"PREVALENCE OF CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIAE ISOLATED FROM VARIOUS CLINICAL SAMPLES- A ONE YEAR CROSS SECTIONAL STUDY"

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LIST OF ABBREVIATIONS

NAME	ABBREVIATIONS
β	Beta
µl	Microliter
A/A	Acid slant/Acid butt
AMC	Amoxicillin-clavulanic acid
AMP	Ampicillin
Amp C	Ampicillin resistant gene
AST	Antibiotic sensitivity test
ATCC	American Type Culture Culuture
CAI	Community Acquied Infections
CLSI	Clinical and Laboratory Standards Institute
CP-KPN	Carbapenemase-producing Klebsiella pneumoniae
CRE	Carbapenem Resistant Enterobactarales
CTX	Cefotaxime
ESBL	Extended spectrum beta lactamases
FO	Fosfomycin
GEN	Gentamicin
HAI	Healthcare Associated Infections
ICU	Intensive care units
IMP	Imipenem

KPC	Klebsiella pneumoiae Carbapenemase
KPN	Klebsiella pneumoniae
MBL	Metallo beta lactamases
mCIM	Modified Carbapenemase inactivation method
MDRO	Multi Drug Resistant Organisms
MHA	Muller Hinton Agar
MHT	Modified Hodge Test
MR	Methyl red
MRP	Meropenem
MRSA	Methicillin Resistant Staphylococcus aureus
NIT	Nitrofurantoin
PCR	Polymerase cahin reaction
PIT	Piperacillin-Tazobactam
R	Resistant
S	Sensitive
SHV	Sulphydryl variable
TOB	Tobramycin
TSB	Trypticase Soy Broth
TSI	Triple sugar iron
VP	Voges Proskauer
VRE	Vancomycin Resistant Enterococci

ABSTRACT

Study Background:

Multi-drug-resistant organisms causing community-acquired and healthcare-associated infections are increasing at a dangerous rate globally. Among them, the Enterobacteriaceae family, primarily Klebsiella pneumoniae, are most commonly responsible for deadly infections. Commonly seen resistance patterns are due to the presence of Extended-spectrum β-Lactamase (ESBL) or acquired cephalosporins (AmpC)-producing organisms that are resistant to all β-Lactams except carbapenems. But recently, it has been observed that there has been an increase in the prevalence of carbapenem-resistant Klebsiella species resulting in treatment failure. Therefore, it is the need of the hour to implement an appropriate, cost-effective, and straightforward phenotypic method for the detection of Carbapenemase-producing Klebsiella pneumoniae for further prevention and spread of CP-KPN.

Objectives:

1. To detect the prevalence of Carbapenemase production in Klebsiella pneumoniae in various clinical samples by using the modified Carbapenemase Inactivation Method.
2. Compare antibiotic sensitivity pattern and Carbapenemase production in hospital-acquired and community-acquired infections among Klebsiella pneumoniae isolates.

Methodology:

A cross-sectional study was conducted in the Department of Microbiology of a tertiary care centre.

A total of 123 isolates of Klebsiella pneumoniae were identified over a period of 12 months. Screening and detection of Carbapenemase production were done using phenotypic methods.

RESULTS:

The present study reports a prevalence of 27% of CP-KPN. Of the 123 samples screened for carbapenemase production, 44 (36%) were found to be positive using the screening method, out of which 33 (27%) isolates were confirmed positive using the modified carbapenemase inactivation method(mCIM).

The antibiogram of isolates from both community-acquired and healthcare-associated infections was recorded, and a comparison was made. Maximum sensitivity was observed with Imipenem(87%) and Meropenem(86%), followed by Gentamicin (85%) in CAI isolates.

In HAI isolates, maximum sensitivity was with Imipenem(48%), Meropenem (45%), followed by Gentamicin(44%).

Conclusion:

Our study showed a higher prevalence of CP-KPN in the area. Production of Carbapenemase mediates antibiotic resistance in clinical isolates. The development of simple and inexpensive screening methods to detect carbapenemase production in laboratories is crucial for optimal treatment of critically ill and hospitalized patients and to control the spread of resistance.

