pH indicator bromothymol blue, also inorganic ammonium salts, which is used as sole source of nitrogen. Citrate is digested by an enzyme called citritase. It breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and Co_2. Formation of Na_2CO_3 and NH_3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH.

The indicator Bromothymol blue becomes blue in alkaline conditions. The color of medium changes from green to blue.

4.2.4.8 SUGAR FERMENTATION TEST

This test checks the ability to ferment different sugars like glucose, sucrose, mannitol, lactose.

- Inoculated tubes were incubated at 37°C for 24 hours.
- Following incubation tubes were examined for the production of acid and gas.

4.2.4.9 CATALASE TEST

Catalase acts as a catalyst in breakdown of hydrogen peroxide into water and oxygen.

$H_2O_2 \text{----------} \rightarrow H_2O + O^+$

4.2.4.9 OXIDASE TEST

Oxidase test is done to check the presence of enzyme cytochrome oxidase. This enzyme is required for reduction of oxygen at the terminal of electron transport chain. This test is usually performed to distinguish between the family *Pseudomonadaceae* (oxidase positive) and *Enterobacteriaceae* (oxidase negative).

4.2.4.10 MOTILITY TEST

This test is done to check whether organism is motile or non motile. Hence, a thin line growth can be considered as negative result and diffused growth (cloudy growth) indicated positive results.

4.2.4.11 UREASE TEST

This test is done to detect an enzyme called urease which is produced by some bacteria
Interpretation-

4.2.4.12 PPA TEST (PHENYLALANINE DEAMINASE)

4.2.4.13 TSI AGAR TEST

It is a multipurpose test performed to detect the production of H_2S and gas. Also the acid formation (fermentation) by the organism.

4.2.4.14 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Mueller Hinton Agar plates of 12cm diameter were used to check the Antimicrobial susceptibility of the test organism by the standard Kirby Bauer disk diffusion method. The diameter of the zones of inhibition of growth was examined according to guidelines provided by CLSI. *E. coli* ATCC 25922 was used as control organism. Antibiotics used are as following, Ceftazidime (30g), Cephotaxime (30g), Ceftriaxone (30g), Cefepime (30g), Amikacin (30g), Ciprofloxacin (5g), Gentamicin (10g), Cefoxitin (30g), Amoxicillin + Clavulinic acid (20/10g), Ampicillin + Sulbactam (10/10g), Pipracillin + Tazobactam(100/10g), Cefoperazone + Sulbactam (75/30g), Polymixin-B (300 units), Tetracyclin (30g), Meropenem (10g), Imipenem (10g) and Tigecycline (15g). All the antibiotic discs were procured from Hi-media, Mumbai. Strains of *E. coli* ad *K. pneumoniae* found to be resistant to Meropenem by disk diffusion test (zone of inhibition < 13mm) were further tested for Carbapenemase production by MHT and Amp C production by Amp C detection test.

4.2.4.15 MODIFIED HODGE TEST

It is a phenotypic test performed to check production of Carbapenemase which can results in reduced effect of carbapenems. 0.5 McFarland suspension of *E. coli* ATCC 25922 was inoculated by swabbing using a sterile cotton swab on Mueller Hinton Agar plate to get a confluent growth. An imipenem disk was used in this test. A 10 mg imipenem disk was placed at the centre of the plate. With help of a 10µl loop, 4-5 colonies were picked from a blood agar plate having a test organism incubated overnight. A straight line was drawn with this loop from the disk to the edges of the Petri plates. Four test organisms were tested at the same time y drawing four adjacent streaks. This plate was incubated overnight at 37ºC.

4.2.4.16 AMP C DETECTION

Amp C production was detected by cefoxitin disk. A Mueller Hinton Agar plate was taken and inoculated with 0.5 McFarland suspension of ATCC *E. coli* 25922. Cefoxitin disk(30mg) was positioned on the plate at least two centimeters away from the edges. A sterile paper disk was placed just beside the cefoxitin disk. Several colonies were inoculated on paper disk. The plate was placed in incubator for incubation in an inverted position. Incubation was done at 37ºC for 18-24 hours.

CHAPTER 5 - RESULTS AND DISCUSSION

5.1 RESULTS

5.1.1 Culture results

5.1.1.1 MacConkey agar

Interpretation-
Lactose Fermenter bacteria colonies appeared- Pink

Non- lactose Fermenter bacteria colonies appeared- Pale

Colony characteristics on MacConkey agar-

- *K. pneumoniae* appeared as mucoid colonies. The colour becomes pink due to the lactose fermentation.
- *E. coli* appear as lactose fermenter having pink colour colonies but dry in nature.

5.1.1.2 BLOOD AGAR

Colony characteristics on blood Agar:

- On blood Agar Klebsiella pneumoniae produces large colonies and E.coli produce comparatively small colonies.
- Colonies of E.coli and Klebsiella pneumoniae are creamy white in colour.
- But colonies of Klebsiella pneumoniae are mucoid while E.coli has dry colonies.

5.1.2 GRAM STAINING

Gram positive organisms appeared- violet

Gram negative organisms appeared- pink in colour.

5.1.3 MICROSCOPY

Klebsiella pneumoniae is a type of Gram-negative bacteria, short, plump, straight rods measures 1-2×0.5 -0.8µm non-motile, usually encapsulated rod-shaped bacteria.

E. coli is also a Gram-negative bacteria, motile, non- spore forming, bacilli, measuring 0.5×2µm in size.

5.1.4 BIOCHEMICAL TEST RESULTS FOR *K. PNEUMONIAE* AND *E. COLI*

5.1.4.1 INDOLE TEST

Interpretation –

K. pneumoniae: Indole negative

E.coli: Indole positive.

5.1.4.2 METHYL RED (MR) TEST

Interpretations – *K. pneumoniae*: Negative

E. coli: Positive.

5.1.4.3 CITRATE UTILIZATION TEST

The indicator Bromothymol blue becomes blue in alkaline conditions. The colour of medium changes from green to blue.

Interpretations –

K. pneumoniae - Positive

E. coli - Negative.

5.1.4.4 SUGAR FERMENTATION TEST

Interpretations –

- Acid and gas – pink colour and bubble formation in Durham's tube.
- Acid but no gas – pink colour and no bubble formation in Durham's tube
- No acid no gas – no change in colour of the tube.

Klebsiella pneumoniae and E.coli ferments the sugar with acid and gas production in durham's tube.

5.1.4.5 CATALASE TEST

Interpretations –

- If bubbles are produced, it correspond to presence of catalase and the organism is catalase positive (*Staphylococci, Enterobacteriaceae*)
- If there's no bubble formation, then organism is catalase negative (*Streptococci*)
- *K. pneumoniae*: positive
- *E.coli*: positive.

5.1.4.6 OXIDASE TEST

Interpretations –

Oxidase positive organisms – blue- purple color (within 10 seconds)

Oxidase negative organisms – no change in color

5.1.4.7 MOTILITY TEST

Interpretations –

Positive test – Cloudy, diffused, mascara brush like growth e.g. *E. coli*.

Negative test – Well defined growth along the stab e.g. *K. pneumoniae*.

5.1.4.8 UREASE TEST

Interpretations –

Positive test – Appearance of Pink colour within 24 hours to 48 hours of inoculation.

Negative test – there's no change in color after 24 to 48 hours. It remains orange yellow.

K. pneumoniae – positive

E. coli - negative

5.1.4.9 PPA TEST (PHENYLALANINE DEAMINASE)

Interpretations –

Positive test – green colour

Negative test – yellow colour

Klebsiella pneumoniae and *E. coli* both are PPA negative.

