SENSITIVITY PATTERN OF KLEBSIELLA PNEUMONIAE ISOLATED FROM SAMPLES OF URINE, PUS, AND RESPIRATORY TRACT SECRETIONS

Internship Training Report

Submitted to

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For the degree of

Master of Science in Clinical Microbiology

Submitted by:
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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Mr. Harpreet Singh (Internal supervisor), Lovely Professional University and Dr. Renuka Bajaj (External Supervisor), MD, Microbiologist, SRL Limited, Fortis Escort Hospital, Amritsar. This work has not been submitted in part or in full in any other university for any degree or diploma.

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CERTIFICATE

This is to certify that ***Ms.NAVJEET KAUR*** bearing **Registration Number 11401865** 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.

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ABSTRACT

Klebsiella pneumoniae is associated with different types of infections, such as, urinary tract infections, blood infections, respiratory tract infections, and wound infections. It is equally responsible for hospital acquired infections and for community acquired infections.In recent times, the most important aspect of *Klebsiella pneumoniae* is emergence of its multidrug resistant strains, particularly those, involved in nosocomial infections.Infections from such strains are very difficult to treat.Antibiotic sensitivity pattern of bacterial strains varies from place to place and from time to time. So the knowledge of the sensitivity pattern of bacterial strains particularly resistant strains of a particilar geographical area is necessary. This study was done to determine the sensitivity pattern of *Klebsiella pneumoniae* isolated from samples of urine, pus, and respiratory tract secretions(collected from the patients of inpatient and outpatient department of the Fortis Escort Hospital, Amritsar). *Klebsiella pneumoniae* was identified by adopting standard microbiological techniques and their sensitivity pattern was obtained by the automated microbiological systems: VITEK 2 COMPACT ,and MICROSCAN autoscan 4. Total 100 strains were isolated from 1566 samples of urine, pus, and respiratory tract secretions. Out of these 100 strains, 30 were isolated from samples of urine, 20 were isolated from samples of pus and 50 were isolated from samples of respiratory tract secretions.

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Amoxi/K clavu	Amoxicillin/Potassium clavunate
ATP	Adenosine Ttriphosphate
BAL	Bronchoalveolar lavage
CLED	Cysteine Lactose Electrolyte Defecient
ESBL	Extended spectrum beta lactamase
GN	Gram negative
ID	Indentification
MDR	Multi drug resistant
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
No.	Number
Pip/Tazo	Piperacillin/Tazobactam
RTI	Respiratory tract infection
Trimeth/Sulfa	Trimethoprim/Sulfamethoxazole

1. INTRODUCTION

Development of antibiotic resistance in microorganisms is a serious public health problem worldwide (1).Resistant bacteria causes infections, which are very difficult to treat, thus infections from such bacteria leads to prolonged hospital stays and high health care cost. Moreover, in many cases, resistant bacteria cause untreatable infections(2).

Problem of antibiotic resistance is alarming in both developed and developing countries(3).Indiscriminate and inadequate prolonged use of antibiotics is responsible for emergence and blooming of antibiotic resistance(4).The worst trend, particularly in developing countries, is the prescription of antimicrobials without carrying out sensitivity studies of microbes (5).

Resistance to antibiotics in microorganisms develops to both older and newer antibiotics. Bacteria are characteristically able to transmit and acquire resistance to antibiotics, due to this, resistant bacteria flourish in large numbers and infection from such bacteria results in high mortality and morbidity rates (6).

Injudicious use of antibiotics has resulted in development and spread of multidrug resistant strains among different groups of microorganisms throughout the world and these strains are responsible equally for hospital acquired infections and for community acquired infections (7).

Mechanisms involved in antibiotic resistance are: 1)prevention of interaction of drug with target organism, 2)decreased uptake due to either an increased efflux or decreased influx of antibiotics, 3) modification or destruction of compound by enzymes like ESBL enzymes.(8).

Among many, Klebsiella pneumoniae is one of the common multidrug resistant bacteria frequently isolated from clinical samples.Friedlander was the one, who isolated it for the first time from the lungs of patient died of pneumonia. To honour him, it was named as Friedlander's bacillus but later on it was renamed as '*Klebsiella pneumoniae'*.It is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe. It belongs to enterobacteriaceae family of microbes. It is the second most common organism of the normal flora of human intestine. Infections caused by *Klebsiella* include urinary tract infections, blood infections, wound infections, respiratory tract infections (pneumonia especially in chronic alcoholics) (9).

Virulence factors of *Klebsiella pneumoniae* are capsular polysacccharides, lipopolysaccharides and iron- scavenging systems(siderophores).*Klebsiella pneumoniae* is resistant to a wide range of antibiotics. Inappropriate use of antibiotics has resulted in resistance

of *Klebsiella pneumoniae* to previously sensitive antibiotics. They are naturally resistant to ampicillin and amoxicillin. Major concern is the increase in the number of Extended Spectrum Beta Lactamases producing strains of *Klebsiella pneumoniae*. These strains are also showing co-resistance to other antibiotics like quinolones and aminoglycosides (10).

The sensitivity pattern of microbes changes from time to time and place to place. Therefore knowledge of sensitivity pattern of microorganisms in a particular place must be updated from time to time for better treatment of the patients (11).

The present study is aimed at finding of sensitivity pattern of *Klebsiella pneumoniae* isolated from three different clinical samples: urine, pus and respiratory tract secretions (sputum, endotracheal secretions and bronchoalveolar lavage).