Keywords: Antibiotic resistance CP-KPN, HAI, mCIM

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INTRODUCTION

Multi-drug-resistant organisms causing community-acquired(CAI) and Healthcare-associated infections(HAI) are increasing at a dangerous rate globally, raising a significant threat for public health and society. The heightened misuse of antibiotics in human medicine, agriculture, and veterinary is primarily contributing to the phenomenon. There is an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital-acquired infections[1]. Among them, Enterobacteriaceae, especially Klebsiella pneumoniae, are most commonly responsible for deadly diseases.[1].

Extended-spectrum β-Lactamase (ESBL) and acquired cephalosporins (AmpC)-producing organisms are resistant to all β-Lactams except carbapenems. Therefore treatment of choice for the infections caused by these organisms are often carbapenems. But recently, it has been found that there is an increase in carbapenem-resistant Klebsiella species resulting in treatment failure[2] Carbapenemase, an enzyme under metallo-β-lactamase (MBL) that break down β-lactam drugs is responsible for the resistance to carbapenem group of antibiotics . Mechanism of carbapenem resistance is mainly due to production of various carbapenemases. A new metallo-β-lactamase, named New Delhi metallo-β-lactamase-1 (NDM-1) encoded by blaNDM-1 gene, first identified in a Swedish patient who had travelled to India[4]. They confer resistance to all beta-lactams including carbapenems, with the exception of aztreonam. Highly mobile elements carry the genes for the same, helping easy dissemination of resistance[5.] MBL gene was detected in *Pseudomonas aeruginosa* for the first time, which now has spread to other species of *Enterobacteriaceae*[6]

Klebsiella pneumoniae is an essential source of transferable antibiotic resistance. Clinical manifestation of this organism can go from colonization of the skin and mucous membrane to severe life-threatening infections, leading to substantial mortality and morbidity.

Carbapenemase resistance is an emerging threat to physicians as well as microbiologists. Thus, the emergence and spread of carbapenem-resistant Klebsiella pneumoniae are significant clinical and public health concerns.

Carbapenem resistance is mediated mainly by the production of Carbapenemase enzymes that are present on the mobile genetic elements, followed by chromosomal mediated porin loss and efflux pumps overexpression. Due to the various resistance mechanism, most of the time, Carbapenemase-producing Klebsiella shows resistance to other groups of drugs also, leading to multidrug-resistant or pan drug-resistant isolates[3]

The root cause of the rise in antimicrobial resistance is multi-factorial. A critical factor in emerging resistance is a lack of good antibiotic stewardship leading to overuse of antimicrobials, inappropriate empiric coverage, and delays in precise diagnoses, as well as de-escalation therapy. As time goes on, there are fewer and fewer antimicrobials available that are effective in treating these infections, and so, the problem further escalates. [4]

Therefore, the screening and detection of Carbapenemase producers, selection of appropriate therapeutic schemes, and implementation are essential to control the rise of hospital and community-acquired infections. Though molecular methods are considered to be the gold standard, they may fail to detect unknown Carbapenemase

genes not included in the gene panel. To overcome all these drawbacks, biochemical tests based on technique designed to identify the hydrolysis of β lactam rings of a carbapenem has been developed[1]

CLSI guidelines 2019 recommend Carba NP and Modified Carbapenemase Inactivation methods (mCIM)to identify Carbapenemase-producing organisms replacing Modified Hodge test.[4]

Although many studies have been conducted to find the infection and resistance pattern of Klebsiella, none of them have mentioned the prevalence of Carbapenemase resistant Klebsiella pneumoniae in the northern part of Karnataka.

Therefore, the current study aimed to assess the prevalence of Carbapenemase-producing Klebsiella pneumoniae using the Modified Carbapenemase Inactivation method (mCIM) and compare the antibiotic sensitivity pattern and Carbapenemase production in hospital-acquired and community-acquired infections.

OBJECTIVES OF THE STUDY

1. To detect the prevalence of Carbapenemase production in Klebsiella pneumoniae in various clinical samples by using the modified Carbapenemase Inactivation Method.

2. Compare antibiotic sensitivity pattern and Carbapenemase production in hospital-acquired and community-acquired infections among Klebsiella pneumoniae isolates.