5.1.4.10 TSI AGAR TEST

Interpretations –

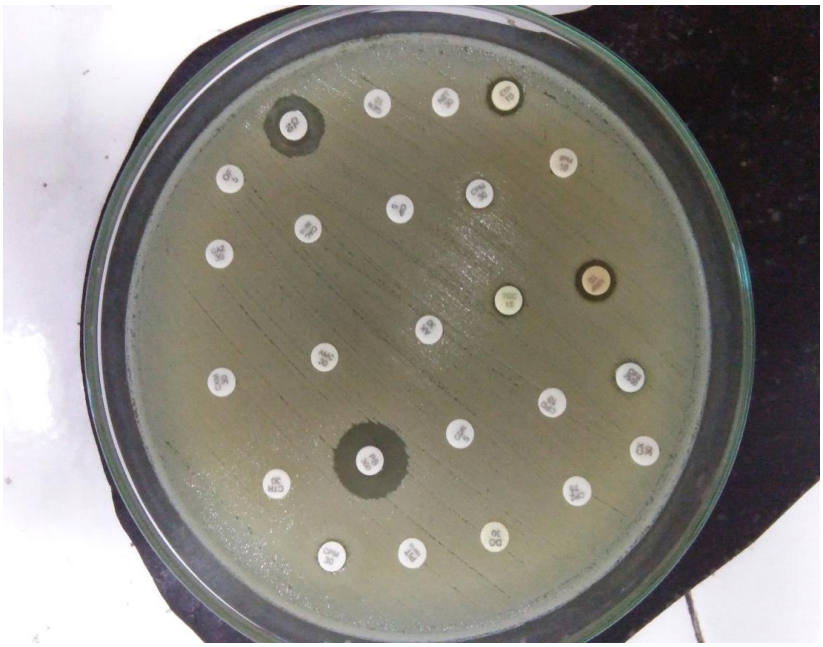
- If the slant of the medium remains pink (alkaline) and butt becomes yellow (acidic) then the organism being tested only ferments glucose.
- Yellow colour of slant and butt indicates acidic conditions i. e. fermentation takes place and the organism is fermenter.
- If the colour of media remains red, then it represents alkaline conditions and no acid formation. So the organism came out to be non fermenter.
- Black colour precipitates were observed in case of production of Hydrogen sulphide (H_2S).

- In case of production of gas the media came apart and cracked.

5.1.4.11 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Zone of inhibition was examined to consider it as resistant or susceptible.

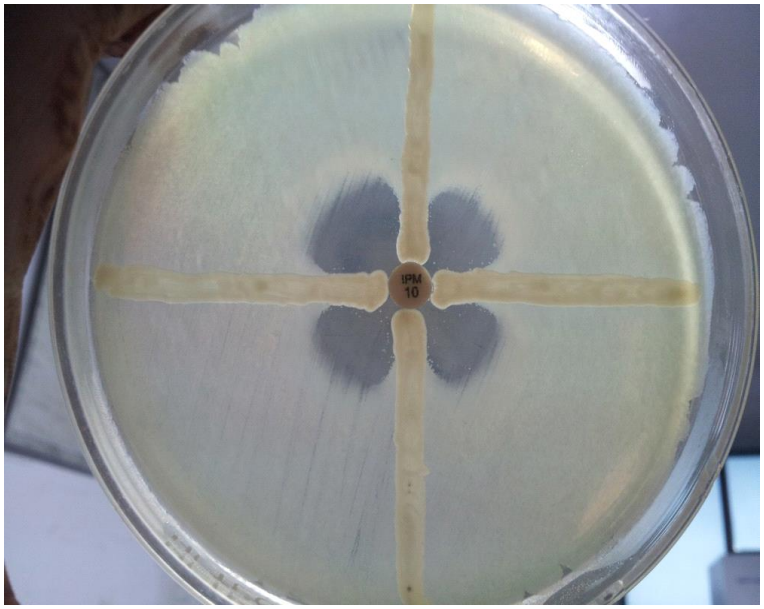
Figure 1 Antimicrobial susceptibility testing



5.1.4.12 MODIFIED HODGE TEST

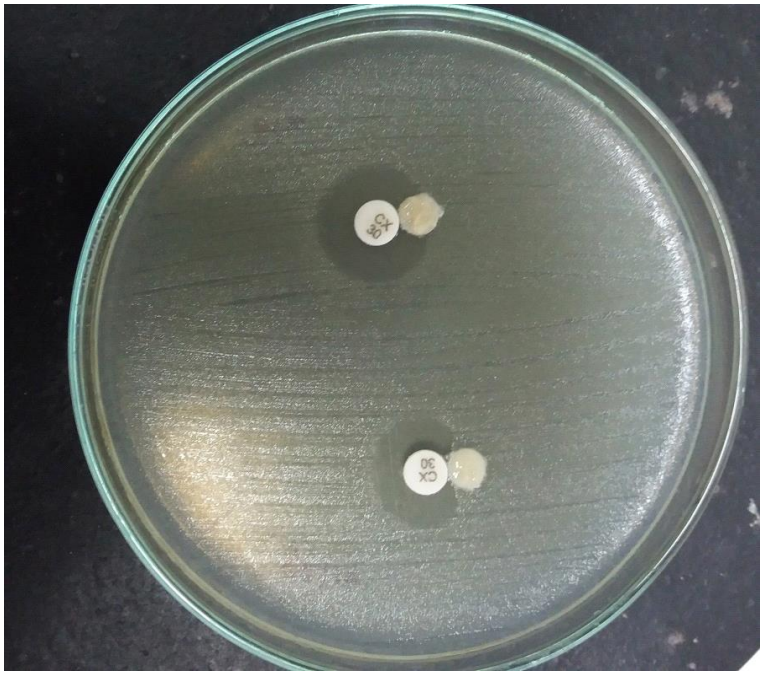
Indentation of *E. coli* ATCC 25922 inside the disk diffusion zone along the test strain growth indicates positive result and no change in zone of inhibition indicate negative results.

Figure 2 Modified Hodge test



5.1.4.13 AMP C DETECTION

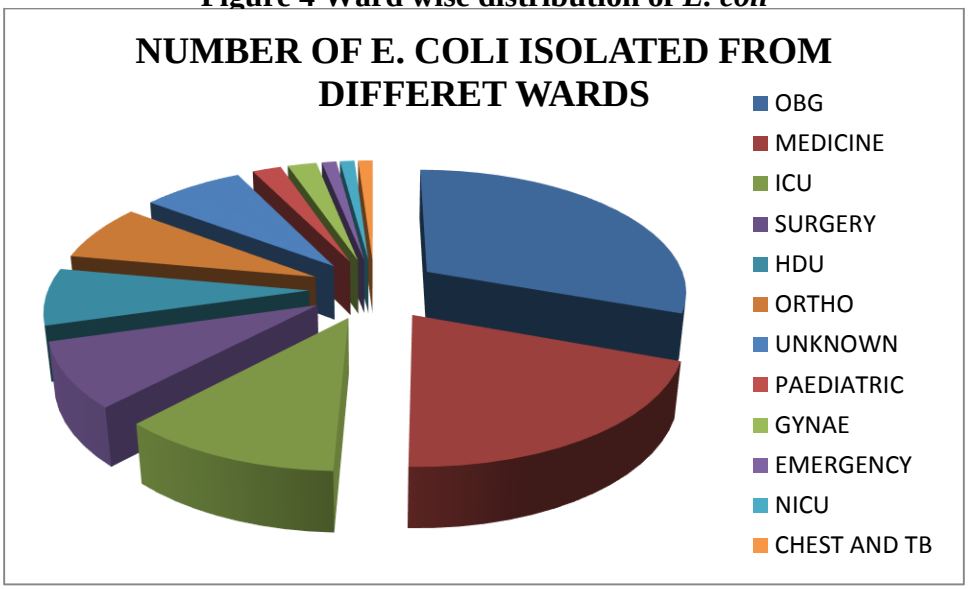
Figure 3 Amp C Detection test



Examination was done to observe the indentation, flattening or distortion of zone of inhibition around cefoxitin disk (positive result), indicating enzymatic inactivation of cefoxitin disk. Absence of change in zone of inhibition indicated no marked inactivation of cefoxitin disk.