2. TERMINOLOGY

ANTIBIOTIC

Antibiotics are the chemical agents that destroy microbes, inhibits their growth, or prevents their pathogenic actions. Antibiotics are widely used in the field of medicine to cure number of diseases. Antibiotics are also known as antimicrobials.

ANTIBIOTIC RESISTANCE

Antibiotic resistance occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply, causing more harm.

ANTIBIOTIC SENSITIVITY

Antibiotic sensitivity or antibiotic susceptibility is the susceptibility of bacteria to antibiotics.

FACULTATIVE ANAEROBE

A facultative anaerobe is an organism that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration. if oxygen is absent

NOSOCOMIAL INFECTION

Nosocomial infections are infections acquired in hospitals and other healthcare facilities. To be classified as a nosocomial infection, the patient must have been admitted for reasons other than the infection. He or she must also have shown no signs of active or incubating infection..

PNEUMONIA

Pneumonia is an infection that inflames the air sacs in one or both lungs. The air sacs may fill with fluid or pus (purulent material), causing cough with pus, fever, chills, and difficulty breathing.

SIDEROPHORES

Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria, and fungi. Siderophores are amongst the strongest soluble Fe^{3+} binding age

3.REVIEW OF LITERATURE

1.Asati Rakesh Kumar in study entitled **" Antimicrobial sensitivity pattern of *Klebsiella pneumonia* isolated from pus from tertiary care hospital and issues related to the rational selection of antimicrobials"** claimed that *Klebsiella pneumoniae* was resistance to different antibiotics . *Klebsiella pneumoniae* was showing more than 50 % sensitivity only for four antimicrobials namely amikacin (88.1), gatifloxacin (77.8 %), gentamicin (57.8 %) and chloramphenicol (55.6 %)(out of 24 tested antibiotics) and more than 20 antimicrobials was showing less than 50 % antibiotic sensitivity. *Klebsiella pneumoniae* was demonstrated 88.8% resistance to cotrimoxazole, cefotaxime, ciprofloxacin, ofloxacin and cefadroxyl and 100 % resistance to polymixin-b, piperacillin, tetracycline..

2. S Ahmed, Al-Junaid NF, Alenzi FQ Matter EH, Bakheet Oel-S in study entitled **"Prevalence, antibiotic susceptibility pattern and production of extended-spectrum beta-lactamases amongst clinical isolates of *Klebsiella pneumoniae* at Armed Forces Hospital in Saudi Arabia"** claimed a high percentage of *Klebsiella pneumoniae* resistant to ampicillin (100%) and tetracycline (92%).

3.**Dr.R.Sarathbabu, Dr.T.V.Ramani, Dr. K.Bhaskara rao, Dr.Supriya Panda** in their study entitled **" Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples**" claimed that majority of the strains were sensitive to amikacin and sensitivity to antibiotics decreases with time.The percentage of sensitivity to amikacin was 75.56% in 2008, 70.37% in 2009,and 66.67% in 2010 for pus samples; 66.67% in 2008, 61.11% in 2009 and 56.92% in 2010 for sputum samples and 78.31% in 2008, 74.44% in 2009 and 71.60% in 2010. In addition **Asati Rakesh Kumar** in his another study entitled "**Antimicrobial Sensitivity Pattern of *Klebsiella Pneumoniae* isolated from Sputum from Tertiary Care Hospital**, **Surendranagar, Gujarat and Issues Related to the Rational Selection of Antimicrobials"** also stated that *Klebsiella pneumoniae* is most sensitive for amikacin followed by gatifloxacin, chloramphenicol, cefipime, ciprofloxacin and cefoperazone plus sulbactam. Considering the antibiotic susceptibility testing, cost, side effects and many other factors, gatifloxacin should be preferred for *K. pneumoniae* infection for RTI. *K. pneumoniae* showed low resistance to older drugs like chloramphenicol. It indicates that routine exposure of bacteria only to newly developed antibiotics eliminated resistance against older out of use antibiotics and present bacterial strains have grown sensitive to these outdated agents.

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4.C.Manikandan and A.Amsath in study entitled"**Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from urine samples**" stated that the most sensitive antimicrobial was amikacin and imipenem followed by gentamicin and tobramycin , ofloxacin), ciprofloxacin. Significant number of isolates were multiresistant to all the antimicrobial agents used. The lowest percentage of susceptibility was manifested against amoxicillin followed by ampicillin , Cotrimoxazole, cephalexin and nalidixic acid , whereas more susceptibility was observed with amikacin/imipenem , followed by gentamicin/ tobramycin. Three aminoglycoside antibiotics, amikacin and gentamicin/ tobramycin were moderately resistant to species of pathogens used.

5.Radji m, Fauziah S, Aribinuko in study entitled **" Antibiotic sensitivity pattern of bacterial pathogens in the intensive care unit of Fatmawati Hospital, Indonesia" stated** that *K. pneumoniae* was resistant to third generation cephalosporins cephalexin,fallowed byother cephalosporins ceftriaxone , ceftazidime, cefpirome and cefotaxime.