REVIEW OF LITERATURE

Enterobacteriaceae family

The family consists of non-sporulating, facultatively anaerobic, Gram-negative rods belonging to the γ-proteobacteria. Their natural habitat is the intestinal tract of humans and animals.[5,6]This causes a plethora of infections in the community and hospitals. Members of this family are involved in almost all types of infectious disease and is one of the most commonly isolated organisms in the labs.[5]

They exhibit general morphological and biochemical similarities.

General Characteristics[7]

1. Gram negative Bacilli
2. They are both aerobic and facultative anaerobes
3. Grows readily in ordinary media
4. Glucose fermented, production of acid and gas or acid only
5. Nitrate reduced to nitrite
6. Catalase positive
7. Oxidase test negative

Classification of Enterobacteriaceae:[8]

The advent of newer technologies has helped enhance the taxonomical study of microorganisms, increasing the number of genera and species.

Edward and Ewing described 11 genera comprising of species belonging to 6 Enterobacteriaceae in 1972[8]. In 1985, Farmer and associates described 22 genera with 69 species and 29 enteric groups[9]

The tribe concept was proposed by Ewing, where he classified family into tribe, genus, and species. Use of tribe concept has a significant impact in the laboratories for easy identification as genera under each tribe possess common properties [10]

Table 1: Edward and Ewing classification of Enterobacteriaceae [5]

TRIBE	GENUS	SPECIES
Tribe I – Escherichiae	Escherichia Shigella	E. coli, E. albertii, E. vulneris S. dysenteriae, S. flexneri, S. boydii, S. sonnei
Tribe II – Edwardsielleae	Edwardsiella	E. tarda, E. hoshinae
Tribe III - Salmonellae	Salmonella	S. enterica, S. bongori
Tribe IV- Citrobactereae	Citrobacter	C. freundii, C. koseri
Tribe V- Klebsiellae	Klebsiella Enterobacter Hafnia Serratia Pantoea	K. pneumoniae, K. oxytoca E. cloacae, E. sakazaki H. alvei S. marcescens, S. liquefaciens P. agglomerans,
Tribe VI – Proteeae	Proteus Morganella Providencia	P. mirabilis, P. vulgaris M. morgagnii P. alcalifaciens, P. stuartii
Tribe VII – Yersiniae	Yersinia	Y.pestis, Y.pseudotuberculosis,Y.enterocolitica
Tribe VIII: Erwinieae	Erwinia	E. persicinus

The clinically relevant Enterobacteriaceae are bisected to opportunistic pathogens, like Citrobacter species, Klebsiella species & other microorganisms, like Shigella, and Salmonella species[8] Klebsiella pneumoniae and Escherichia coli are commonly isolated Enterobacteriaceae species in the humans, causing diseases including Upper Respiratory tract Infections, Urinary Tract Infections, Blood Stream Infections (BSIs). [12]

Klebsiella species

Klebsiella species is divided into four subspecies according to Cowen's classification. [13]

The pathogen generally colonizes the mucosal surface of the oropharynx and alimentary canal. When a pathogen invades, it shows various range of virulence and antibiotic resistance.

In the current scenario, Klebsiella pneumoniae pneumonia is considered the most common cause of healthcare-associated infections.

Classification:(6)

Klebsiella species belongs to tribe Klebsiellae Enterobacteriaceae family

Family: Enterobacteriaceae

Species: Klebsiella pneumonia

Klebsiella oxytoca

Klebsiella planticola

Klebsiella ornitholytica

Klebsiella terrigena

Genus Raoutella(new)

Subspecies: Klebsiella pneumoniae

Klebsiella aerogenes

Klebsiella ozaenae

Klebsiella rhinoscleromatis

Characteristics of Klebsiella in culture[12]

Members of the Klebsiella group are short, stumpy straight rods about 1-2 μm in length and 0.5-0.8μm in diameter, with parallel sides and rounded ends compared to other Enterobacteria.