The study was conducted at Punjab Institute of Medical Sciences, Jalandhar. A total of 50 *K. pneumoniae* and 95 *E. coli* were isolated from the various OPD and IPD patients from different clinical samples in the time period of January 1, 2016 to April 30, 2016. Highest number of *K. pneumoniae* were isolated from ICU (24%) while yield of *E. coli* was maximum from OBG (obstetric gynae) (30.5%).(Table No. 1) Out of total of 95 isolates of E. coli maximum were obtained from urine(n=62)followed by pus (n=11), sputum (n=8), HVS (n=4), wound swab (n=3), tissue (n=2), stool (n=1), ETT secretion (n=1), necrotic soft tissue (n=1) and bile catheter tip (n=1). However, the maximum number of *K. pneumoniae* isolates were obtained from pus (n=26), followed by urine (n=18), wound swab (n=16), sputum (n=14), ET secretion (n=10), HVS (n=6), throat swab (n=4), catheter tip (n=2), tracheal secretion (n=2), blood (n=2). Among 95 *E. coli* isolates 47(49.5%) were resistant to Meropenem and 12(12.63%) were resistant to imipenem out of 50 isolates of *K. pneumoniae* 27(54%) were Meropenem resistant by disc diffusion method. out of 50 isolates of Klebsiella pneumoniae 12 (24%) were imipenem resistant. The maximum resistance in case of *K. pneumoniae* was found in ICU, then in orthopaedics, surgery, medicine, OBG, HDU , paediatric, unknown, emergency respectively. Modified hodge test positive done for the detection of carbapenemases was shown positive by 16 isolates in total while Amp C β-lactamases productionwas shown by 10 isolates by Amp C disk test.

Figure 4 Ward wise distribution of *E. coli*

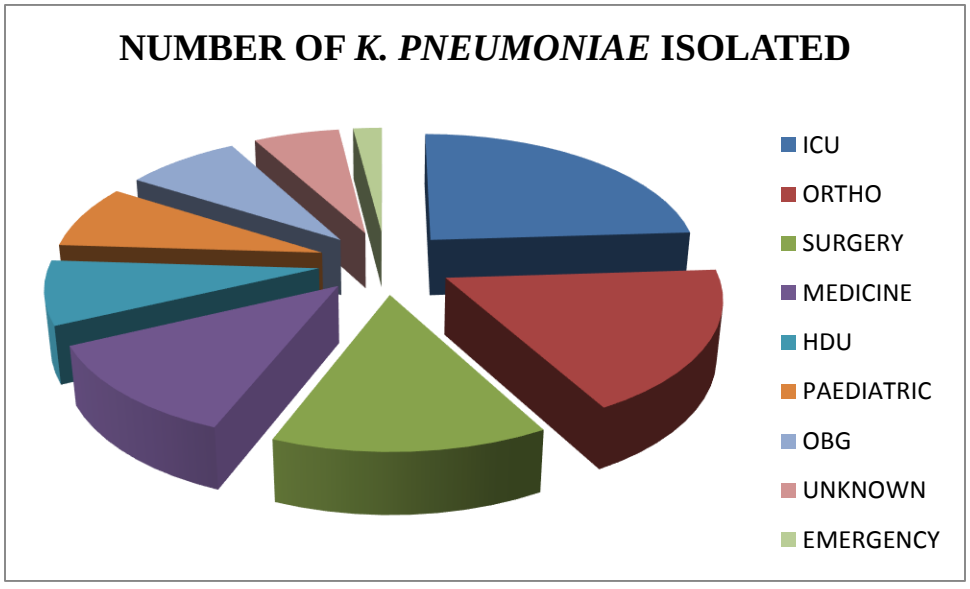


5.1.1 WARD WISE DISTRIBUTION OF *E. COLI K. PNEUMONIAE*

Table 1 Ward wise distribution of *E. coli K. pneumoniae*

WARD	NUMBER OF *K. PNEUMONIAE* ISOLATED (%)	NUMBER OF *E.COLI* ISOLATED (%)
ICU	12(24%)	11(11.5%)
ORTHOPAEDICS	9(18%)	7(7.4%)
SURGERY	7(14%)	8(8.4%)
MEDICINE	6(12%)	19(20%)
HDU	4(8%)	7(7.4%)
PAEDIATRIC	4(8%)	2(2.1%)
OBG	4(8%)	29(30.5%)
UNKNOWN	3(6%)	7(7.4%)
EMERGENCY	1(2%)	1(1%)
NICU	-	1(1%)
CHEST AND TB	-	1(1%)
GYNAE	-	2(2.1%)
Total	50	

Figure 5 Ward wise distribution of *K. pneumoniae*



5.1.2 SAMPLE WISE DISTRIBUTION OF *E. COLI* AND *K. PNEUMONIAE* ISOLATES.

Table 2 Sample wise distribution of *E. coli* and *K. pneumoniae* isolates.

SAMPLE	NUMBER OF *K. PNEUMONIAE* ISOLATED (%)	NUMBER OF *E.COLI* ISOLATED (%)
PUS CULTURE	13(26%)	11(11.6%)
URINE	9(18%)	62(65.3%)
WOUND SWAB	8(16%)	3(3.2%)
SPUTUM	7(14%)	8(8.42%)
ET SECTRETION	5(10%)	1(1%)
HVS	3(6%)	4(4.2%)
THROAT SWAB	2(4%)	-
CATHETER TIP	1(2%)	-
TRACHEAL SECRETION	1(2%)	-
BLOOD	1(2%)	-
TISSUE	-	2(2.1%)
STOOL	-	1(1%)
NECROTIC SOFT TISSUE	-	1(1%)
BILE	-	1(1%)
CATHETER TIP	-	1(1%)
Total	50	