6.Guillermo V. Sanchez, Ronald N. Master, Richard B. Clark, Madiha Fyyaz, Padmaraj Duvvuri, Gupta Ekta, and Jose Bordonn in their study entitled**, " *Klebsiella pneumoniae* Antimicrobial Drug Resistance, United States, 1998–2010"** stated that, the proportion of *K. pneumoniae* isolates resistant to carbapenems was lower than those previously reported . In 2010, resistance rate of 4.3% for imipenem was observed but **Shilpa K , Ruby Thomas and Allavarapu Ramyashree iK.N.Ravichitra1 , P.Hema Prakash , S. Subbarayudu and U. Sreenivasa Rao** in thir study entitled " **Isolation and antibiotic sensitivity of *Klebsiella pneumoniae* from pus, sputum and urine samples"** claimed imipenam as the most sensitive antibiotic against K. pneumonia fallowed by cefaprazone and sulbactam. Sensitivity to quinolones and aminoglycosides antibiotics was moderate**.**

8. **Archana Singh Sikarwar and Harsh Vardhan Batra, in a study entitled " Prevalence of antimicrobial drug resistance of Klebsiella pneumoniae in India"** stated that *K.pneumoniae* strains from clinical cases were found highly susceptible to quinolones and aminoglycoside, amikacin and gentamycin. At the same time over 60% strains were found resistant to chloramphenicol and tetracycline. Twenty-eight to 76% of them were resistant to cephalosporins (ceftizoxime and cefotaxime). Cephalosporins have been widely used as monotherapy and in combination with aminoglycosides for the treatment of *Klebsiella* infection. Plasmid encoded resistance to broad spectrum cephalosporins is becoming a widespread phenomenon in clinical medicine. These antibiotics are inactivated by an array of different extended spectrum beta lactamases (ESBLs) which have evolved by stepwise mutation of TEM/SHV type beta genes.

4. OBJECTIVES

1. To determine the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from samples of urine, pus, and respiratory tract secretions

2. To determine the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from samples of urine

3. To determine the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from samples of pus

4. To determine the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from samples of respiratory tract secretions

5. MATERIAL AND METHODS

2.1 PLACE: - This study was conducted in Microbiology Section of SRL Ltd, Fortis Escort hospital, Amritsar from January 1 to April 30, 2016.

2.2 TARGETED SPECIMENS: - Specimens targeted for this study was urine, pus, and respiratory tract secretions (sputum, endotracheal secretions, and bronchoalveolar lavage). These specimens were collected from both inpatient and outpatient department of the hospital by adopting universal safety precautions.

2.3 DATA COLLECTION: - The information about sensitivity pattern of total 100 strains isolated from the targeted specimens was collected from antibiotic sensitivity reports of those strains. Out of total 100 strains, 30 were isolated from urine samples, 20 from pus samples and 50 from respiratory tract secretions.

6. CHARACTERIZATION OF BACTERIAL ISOLATES

Targeted specimens were inoculated aseptically on Blood, CLED, Chocolate, and MacConkey agar plates and in Thioglycollate broth. Urine samples were inoculated on CLED agar plates only. Sputum samples were inoculated on Blood, MacConkey and Chocolate agar plates. Blood, MacConkey, and Chocolate agar plates, and Thiogycollate broth were used to inoculate bronchoalveolar lavage and endotracheal secretions. Specimens of pus were inoculated on Blood, and MacConkey agar plates, and Thioglycollate broth. All the inoculated media subjected to 24 hour's incubation at 37^0 C (12,13).

Klebsiella pneumoniae's identification was done by observing it's microscopic and culture characteristics. Microscopic examination reveals gram negative, short, stout, blunt rods. Large dome shaped colonies of the organism seen on Blood, and Chocolate agar plates. Lactose fermenting mucoid colonies were seen on the MacConkey and CLED agar plates. Growth of Klebsiella pneumoniae was seen throughout the Thioglycollate medium but it was more concenterated at the top of the medium. Biochemical characters includes positive Voges-Proskauer test, positive citrate utilization, positive urease test,acid and abdundant gas production from glucose, lactose, sucrose, maltose, and mannitol sugar fermentation test (14, 15).

7. ANTIBIOTIC SENSITIVITY TESTING

All the 100 isolated strains were screened for antimicrobial sensitivity. The antibiotics that were tested for all the strains included Amikacin, Amoxicillin/ Potassium clavunate, Ampicillin, Cefepime, Cefotaxime, Cefuroxime, Ciprofloxacin, Colistin, Ertapenem, Imipenem, Meropenem, Gentamicin, Trimethoprim/Sulfamethoxazole, Piperacillin/Tazobactam. Nitrofurantoin was tested for strains isolated from urine and pus samples.Tigecycline was tested for strains isolated from samples of pus, BAL, and sputum. The sensitivity pattern of the isolated strains was obtained by using antibiotic sensitivity testing Automated Microbiological Systems: Vitek 2 Compact and Microscan autoscan 4.Both the systems performs mainly two functions i.e. 1) Identification of the isolated strain and 2) Determination of antibiotic sensitivity of the isolated strain. Vitek performs one more function i.e. Resistance mechanism detection. Vitek uses an Advanced Colorimetry Technology for identification of the organism and includes an Advanced Expert System that analyse MIC (minimum inhibitory concentration) patterns and detects phenotypes for most of the organisms. The system reads the latest generation Vitek test cards containing 64 wells. Currently four reagent cards available for the identification of different organism classes: 1) Gram-negative fermenting and non-fermenting bacilli, 2) Gram-positive cocci and non spore forming bacilli, 3) Yeast and yeast like organisms, and 4) Gram-positive spore forming bacilli. The GN card is used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting Gram-negative bacilli. The GN card is based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistances. Final identification results avalaible in approximately 10 hours or less (16,17). MicroScan autoscan-4 system also, has its applications in indentification and antimicrobial sensitivity testing of the isolated organism. This system has simple testing process with automated processing. MicroScan system includes Pentium desktop computer, Reports printer, LabPro alert software, Turbidity meter. MicroScan reads conventional combo panels, conventional MIC only panels, and conventional ID only panels for identification and antimicrobial susceptibility testing of the organism. MicroScan is an excellent supplemental system for difficult organism such as MRSA (methicillin-resistance Stapylococcus aureus) and drug- resistant gram negative bacilli. Major benefits of both these microbiological systems are quick results, decreased manual workload and reduction in waste(18).