They are non-motile and are capsulated, which can be demonstrated with Gram's stain [8]. With the identification of the presence or absence of capsular (K) somatic(O) and slime(M) antigens, Klebsiella strains have been divided into four smooth and four rough forms[6]

Smooth forms	**Rough forms**
1. MKO	MKR mucoid capsulated
2. KO	KR non-mucoid capsulated
3. MO	MR mucoid non-capsulated
4. O	R non-mucoid non-capsulated

When more capsular materials are produced, the colony development on agar is sumptuous, greyish white, mucoid colonies. It is because of the presence of high water content -92%(Toenniessen1921)-in capsule [14]

Pathogens can be inactivated by moist heat at 55ºC in 30 min and can survive for months. [8]At room temperature, cultures can be viable for weeks and sometimes for months.

These organisms are facultative anaerobes. No hemolysis of horse or sheep red blood cells on blood agar and produce lactose fermenting large mucoid glistening colonies due to the presence of polysaccharide capsule (K-antigen) in MacConkey agar. The ideal temperature for growth is at 37ºC, minimum and maximum are 12ºC and 43ºC respectively .[15]

Virulence factors and Pathogenicity[16]

The pathogenicity of Klebsiella pneumoniae is associated with virulence factors:[17]

1) Capsular Antigens
2) Adhesins
3) Siderophores
4) Lipopolysaccharides (Endotoxins).

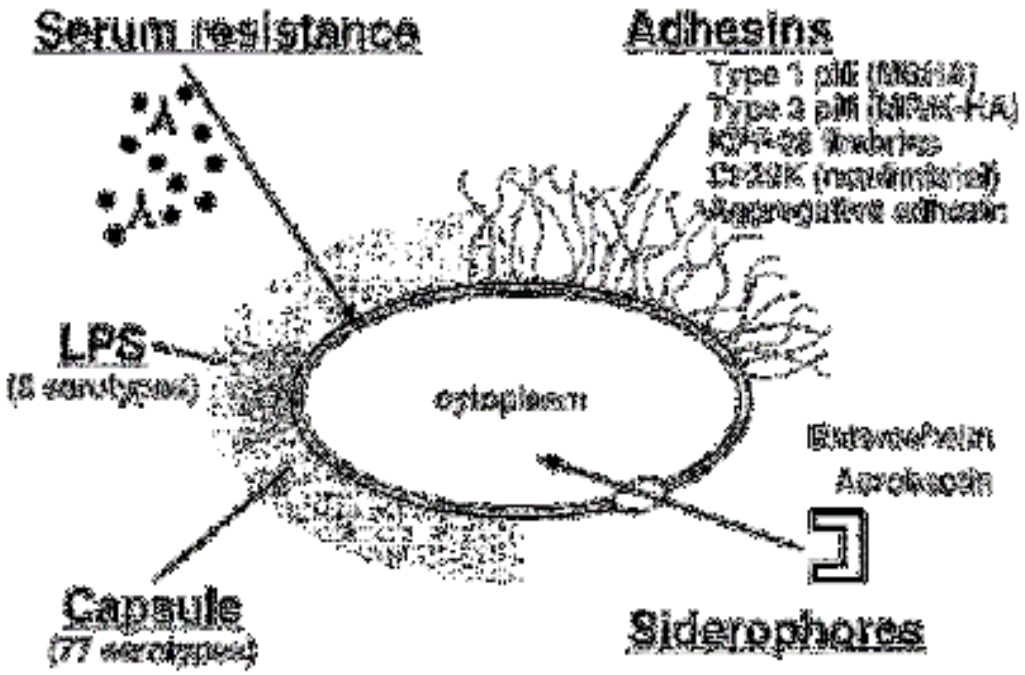


Figure 1: Virulence factors associated with Klebsiella species

The virulence of Klebsiella species is mainly because of the presence of capsule since it protects the pathogen getting from phagocytosed and prevents the organism's death with the production of bactericidal factors.[18]

Typing of Klebsiella isolates: [6]

Determination of clonality of strains is necessary for epidemiological study purposes.