Figure 6 Sample wise distribution of *E. coli*

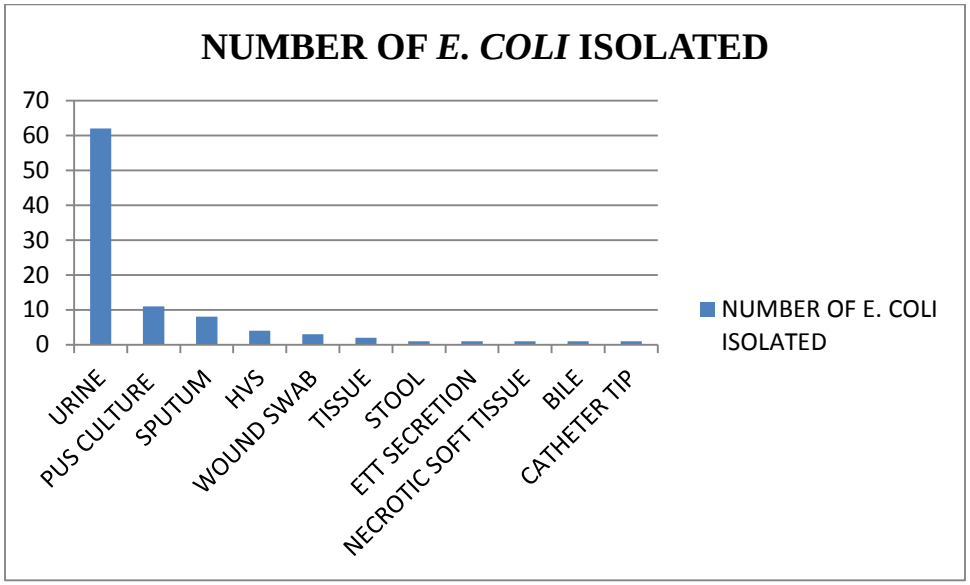
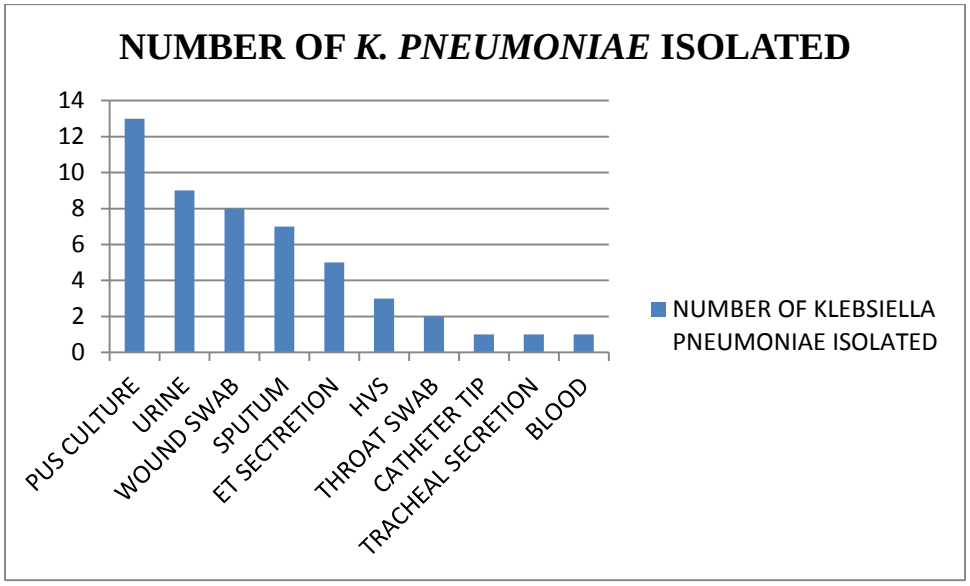


Figure 7 Sample wise distribution of *K. pneumoniae*



5.1.3 RESISTANCE PATTERN OF *E. COLI* AND *K. PNEUMONIAE* ISOLATES TOWARDS DIFFERENT ANTIBIOTICS.

Table 3 Resistance pattern of *E. coli* and *K. pneumoniae* isolates towards different antibiotics.

DRUGS	*K. pneumoniae* (RESISTANCE in %)	*E.COLI* (RESISTANCE in %)
AMINOGLYCOSIDES		
Ofloxacin	31(62)	25(75.7)
Gentamycin	28(56)	48(50.5)
Amikacin	28(56)	51(51.5)
Netilmicin	26(52)	32(33.6)
FLUROQUINOLONES		
Ciprofloxacin	29(58)	64(67.36)
β LACTAMS		
Tigecyclin	7(14)	3(3.15)
Cefpodoxime	44(88)	85(89.4)
Cefoperazone	36(72)	75(80)
Cefotaxime	36(72)	65(68.4)
Ceftazidime	37(74)	58 (61)
Cefoxitin	42(84)	79(83.1)
Cefepime	37(74)	71(74.7)
β LACTAM/β LACTAMASE INHIBITORS		
Piperacillin/tazobactam	30(60)	34(35.78)
Cefoparazone/sulbactam	30(60)	38(40)
Amoxicillin/clavulanate	40(80)	84(88.4)
CARBAPENEMS		
Meropenem	27(54)	47(49.4)
Imipenem	12(24)	12(12.63)
Ertapenem	19(38)	
OTHERS		
Polymixin-B	3(6)	9(9.47)

Among 47 isolates of *K. pneumoniae* 25 were resistant to Meropenem and among 95 isolates of *E. coli* 47 were Meropenem resistant by disk diffusion method.

5.1.4 CARBAPENEM RESISTANCE IN *E. COLI* AND *K. PNEUMONIAE*

Table 4 Carbapenem resistance in *E. coli* ad *K. pneumoniae*

Organism	Total number	Carbapenem resistant (%)
E. coli	95	47(49.4%)
K. pneumoniae	47	25(53.1%)

5.1.5 MODIFIED HODGE TEST (MHT) RESULTS

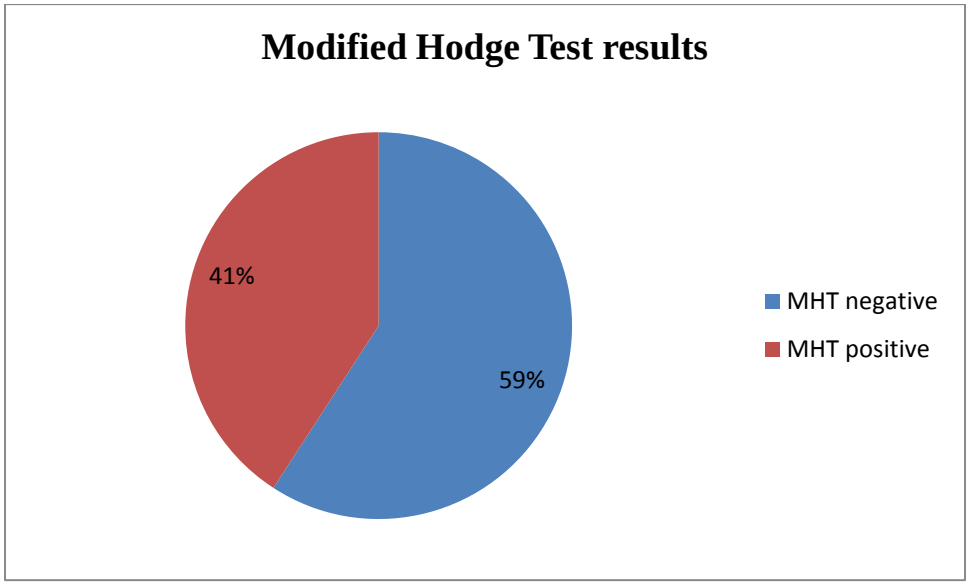
Modified Hodge Test was performed with imipenem disk (10mg) to check the production of Carbapenemase enzyme by various isolates of *K. pneumoniae* and *E.coli*. Clover leaf type indentation in the zone of inhibition was considered as positive.

Figure 8 Modified hodge test results.

Table 5 Modified Hodge Test results.

	Number of isolates	Percentage
MHT positive	87	61.53%

Figure 9 Proportion of MHT positive and negative isolates



5.1.6 AMP C TEST RESULTS

Amp C test was performed with cefoxitin disk (10mg) to check the production of Amp C enzyme by various isolates of *K. pneumoniae* and *E.coli*. An indentation in the zone of inhibition on the side of inoculated test strain was considered as positive result.