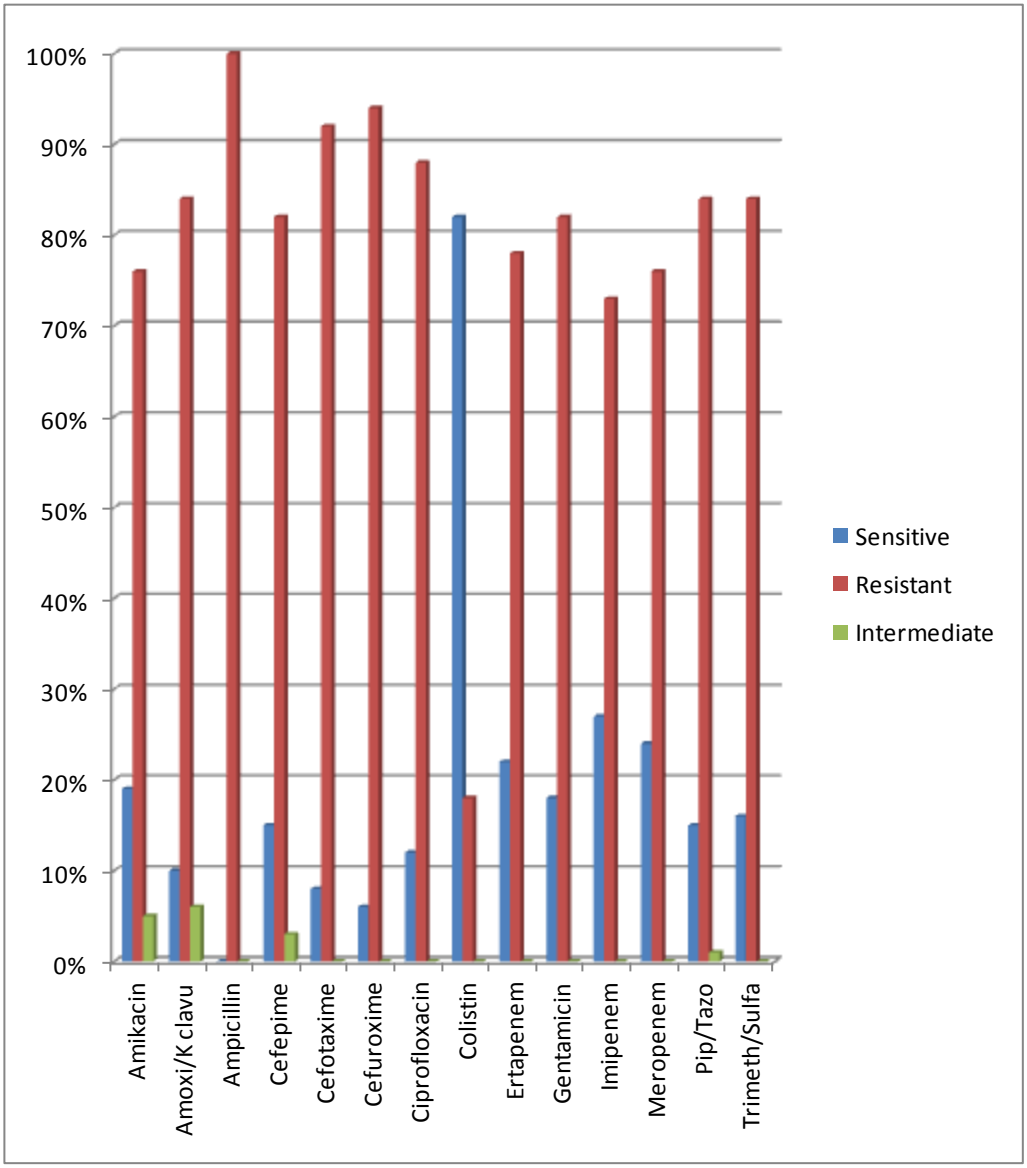
8. DATA ANALYSIS

8.1 COLLECTIVE DATA ANALYSIS

Antibiotic	Sensitive	Resistant	Intermediate
Amikacin	19%	76%	05%
Amoxi/K clavu	10%	84%	06%
Ampicillin	00%	100%	00%
Cefepime	15%	82%	03%
Cefotaxime	08%	92%	00%
Cefuroxime	06%	94%	00%
Ciprofloxacin	12%	88%	00%
Colistin	82%	18%	00%
Ertapenem	22%	78%	00%
Gentamicin	18%	82%	00%
Imipenem	27%	73%	00%
Meropenem	24%	76%	00%
Pip/Tazo	15%	84%	01%
Trimeth/Sulfa	16%	84%	00%

TABLE 1 SHOWING ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF URINE, PUS, AND RESPIRATORY TRACT SECRETIONS (SAMPLE SIZE=100)

CHART 1 ILLUSTRATING THE ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF URINE, PUS, AND RESPIRATORY TRACT SECRETIONS