Different methods are available for this, such as:

- Biotyping
- Serotyping
- Phage typing
- Bacteriocin (Klebocin) typing
- Molecular typing

INTRINSIC RESISTANCE OF KLEBSIELLA SPECIES:

Klebsiella species have an inherent resistance to Ampicillin, Ticarcillin, and Piperacillin because of chromosomal SHV-1-production. [19]

A combination of intrinsic resistance combined with resistance to commonly used antiseptics in pathogens, like Klebsiella pneumoniae, might favor the selection and spread of resistant organisms in the environment of healthcare facilities

Colonization in humans and spread in healthcare facilities

Klebsiella pneumoniae and other subspecies of Klebsiella are common colonizers of the skin, intestinal tract, and the naso-oropharynx. [14,20]

Carrier rates for these pathogens are on the lower side compared to other colonizers in the human body. Still, they are found to be increased in the case of admitted or patients treated with broad-spectrum antibiotics for a long time.[15,21]. Also, Klebsiella pneumoniae has been isolated from the hands of health care workers, which can be a causative factor for the spread of these pathogens in the healthcare facilities leading to outbreaks in intensive care units or any high-risk wards.

Infections caused by Klebsiella pneumoniae

They were known to cause pneumonia or Friedlander's pneumonia, which occurred in the community mainly in immunocompromised patients.

Nowadays, Klebsiella pneumoniae is primarily known to cause healthcare-associated infections along with other common ailments.[22] Common types of infections caused by Klebsiella pneumoniae in health care facilities are mainly, blood stream infections, surgical site infections, ventilator associated pneumoniae and urinary tract infections. Procedures like catheterization and/or insertion of central/peripheral lines also plays a key role in the colonization and transmission of these organisms within the health care facility.

Immunocompromised patients are mainly at risk of opportunistic Klebsiella-associated infections. The current rate of healthcare-associated Klebsiella pneumoniae infections is found to be higher in patients who are carriers when compared to non-carriers. Treatment with antibiotics interferes with the development of normal intestinal flora, especially with Ampicillin, has been associated with increased Klebsiella carrier rates in patients who are admitted to ICUs [23]

Resistance to commonly used antibiotics is considered an emerging threat as it leads to limited treatment options for infections caused by these pathogens.

Beta Lactam Antibiotics

In 1928, Alexander Fleming discovered the first beta-lactam, penicillin; different antibiotics were discovered after that. The Beta-lactam class of antibiotics consists of the largest group; they became widely popular due to their bactericidal action and lack of toxicity to humans. [8]

Structure of beta-lactams [6]

Beta-lactam antibiotics contain a four-membered beta-lactam ring containing nitrogen at the core of their structure.