Figure 10 Amp C detection test results

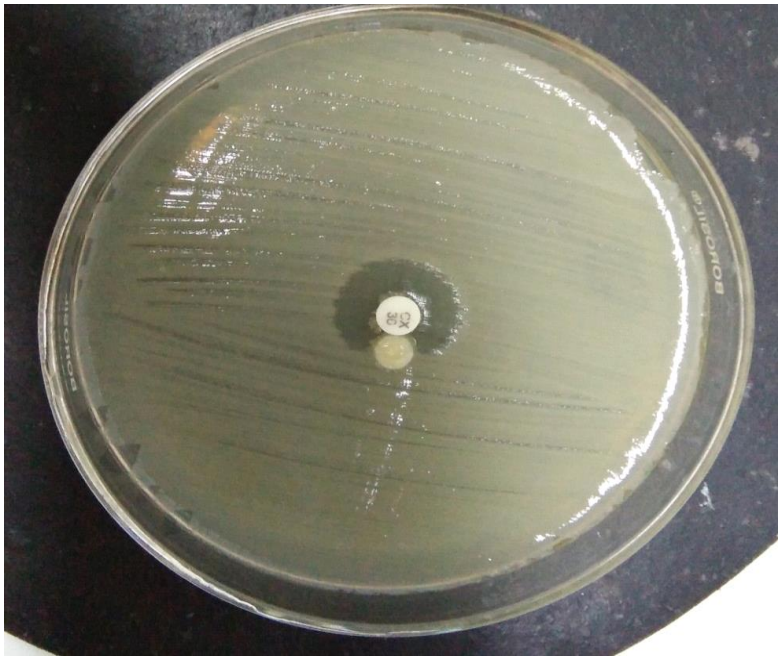
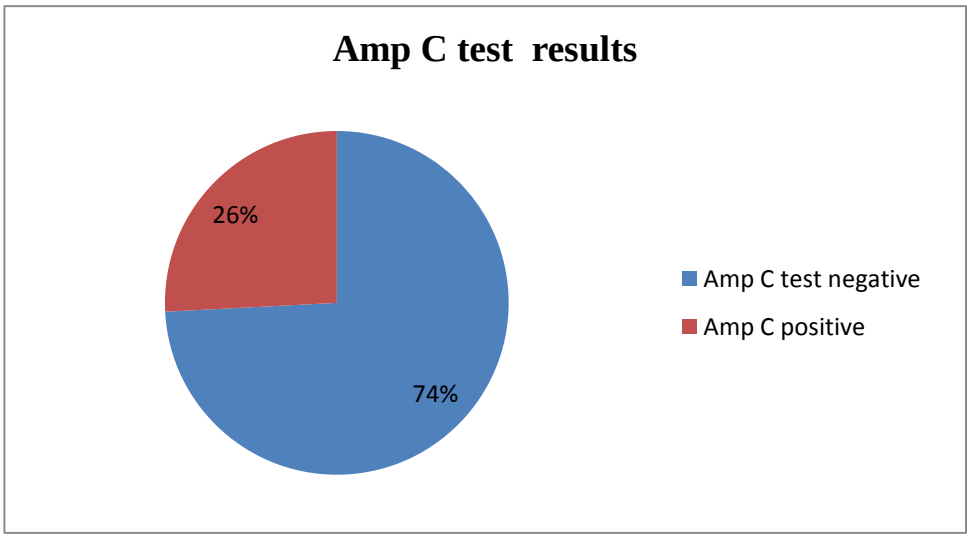


Table 6 Amp C test results.

	Number of isolates	Percentage
Amp C producers	55	38.46%

Figure 11 Proportion of Amp C positive and negative isolates



5.2 DISCUSSIONS

In our study Maximum number of *E. coli* was isolated from Obstetric Gynae ward, accounting for 30.5% (n=29) of all the wards. After OBG ward, medicine ward accounted for 20% (n=19). 11.5% (n=11) of *E. coli* were isolated from ICU. Surgery ward rated 8.4% (n=8). HDU and orthopaedics had the same percentage of *E. coli* isolates, namely 7.4% (n=7).ward information of 7.4% (n=7) *E. coli* was not known. Paediatric ward and gynae ward accounted for 2.1% (n=2), followed by emergency, NICU and Chest and TB all having the same percentage i.e. 1% (n=1).

The highest number of *K. pneumoniae* was reported in ICU. Prolonged stay and prolonged administration of antibiotics contributes to high resistance pattern in ICUs. 24% (n=12) of *K. pneumoniae* was found in ICU, followed by 18% (n=9) in Orthopaedics. Surgery ward accounted for 14% (n=7) of the *K. pneumoniae* isolates. HDU, paediatric, OBG wards gave the same percentage of K. pneumoniae i.e. 8% (n=4). 6% of K. pneumoniae was those whose wards were not known. Lastly, emergency ward yielded only 2% (n=1) of *K. pneumoniae*.

The highest percentage of *E. coli* was observed in urine samples being 65% (n=62). 11.6% (n=11) of *E. coli* were obtained from pus samples. Followed by sputum samples with a percentage of 8.42% (n=8). All other samples had very low proportion as compared to above mentioned samples. As illustration, only 3.2% (n=3) of *E.coli* was isolated from wound swab, 2.1% (n=2) from tissue samples, and 1% (n=1) from ETT secretion, necrotic soft tissue, bile and catheter tip samples respectively.

In case of *K. pneumoniae* pus culture dominated the other samples with a percentage of 26% (n=13). Urine yielded 18% (n=9) of *K. pneumoniae*. Moreover, 16% (n=8) of K. pneumoniae were isolated from wound swabs and 14% (n=7) were obtained from sputum samples. ET secretion contributed 10% (n=5) of *K. pnemoniae* isolates. 6% (n=3) of *K. pnemoniae* were obtained from HVS and 4% (n=2) were isolated from throat swab. Moreover, catheter tip, tracheal secretion and blood had same percentages i.e. 2% (n=1). Whereas, Patricia et al, in 2013 found 47% of *K. pneumoniae* from urine sample and 26% from sputum (76).

Resistance in case of other antibiotics was also recorded for *E. coli* and *K.pneumoniae* isolates.

A variable resistance pattern was seen in *E. coli* towards aminoglycosides in the study. A resistance of 33.6% to 75.7% was noted in case of Ofloxacin, Gentamycin, Amikacin and Netilmicin. Ciprofloxacin among flouroquinolones showed a resistance of 67.36%. A drug from class glycylcycline named Tigecycline showed a resistance of 9%. Among β lactam drugs the the rates of resistance was found to be 61% to 89.4%. The resistance towards β lactam and β lactamase inhibitors was 35% to 88.4%. While, resistance towards Carbapenems was lower than other classes of antibiotics. It was 49.4% in case of Meropenem and 12.63% in case of Imipenem. And resistance was very low in case of Polymixin-B i.e.9.47%. While Priyadarshini Shamugam in 2013 reported 93.4% strains of family *Enterobacteriaceae* resistant to Meropenem ad 73.9% to Imipenem which is much higher the study conducted in our hospital (77). However, Patricia in 2013 reported 100% resistance towards Imipenem ad Meropenem.