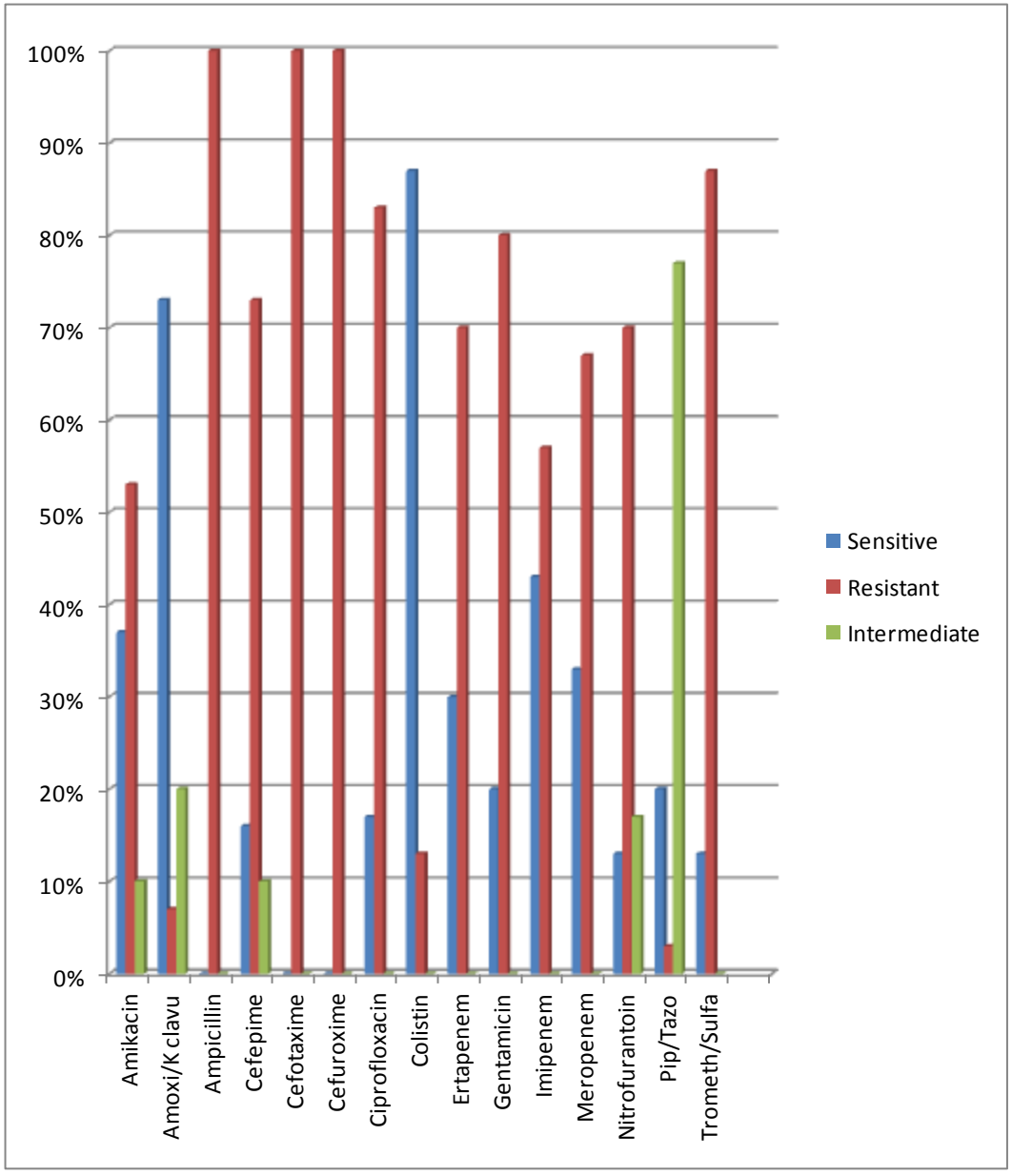


8.2 DIFFERENTIAL DATA ANALYSIS

Antibiotic	Sensitive	Resistant	Intermediate
Amikacin	37%	54%	10%
Amoxi/K clavu	73%	7%	20%
Ampicillin	0%	100%	0%
Cefepime	17%	73%	10%
Cefotaxime	0%	100%	0%
Cefuroxime	0%	100%	0%
Ciprofloxacin	17%	83%	0%
Colistin	87%	13%	0%
Ertapenem	30%	70%	0%
Gentamicin	20%	80%	0%
Imipenem	43%	57%	0%
Meropenem	33%	67%	0%
Nitrofurantoin	13%	70%	17%
Pip/Tazo	20%	3%	77%
Trimeth/ Sulfa	13%	87%	0%

TABLE 2 SHOWING ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF URINE (SAMPLE SIZE=30)