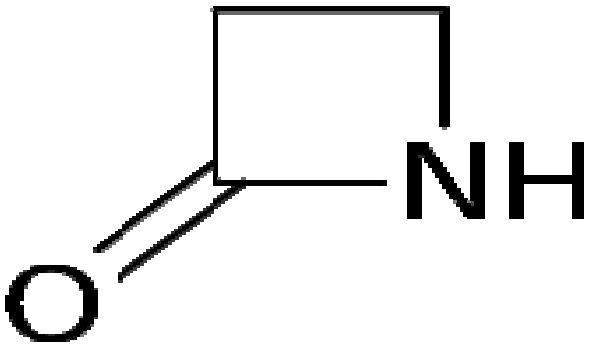


Figure 2: Structure of beta-lactam ring

Classification of Beta-lactam antibiotics

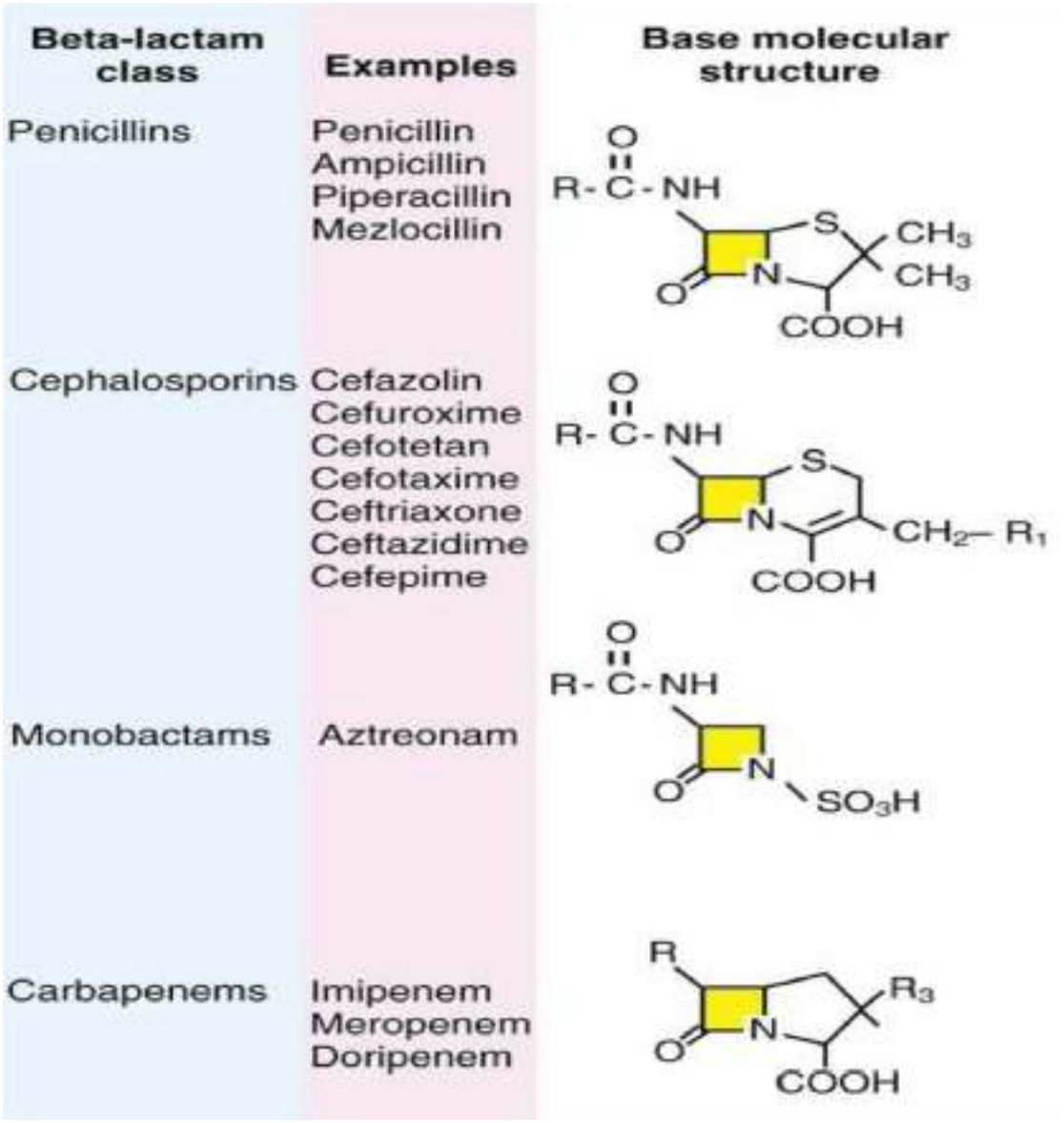


Figure 3: β lactam antibiotics

Mechanism of action(MOA)

B-lactam group acts by interfering with the formation of peptidoglycan. The structural similarity between the Penicillin molecule and the Dalanine-D-alanine terminus of the peptidoglycan chain was responsible for the antibacterial action of the compound [12]

Once penicillin gets attached to the penicillin-binding protein (PBP), it blocks the transpeptidation, thus terminating further elongation of the peptidoglycan.

The effectiveness of beta-lactams against different types of bacteria can vary widely because of differences in PBP content, natural structural characteristics, and their common antimicrobial resistance mechanisms among bacteria. [8]

Resistance mechanism to Beta -lactams

1. Enzyme production, i.e., beta-lactamases that bind and hydrolyze these drugs, are the standard mechanism of bacterial resistance to beta-lactams.[12]

2. Another vital mechanism for enteric Gram-negative bacteria resistance is alterations in penicillin-binding protein. [13]

The level of antibiotic resistance possessed by particular β-lactamases in

bacterial population is determined by several variables [12, 8]

1. Affinity of the enzyme for an antibiotic.
2. Susceptibility of target protein, i.e., PBP to the antibiotic.
3. Quantity of beta-lactamases produced by the bacterial cell.
4. Efficiency of the β-lactamases in hydrolyzing the antibiotic.
5. Diffusion rate of the antibiotic into the cell.