K. pneumoniae showed a bit different resistance pattern then *E. coli*. Among aminoglycosides 55.3% to 65.7% of *K. pneumoniae* were found resistant. Resistance against ciprofloxacin came

out to be 61.7%.15.7% of the total were resistant to Tigecycline. Resistance towards β lactam drugs was higher in *K. pneumoniae*. It was 76.5% to 93.6%. Resistance towards β lactam and β lactamase inhibitor was also found to be higher than *E. coli*. In this study it was found to be 63.8% to 85.1%. Resistance towards carbapenems was also higher *K. pneumoniae*. It was 53.1% against Meropenem, 25.5% against Imipenem and 40% against Ertapenem. Only 6.38% of *K. pneumoniae* were resistant towards Polymixin-B.

In our study, 53.1% of *K. pneumoniae* was resistant to Carbapenems but according to study conducted in 2013 by CDC reported only 11% of strains of *K. pneumoniae* resistant to Carbapenems. Whereas, In case of *E. coli* 49.4% were resistant to Carbapenems but CDC reported only 2% of them to be resistant to Carbapenems.

Out of 95 strains of E. coli 48(50.5) were susceptible to Meropenem with MIC < 4, only 1 was intermediate with MIC =8 and 46 were resistant with MIC>16. And among 47 isolates of *K. pneumoniae* 25 were susceptible to Meropenem having MIC <4, none were intermediate with MIC =8 and 22 were resistant with MIC >16.

CLSI taken a step in 2008to improve the detection of Carbapenemase producing *Enterobacteriaceae*. CLSI ascertained that *Enterobacteriaceae* having elevated MICs (2-4 mg/ml) and less disk diffusion zone should be tested for Carbapenemase production (30).

CLSI tested the Carbapenem breakpoints and recommended new lowered Carbapenem breakpoints in 2010 for imipenem, Meropenem and Ertapenem and made new breakpoints for doripenem (52).

New Carbapenem breakpoints established in 2010 are not used currently as FDA has not changed the breakpoints. So the clinicians and clinical laboratories rely on the older techniques and use Carbapenem breakpoints in collaboration with phenotypic confirmatory tests to detect Carbapenemase production in *Enterobacteriaceae*.

Previous breakpoints for Ertapenem were as follows, MIC ≤2 were considered as susceptible strains, MIC =4 were considered as intermediate strains and MIC ≥8 were considered as resistant. Revised breakpoints for Ertapenem included MIC ≤1 as susceptible, MIC =2 as intermediate strains and ≥4 as resistant. Similarly, for imipenem previous breakpoints were MIC ≤2 as susceptible, MIC=4 as intermediate and MIC ≥8 as resistant and new breakpoints are MIC ≤0.25 as susceptible, MIC=0.5 as intermediate and MIC ≥1 as resistant. In case of Meropenem, the breakpoints designed previously were MIC ≤4 as susceptible, MIC=8 as resistant and new MIC ≥16 as resistant. And revised breakpoints are MIC≤1 as sensitive, MIC=2 as intermediate and MIC ≥4 as resistant.30 in my study I have used older breakpoints of Meropenem.

In study conducted in our hospital 61.53% of isolates were MHT positive when performed with imipenem disk (10mg) and indicated the production of Carbapenemases. But Priyadarshini et al in 2013 noted 82.6% strains to be MHT positive (77).

+++++++On the other hand, only 38.46% of these isolates were Amp C producers. An amp C positive sample indicates the presence of extended spectrum β – lactamases (ESBL). The

organisms having these enzymes degrade the β – lactam drugs (till third generation cephalosporins).

CHAPTER 6 – SUMMARY AND CONCLUSIONS

This resistance is well known in both *K. pneumoniae* and *E. coli*. This topic was chosen because of the increasing trend in the resistance shown by the members of the family Enterobacteriaceae A Carbapenemase namely KPC is also invented In accordance with the bacteria *K. pneumoniae*. The new mechanisms of resistance discussed In the report helps these bacteria to survive through the most effective and the drugs of the highest known effect on these bacteria. The two organisms chosen are the commonly isolated bacteria in our hospital. Moreover, these bacteria are found to be more resistant to carbapenems than others. And more interestingly a significant resistance is found in the study conducted. The scenario of resistant *K. pneumoniae* and *E. coli* are becoming more common and troublesome for the people of the community. Intense care is needed for the prevention of the spread of these resistant organisms. There are merely other options after carbapenems except Tigecycline, Polymyxine- and Colistine. These drugs are not considered a good choice because of their association with higher mortality rates after their consumption. So there is a requirement of next line of drugs after carbapenems as there is nothing in the pipeline after them. Moreover, no drugs should be given without performing antibiotic susceptibility testing. Drugs of lower classes should be employed rather than straight away usage of carbapenems. Proper counselling, conferences and awareness about usage of drugs in a proper manner should be held.

CHAPTER 7 – BIBLIOGRAPHY

1. Albers-Schonberg G., et al. 1976. Abstr. 16th Intersci. Conf. Antimicrob. Agents Chemother.
2. Kropp, H., et al. et al. 1976. Abstr. 16th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 229. American Society for Microbiology, Washington, DC
3. Singleton P. Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley. 1999; pp. 444–454. ISBN 0-471-98880-4
4. Yigit H, queenan AM, Anderson CJ, et al. Novel Carbapenem-hydrolysing beta-lactamases, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae, Antimicrob Agents Chemother 2001;45:1151-61.
5. Limansky AS, Mussi MA, Viale AM. Loss of a 29-kilodalton outer membrane protein in Acinetobacter baumannii is associated with imipenem resistance. J ClinMicrobiol. 2002 Dec;40(12):4776-8.
6. Jones RN, Rhomberg PR, Varnam DJ, et al. A comparison of the antimicrobial activity of meropenem and selected broad-spectrum antimicrobials tested against multi-drug resistant Gram-negative bacilli including bacteraemic Salmonella spp.: initial studies for the MYSTIC programme in India. Int J Antimicrob Agents 2002;20:426-31
7. Lee KW, Kim MY, Kang SH, et al. Korean nationwide surveillance of antimicrobial resistance in 2000 with special reference to vancomycin resistance in enterococci, and expanded-spectrum cephalosporin and imipenem resistance in gram-negative bacilli. Yonsei Med J 2003;44:571-8.
8. Ryan, KJ; Ray, CG, eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN 0-8385-8529-9
9. Woodford N, Tierno P, Young K, et al. Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A b-lactamase, KPC-3, in a New York medical center. Antimicrob Agents Chemother 2004;48(12):4793–4799
10. Naas t, Nordmann P,Vedel G, poyart C. Plasmid-medited carbapenem-hydrolysing beta-lactamase KPC in klebsiella pneumoniae isolate drom france. Antimicrob Agents Chemother. 2005 Oct; 49(10): 4423–4424
11. Bratu S, Mooty M, Nichani S,, et al. Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob Agents Chemother. 2005 Jul;49(7):3018-20.
12. Mena, A., et al. 2006. Characterization of a Large Outbreak by CTX-M-1-ProducingKlebsiella pneumoniae and Mechanisms Leading to In Vivo Carbapenem Resistance Development. J Clin Microbiol. 2006 Aug; 44(8): 2831–2837.doi: 10.1128/JCM.00418-06
13. Ling TK, Xiong J, Yu Y, et al. Multicenter antimicrobial susceptibility survey of gram-negative bacteria isolated from patients with community-acquired infections in the People's Republic of China. Antimicrob Agents Chemother 2006;50:374-8.