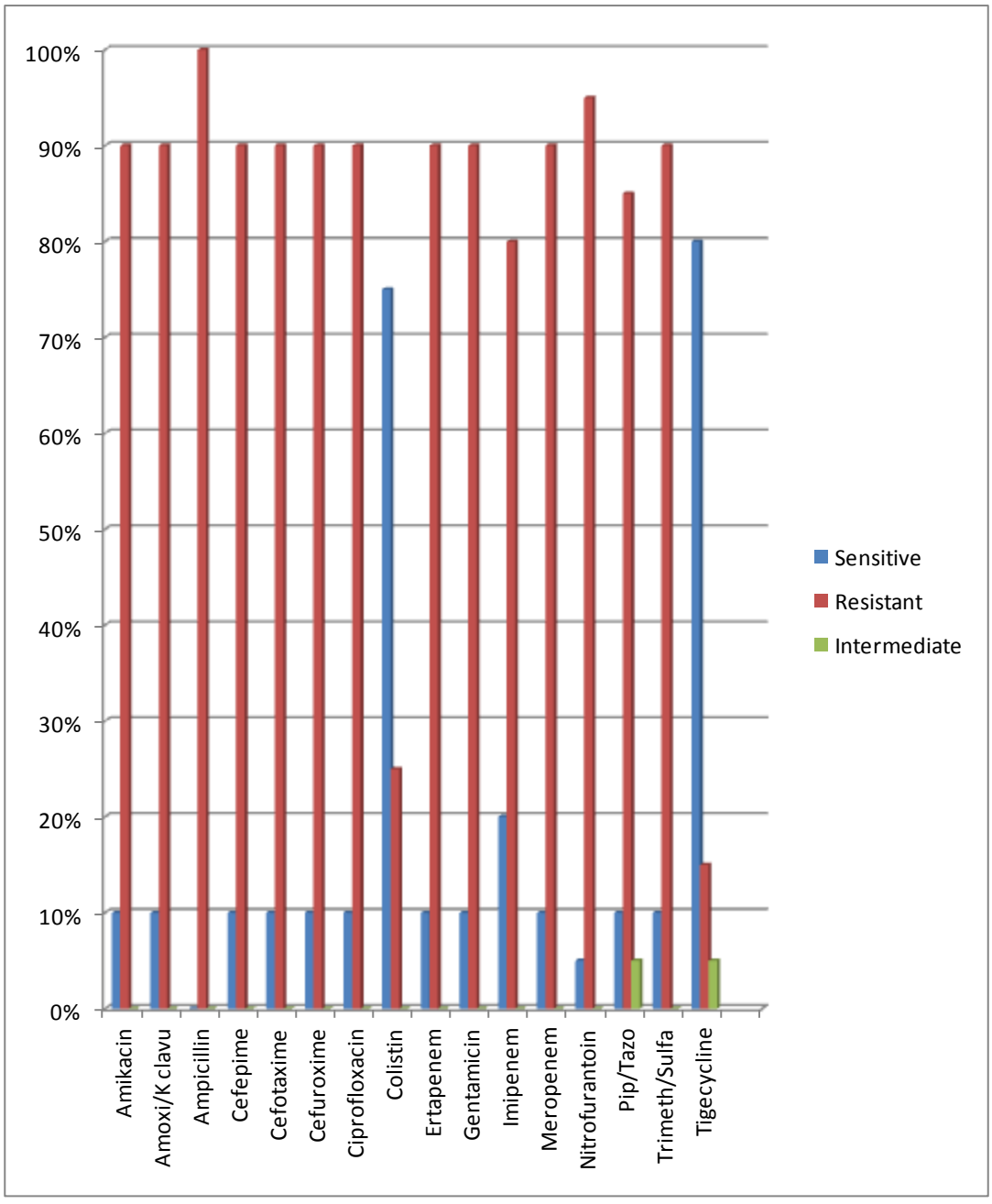
CHART 2 ILLUSTRATING THE ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF URINE



Antibiotic	Sensitive	Resistant	Intermediate
Amikacin	10%	90%	0%
Amoxi/K clavu	10%	90%	0%
Ampicillin	0%	100%	0%
Cefepime	10%	90%	0%
Cefotaxime	10%	90%	0%
Cefuroxime	10%	90%	0%
Ciprofloxacin	10%	90%	0%
Colistin	75%	25%	0%
Ertapenam	10%	90%	0%
Gentamicin	10%	90%	0%
Imipenam	20%	80%	0%
Meropenam	10%	90%	0%
Nitrofurantoin	5%	95%	0%
Pip/Tazo	10%	85%	5%
Trimeth/ Sulfa	10%	90%	0%
Tigecycline	80%	15%	5%

TABLE 3 SHOWING ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF PUS (SAMPLE SIZE=20)