14. Ling TK, Xiong J, Yu Y, et al. Multicenter antimicrobial susceptibility survey of gram-negative bacteria isolated from patients with community-acquired infections in the People's Republic of China. Antimicrob Agents Chemother 2006;50:374-8.
15. Al-Tawfiq JA. Increasing antibiotic resistance among isolates of Escherichia coli recovered from inpatients and outpatients in a Saudi Arabian hospital. Infect Control Hosp Epidemiol 2006;27:748-53.
16. Leavitt A, Navon- Venezia. S, Chemelnitsky I, Schwaber MJ, carmeli Y. Emergence of KPC-2 and KPC-3 in Carbapenem-resistant Klebsiella pbeumoniae strains in an Israili hospital. Antimicrob Agents Chemother 2007;51:3026-9.
17. Queen AM, Bush K. Carbapenemases: the versatile beta-lactamases Clin Microbiol Rev 2007; 20:440-58.
18. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007 Jul;20(3):440-58.
19. Rizvi MF, Hasan Y, Memon AR, et al. Pattern of nosocomial infection in two intensive care units of a tertiary care hospital in Karachi. J Coll Physicians Surg Pak 2007;17:136-9
20. Shahcheraghi F, Moezi H, Feizabadi MM. Distribution of TEM and SHV beta-lactamase genes among Klebsiella pneumoniae strains isolated from patients in Tehran. Med Sci Monit 2007;13:BR247-50.
21. Zhu, D.M., Zhang, Y.Y., Wang, F. Surveillance of bacterial resistance in Shanghai hospitals during 2006. Chin J Infect Chemother. 2007;7:393–399.
22. Zhu, D., Zhang, Y. & Wang, F. (). Surveillance of bacterial resistance from hospitals in Shanghai in 2007. Chin J Infect Chemother 2007;8, 401–410.
23. Second meeting of the Subcommittee of the Expert Committee on the Selection and use of essential medicines. Use of carbapenems in children. Geneva, 29 September to 3 october 2008.
24. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. J Infect Dev Ctries 2008;2:354-8.
25. Khorvash F, Mostafavizadeh K, Mobasherizadeh S, et al. Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of surgical site infection (SSI); A 1 year of surveillance. Pak J Biol Sci 2008;11:1940-4.
26. Hidron AI1, Edwards JR, Patel J, NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol. 2008 Nov;29(11):996-1011. doi: 10.1086/591861
27. Al-Zarouni M, Senok A, Rashid F, et al. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med Princ Pract 2008;17:32-6.
28. Mehrgan H, Rahbar M. Prevalence of extended-spectrum beta-lactamase-producing Escherichia coli in a tertiary care hospital in Tehran, Iran. Int J Antimicrob Agents 2008;31:147-51.
29. Xiao YH, Wang J, Li Y. Bacterial resist ance surveillance in China: a report from Mohnarin 2004-2005. Eur J Clin Microbiol Infect Dis 2008;27:697-708.

30. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 18th informational supplement. CLSI document M100-S18. Wayne, PA: CLSI, 2008.
31. Khorasani G, Salehifar E, Eslami G. Profile of microorganisms of antimicrobial resistance at a tertiary care referral burn Centre in Iran: emergence of citrobacter freundii as a common microorganism. Burns 2008:34:947-52.
32. Jamal WY, Al Hashem G, Khodakhast F, et al. Comparative in vitro activity of tigecycline and nine other antibiotics against gram-negative bacterial isolates, including ESBLproducing strains. J Chemother 2009;21:261-6.
33. Borer A, Saidel-Odes L, Riesenberg K, et al. Attributable mor- tality rate for carbapenem-resistant Klebsiella pneumoniae bac- teremia. Infect Control Hosp Epidemiol 2009;30(10):972–976.
34. Weisenberg S, Morgan D, Espinal-Witter R, et al. Clinical out- comes of patients with Klebsiella pneumoniae carbapenemase– producing K. pneumoniae after treatment with imipenem or meropenem. Diagn Mircrobiol Infect Dis 2009;64(2):233–235.
35. Centers of Disease Control and prevention Guidance for Control of Infections with Carbapenem-Resistant or Carbapenemase-Producing Enterobacteriaceae in Acute Care Facilities.March 20, 2009 / 58(10);256-260.
36. Wiener-Well Y[1], Rudensky B, Yinnon AM,,et al. Carriage rate of carbapenem-resistant Klebsiella pneumoniae in hospitalised patients during a national outbreak. J Hosp Infect. 2010 Apr;74(4):344-9. doi: 10.1016/j.jhin.2009.07.022. Epub 2009 Sep 26.
37. Ahmad S, Al-Juaid NF, Alenzi FQ, et al. Prevalence, antibiotic susceptibility pattern and production of extended-spectrum beta-lactamases amongst clinical isolates of Klebsiella pneumoniae at Armed Forces Hospital in Saudi Arabia. J Coll Physicians Surg Pak 2009;19:264-5.
38. AI-Dhaheri AS, AI-NIyadi MS, AI-Dhaheri AD, et al. Resistance patterns of bacterial isolates to antimicrobials from 3 hospitals in the United Arab Emirates. Saudi Med J 2009:30:618-23.
39. Rhomberg PR1, Jones R Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999-2008). Diagn Microbiol Infect Dis. 2009 Dec;65(4):414-26. doi: 10.1016/j.diagmicrobio.2009.08.020.
40. Bazzaz BS, Naderinasab M, Mohamadpoor AH, et al. The prevalence of extended-spectrum beta-lactamaseproducing Escherichia coli and Klebsiella pneumoniae among clinical isolates from a general hospital in Iran. Acta Microbiol Immunol Hung 2009;56:89-99.
41. Polwi Chai P, Dejsririlert S, Panpetch S, et al. Antimicrobial resistance of Escherichia coli isolated from urine in Thailand from 2000to 2005. J Med Asscoc Thai 2009;92 suppl4:s59-679
42. Hussain K, sprecher H, Mashiach T, Oren I, Kassis I, Finkelstein R. carbapenem resistance among klebsiella isolates: Risk factors, molecular characteristics, and susceptibility patterns. Infect Control Hosp Epidemiol 2009; 30:666-71.
43. Elemam A, Rahimian J, Mandell W. Infection with panresistant Klebsiella Pneumoniae. A report of 2 cases and a brief review of literature. Clin Infect Dis 2009; 49:271-4

44. Health Protection Agency. Multi-resistant hospital bacteria linked to India and Pakistan. Health Protection Report 2009; 3 : 3-4.
45. Navon-venezia S, Leavitt A, Schwaber MJ, et al. First report on a hyper epidemic clone of KPC-3 producing Klebsiella pneumoniae in Israel genetically related to a strain causing outbreaks in United States. Antimicrob Agents Chemother 2009;53:818-20
46. Rodriguez- Martinez, J. M;L. Poirel, and P. Nordmann.. Extended spectrum cephalosporinases in Pseudomonas aeruginosa, Antimicrob. Agents chemother 2009;53:1766-1771
47. Ali SA, Tahir SM, Memon AS, et al. Pattern of pathogens and their sensitivity isolated from superficial surgical site infections in a tertiary care hospital. J Ayub Med Coll Abbottabad 2009;21:80-2.
48. Lledo W, Hernandez M, Lopez E. Guidance for control of in- fections with carbapenem-resistant or carbapenemase-produc- ing Enterobacteriaceae in acute care facilities. MMWR Morb Mor- tal Wkly Rep 2009;58(10):256–260.
49. AI-Dhaheri AS, AI-NIyadi MS, AI-Dhaheri AD, et al. Resistance patterns of bacterial isolates to antimicrobials from 3 hospitals in the United Arab Emirates. Saudi Med J 2009:30:618-23.
50. Mulvey MR, Simor AE. Antimicrobial resistance in hospitals: how concerned should we be? CMAJ 2009;180(4):408–415.
51. Mehrgan H, Rahbar M, Arab-Halvaii Z. High prevalence of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a tertiary care hospital in Tehran, Iran. J Infect Dev Ctries 2010;4:132-8.
52. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twentieth informa- tional supplement (June 2010 update). CLSI document M100-S20-U Vol 30. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
53. Carvalhaes, C. G., Pica˜o, R. C., Nicoletti, A. G., Xavier, D. E. & Gales, A. C. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in Klebsiella pneumoniae: be aware of false positive results. J Antimicrob Chemother 2010;65, 249–251.
54. Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C. G. & other authors. Emergence of a new antibiotic resistance mechan- ism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010;10, 597–602.
55. Hirsch, E. B. & Tam, V. H. Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother 2010;65, 1119– 1125.
56. Chu YZ, Tian SF, Chen BY, et al. Pharmacokineticpharmacodynamic profiling of four antimicrobials against gram-negative bacteria collected from Shenyang, China. BMC Infect Dis 2010;10:171-5
57. Rolain, J. M., Parola, P. & Cornaglia, G. New Delhi metallo-b- lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect 2010;16, 1699–1701..