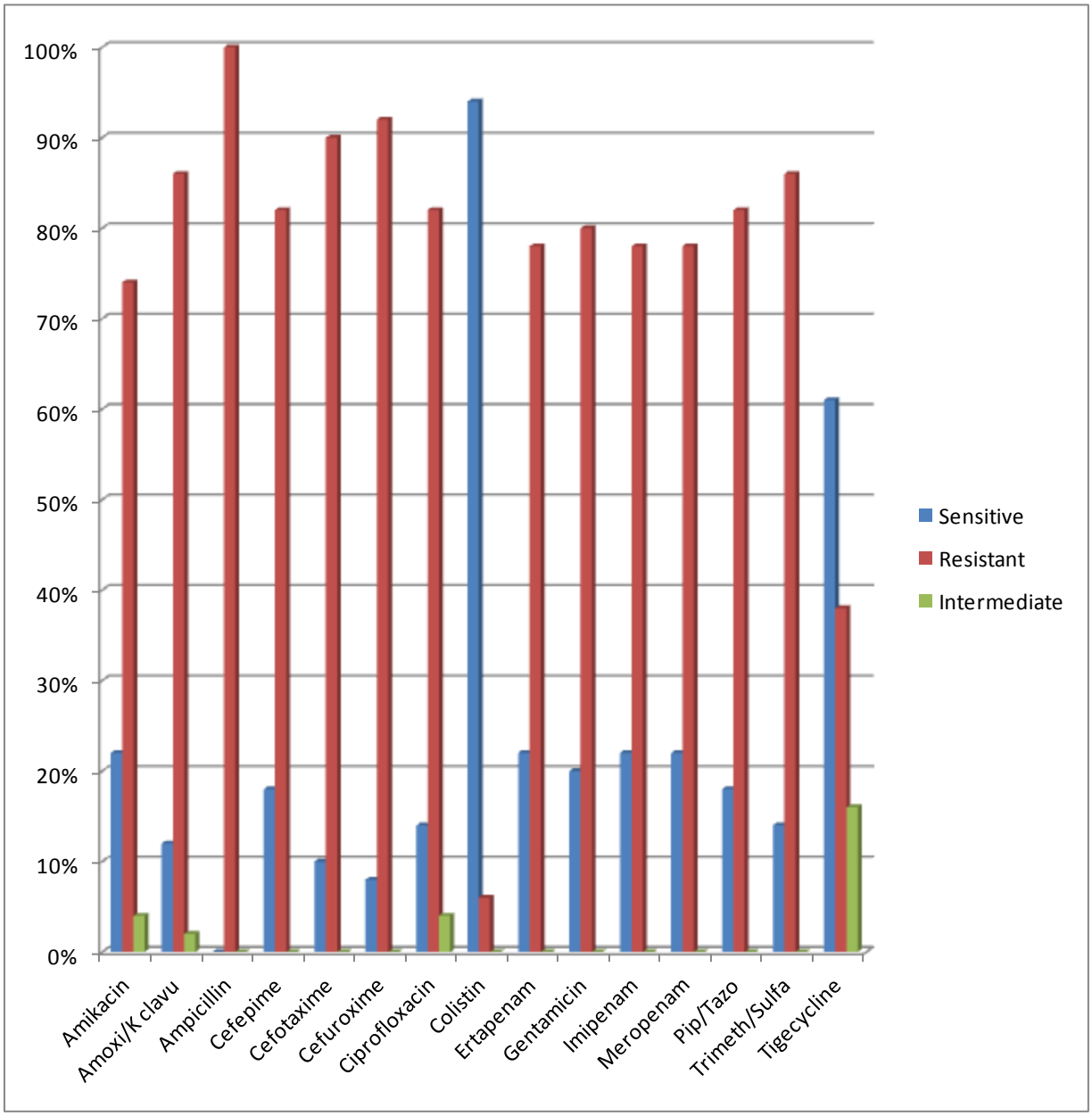
CHART 3 ILLUSTRATING ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF PUS



Antibiotic	Sensitivity	Resistant	Intermediate
Amikacin	22%	74%	4%
Amoxi/K clavu	12%	86%	2%
Ampicillin	0%	100%	0%
Cefepime	18%	82%	0%
Cefotaxime	10%	90%	0%
Cefuroxime	8%	92%	0%
Ciprofloxacin	14%	82%	4%
Colistin	94%	6%	0%
Ertapenam	22%	78%	0%
Gentamicin	20%	80%	0%
Imipenam	22%	78%	0%
Meropenam	22%	78%	0%
Pip/Tazo	18%	82%	0%
Trimeth/ Sulfa	14%	86%	0%
Tigecycline	61%	38%	16%

TABLE 4 ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF RESPIRATORY TRACT SECRETIONS (SAMPLES SIZE= 50)

CHART 4 ANTIBIOTIC SENSITIVITY PATTERN OF STRAINS ISOLATED FROM SAMPLES OF RESPIRATORY TRACT SECRETIONS



9. RESULTS

The data is analyzed collectively and differentially with respect to various targeted samples. Collective data analysis includes analysis of antibiotic sensitivity pattern of all strains (100) isolated from all the targeted samples (urine, pus, and respiratory tract secretions). Differential data analysis includes analysis of antibiotic sensitivity pattern of strains with respect to various targeted samples.

9.1 RESULT OF COLLECTIVE DATA ANALYSIS

It shows that 82% strains of *Klebsiella pneumoniae* is sensitive to colistin. 27% of strains are sensitive to imipenem. Along with colistin and imipenem, sensitivity to other antibiotics is shown in table-1 and illustrated in chart -1. Few strains shows intermediate response to some antibiotics which is also shown in the same table and same chart.

9.2 RESULT OF DIFFERENTIAL DATA ANALYSIS

9.2.1 ANTIBIOTIC SENSITIVITY OF STRAINS ISOLATED FROM URINE SAMPLES

87% strains are sensitive to colistin, fallowed by amoxi/K clavu, to which 73% isolates are sensitive. 43% isolates are sensitive to imipenem . Along with these antibiotics, sensitivity to other antibiotics is shown in table no.2 and illustrated in chart no. 2.

9.2.2 ANTIBIOTIC SENSITIVITY OF STRAINS ISOLATED FROM PUS SAMPLES

80% strains are sensitive to tigecycline fallowed by colistin, to which 75% strains are sensitive. Along with tigecycline and colistin, sensitivity to other antibiotics is shown in table- 3 and illustrated in chart- 3.

9.2.3ANTIBIOTIC SENSITIVITY OF ISOLATES ISOLATED FROM RSPIRATORY TRACT SECRETION SAMPLES

94% strains are sensitive to colistin. 46% strains, isolated from BAL and sputum samples are sensitive to tigecycline. Sensitivity to other antibiotics is shown in table-4 and illustrated in chart- 4.

9.3 DATA INTERPERATATION

Collective data analysis shows that maximum number of strains are sensitive to colistin mainly and these strains are least sensitive to rest of the tested antibiotics(beta lactam antibiotics, beta lactamase inhibitors, Aminoglycosides). All strains are resistant to ampicillin.