58. Krisztina M. Papp-Wallace,[1,2] Andrea Endimiani,[1,2,3] Magdalena A. Taracila,[2] and Robert A. Bonomo[1,2,4,5,*] Carbapenems: Past, Present, and Future. Antimicrob Agents Chemother. 2011 Nov; 55(11): 4943–4960.doi: 10.1128/AAC.00296-11
59. Emamghorashi F, Farshad S, Kalani M, et al. The prevalence of O serogroups of Escherichia coli strains causing acute urinary tract infection in children in Iran. Saudi J Kidney Dis Transpl 2011;22:597-601.
60. Xiao YH, Shen P, Wei ZQ. Mohnrin report of 2010: surveillance of bacterial resistance in China. Chin J Nosocomiol 2011;21:4896-902.
61. Kuzucu C, Yetkin F, Görgeç S, et al. Investigation of the susceptibilities of extended-spectrum beta-lactamaseproducing Escherichia coli and Klebsiella spp. strains to ertapenem and other carbapenems. Mikrobiyol Bul 2011;45:28-35.
62. Chen BH, Zhang XJ, Zhao Y, et al. National Antimicrobial Resistant Investigation Net Mohnarin) 2009 annual report: Antimicrobial resistance surveillance in intensive care units. Chin J Clin Pharmacol 2011;27:483-9.
63. Neil Gupta1,2,Brandi M. Limbago2,Jean B. Patel2, andAlexander J. Kallen. Carbapenem-ResistantEnterobacteriaceae: Epidemiology and Prevention. Clin Infect Dis. (2011) 53 (1): 60-67. doi: 10.1093/cid/cir202
64. Gao L, Li Y. Mohnarin 2009 report: Bacterial drug resistant surveillance of non-ICU inpatients. Chin J Clin Pharmacol 2011;27:373-9.
65. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase- producing Enterobacteriaceae. Emerg Infect Dis 2011;17(10): 1791–1798.
66. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resis- tant Enterobacteriaceae: epidemiology and prevention. ClinInfect Dis 2011;53(1):60–67.
67. Tzouvelekis LS[1], Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. ClinMicrobiol Rev. 2012 Oct;25(4):682-707. doi: 10.1128/CMR.05035-11.
68. Pathak A, Marothi Y, Kekre V, et al. High prevalence of extended-spectrum β-lactamase-producing pathogens: results of a surveillance study in two hospitals in Ujjain, India. Infect Drug Resist 2012;5:65-73.
69. Fupin Hu,1 3 Shudan Chen,1,23 Xiaogang Xu,1 Yan Guo,1 Yang Liu,1 Demei Zhu1 and Yingyuan Zhang Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai, China Journal of Medical Microbiology (2012), 61, 132–136
70. blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae in a Tertiary Care Hospital. DOI: 10.7860/JCDR/2013/7759.3747.
71. Nicola Petrosillo; Maddalena Giannella; Russell Lewis; Pierluigi Viale. Treatment of Carbapenem-resistantKlebsiella Pneumoniae Expert Rev Anti Infect Ther. 2013;11(2):159-177.
72. Lee DS, Choe HS, Lee SJ, et al. Antimicrobial Susceptibility pattern and epidemiology of female urinary tract infections in south Korea, 2010-2011. Antimicrob agents. Chemother 2013;57:5384-93.

73. Shakya P, Barrett P, Diwan Y, et al. Antibiotic resistance among Escherichia coli isolates from stool samples of children aged 3 to 14 years from Ujjain, India. BMC Infect Dis 2013;13:477-82.
74. Chen HB, Zhao ChQ, Wang H, et al. Ananalysis ofresistance ofnosocomial infection pathogens isolated from 13 teaching hospitals in 2011. Zhonghua Nei Ke Za Zhi 2013;52:203-12
75. Shree N, Arora BS, Mohil RS, et al. Bacterial profile and patterns of antimicrobial drug resistance in intraabdominal infections: current experience in a teaching hospital. Indian J Pathol Microbiol 2013;56:388-92
76. Patricia Marquez, Daw Terashita, David Dassey, Lauree Mascola. Population based incidence of carbapenem resistant *Klebsiella pneumoniae* along the Continuum of Care, Los Angeles County. Infect Control Hosp Epidimiol 2013;34(2)144-150.
77. Priyadrshini Shanmugam, Jeya Meenakshi Sundram, Perumal Jayraman, blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae in a Tertiary Care Hospital. DOI: 10.7860/JCDR/2013/7759.3747.
78. Xu Y, Gu B, Huang M, Liu H, Xu T, Xia W, Wang T.Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. J Thorac Dis. 2015 Mar;7(3):376-85.

APPENDIX

Appendix- I

LIST OF ABBREVIATIONS

CLSI Clinical and Laboratory Standard Institute

CSF Cerebrospinal Spinal Fluid

CRKP Carbapenem Resistant Klebsiella pneumoniae

DNA Deoxyribonucleic Acid

ESBL Extended Spectrum Beta Lactamases

FDA Food and Drug Association

HCL Hydrochloric Acid

ICU Intensive Care Unit

IPD In patient Department wards.

KPC *Klebsiella Pneumoniae* Carbapenemase

MIC Minimum Inhibitory Concentration

MBL Metallo Beta Lactamases

MHT Modified Hodge Test

NDM-1 New Delhi Metallo-β-lactamases

NICU Neonatal Intensive Care Unit

NCCLS National Committee for Clinical Laboratory Standards

OPD Out Patient Department

PCR Polymerase Chain Reaction

PPA Phenyl Pyruvic Acid

TSI Triple Sugar

VP Voges Proskauer

Appendix- II
LIST OF MATERIALS

LIST OF EQUIPMENTS USED

- Laminar air hood
- Autoclave
- Binocular microscope
- Hot air oven
- Bio- safety cabinet- II
- Incubator
- Refrigerator
- Electronic weighing balance

GLASS WARES AND PLASTIC WARES

- Petri plates
- Tubes
- Glass slides
- Glass rods
- Conical flask
- Beakers
- Droppers

OTHERS

- Cotton swabs
- Antibiotic disks provided by hi- media
- Sterile filter paper disks
- Racks
- Face mask
- Gloves
- Spatula
- Forceps