Differential data analysis shows that the strains isolated from samples of urine are sensitive to colistin and amoxi/ K clavu mainly. Few strains are resistant to these drugs, even. These strains are not significantly sensitive to imipenem. Maximum number of strains are resistant to rest of the tested antibiotics.All strains isolated from urine samples are resistant to ampicillin, cefotaxime, and cefuroxime.Only tigecycline and colistin are effective against strains isolated from pus samples.Rest of the tested antibiotics are least effective. Again, all strains isolated from pus samples are resistant to ampicillin.

Only colistin is effective against strains isolated from samples of respiratory tract secretions. Tigecycline is significantly effective against strains isolated from samples of BAL and sputum. Most of the strains are resistant to all other tested antibiotics and like other samples, all strains isolated from respiratory tract secretions are resistant to ampicillin.

10. DISCUSSION

K. pneumonia has been responsible for number of infections such as UTI, blood infections, respiratory tract infections, wound infections. These infections can occur both in hospital settings and in community settings. It is one of the important microbe to cause hospital acquired infections. Among many, it has emerged as one of the MDR bacteria due to injudicious use of antibiotics. Mechanisms involved in antibiotic resistance are: 1)prevention of interaction of drug with target organism, 2)decreased uptake due to either an increased efflux or decreased influx of antibiotics, 3)enzymatic modification or destruction of compounds.

Infections from MDR strains are not only difficult to treat but are untreatable in some cases. Antibiotic sensitivity of these strains varies according to time and place. So the determination of antibiotic sensitivity of these strains of a particular geographical area is essential.

This study reveals that maximum number of strains of K. pneumonia are resistant to most of the antibiotics. Even the antibiotics with extended spectrum of activity such as aminoglycosides, beta lactamase inhibitors are least effective against these strains. In this study maximum number of strains are sensitive to colistin, a polypeptide, toxic to human body if used systemically.Few strains are resistant to colistin even.

So this is not a alarming situation now, rather, a challenging situation where health care professionals finding it hard to treat infections from such strains. So extensive efforts and research is needed to overcome this situation. One of the important effort is doing of studies to obtain knowledge of common organisms responsible for the various infections and finding out their sensitivity pattern to treat the patient approropriately and to discourage the development of the MDR strains further.

11. CONCLUSION

The resistant strains of Klebsiella pneumoniae are flourishing in large numbers. Even antibiotics with extended spectrum of activity are not working against these strains. The last resort to combat infections from resistant strains is colistin, a polypeptide toxic to humans if used systemically. So the use of antibiotics without carrying out sensitivity studies must be prohibited to discourage the development of antibiotic resistance further.

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INDEX

Appendix I

A. LIST OF MATERIALS

1. Equipment

- Autoclave.
- Bunsen burner.
- Incubator.
- Microscope.
- Refrigerator.
- Weighing machine.

2. Glass wares

- Petri plates.
- Test tubes.
- Slides.
- Conical flask.
- Cover slips

3. Others

- Cottons.
- Scissors.
- Forceps.
- Inoculating loops
- Face mask.
- Gloves

APPENDIX-II

B. GRAM STAIN

1.PREPARATION OF SMEAR:

1. A clean glass slide was taken and a drop of 0.9% NaCl(normal saline) wasplaced on it.
2. Using sterile technique, a loopful of clinical specimen or bacterial suspension was placed,mixed with normal saline and spread on the slide to make thin smear
3. The smear was allowed to dry in the air or the smear was fixed to the slide by gentle heating over a flame.

2. REAGENTS:

1. Primary stain: crystal violet – 1g crystal violet in 50ml of absolute alcohol
2. Mordant: gram's iodine – 0.6g iodine crystals+1.2g of pottasium iodide in 80ml of dis.water.
3. Decolorizing agent : acetone
4. Counterstain: safranin - 1g in 100ml of dis.water.

Appendix-III

C. COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF CULTURE MEDIA

1.MACCONKEY AGAR :

PRINCIPLE:

The inhibitory action of crysal violet on the growth of gram positive organisms allow the isolation of gram negative bacteria. Incorporation of the carbohydrate lactose, bile salts and the pH indicator neutral red permits the differentiation of enteric bacteria on the basis of their ability to ferment lactose. Bacterial colonies that can ferment lactose (E.coli) turn the medium red due to response of the pH indicator to the acidic environment created by the fermentation. Organism that do not ferment lactose (salmonella) do not cause a color change.

COMPOSITION:

INGREDIENTS	**gm/lt**
Peptic digest of anilal tissue	17.00
Proteose peptone	3.00
Lactose	10.00
NaCl	5.00
Neutral red	0.03
Bile salts	1.50
Agar	15.00

Final pHwas adjust the to 7.1+/-0.2. (25^0c)

METHOD:

51.53gms of media was dissolved in 1000ml of d.waterandwas heated to boiling to dissolve the medium completely. It was sterlize by autoclaving at 15lb pressure 121^0c for 15mins. Then it was cool to 45-50^0c. then it was mixed and poured into the petridishes.

3. Mueller -Hinton Agar:

Composition

INGREDIENTS	**gm/lt**
1. Beef, infusion from	300.00
2. Casein acid hydrolysate	17.50
3. Starch	1.50
4. Agar	17.00

Final pH was adjust to 7.3 +/-0.1

METHOD:

38.0g of media was dissolved in 1000ml of d.H_20 and was heated to boiling to dissolve the medium completely. It was sterlize by autoclaving at 15lbs pressure 121^0c for 15mins. Then it was cool to 45-50^0c. mixed and was poured into sterile petriplates.