Beta Lactamases:

They are a family of enzymes, playing a crucial role in the development of resistance to existing antibiotics. The role of these enzymes is to restructure the peptidoglycan during bacterial cell growth. They inactivate any beta-lactam, antibiotics, or group of antibiotics[6]

Classification of beta-lactamases:

Various researchers have proposed many schemes of classification. One of the widely accepted classification schemes was proposed by Bush in 1989.[24] Another classification based on the molecular structure was proposed by Ambler, which includes only those enzymes that have been characterized .[25 26]

Bush – Jacoby – Medeiros Classification.[24]

This classification integrates functional and molecular characteristics.

Bush–Jacoby (2010)[a]	Ambler Molecular Class	Enzymes	Active Site	Antibiotic Substrate(s)	Enzyme Inhibition[b]	Found in
Group 1 Cephalosporinase	C	Amp C, ACT-1, CMY-2, FOX-1, MIR-1	Serine	Hydrolyzes cephalosporins including cephamycins	PBA, DPA, Cloxacillin	Enterobacteriaceae, *Acinetobacter* spp.
Group 1e Cephalosporinase	C	GC1, CMY-37	Serine	Increased hydrolysis of ceftazidime and other oxymino-β-lactams[c]	Not inhibited by CV or FTZ	Enterobacteriaceae
Group 2a Penicillinase	A	PC1	Serine	Increased hydrolysis of penicillin	CV or FTZ	*S. aureus*
Group 2b Penicillinase	A	TEM-1, TEM-2, SHV-1	Serine	Penicillin, first-generation cephalosporins	CV or FTZ	Enterobacteriaceae
Group 2be ESBLs	A	TEM-3, SHV-2, CTX-M, PER-1, VEB-1	Serine	Increased hydrolysis of oxymino-β-lactams[c]	CV or FTZ	*E. coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *C. freundii*, *Salmonella* spp., *Morganella* spp. (CTX-M)
Group 2ber ESBLs	A	TEM-50	Serine	Increased hydrolysis of oxymino-β-lactams[c] + resistance to inhibitors	Not inhibited by CV or FTZ	Enterobacteriaceae
Group 2c	D	OXA-01, OXA-10		Hydrolysis of oxacillin or cloxacillin	Variable with CV or FTZ	Enterobacteriaceae
Group 2de ESBLs	D	OXA-11, OXA-15	Serine	Hydrolysis of oxacillin or cloxacillin + oxymino-β-lactams[c]	Variable with CV or FTZ	*P. aeruginosa*
Group 2df Carbapenemase	D	OXA-23, OXA-48	Serine	Hydrolyzes carbapenems	Variable with CV or FTZ	*A. baumannii*, Enterobacteriaceae
Group 2e ESBL	A	Cep A	Serine	Hydrolyzes cephalosporins	CV but not cefotetan	Proteae
Group 2f Carbapenemases	A	KPC-2, SME-1, IMI-1	Serine	Hydrolyzes carbapenems, oxymino-cephalosporins[c] and cephamycins[d]	Variable with CV or FTZ	Enterobacteriaceae
Group 3 Metallo-Carbapenemases	B	IMP-1, VIM-2, IND-1, L1	Zinc	Hydrolysis of carbapenems but not monobactams	EDTA	*P. aeruginosa*, *A. baumannii*
Group 4	Not included	Unknown				

[a] Updated Bush–Jacoby classification adapted from reference 90.
[b] β-Lactamase inhibitors: PBA, phenylboronic acid; DPA, dipicolinic acid; CV, clavulanic acid; TZ, tazobactam; EDTA, ethylenediaminetetraacetic acid.
[c] Oxymino-cephalosporins = ceftotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam.
[d] Cephamycins = cefoxitin and cefotetan.

Figure 4: Bush–Jacoby β-Lactamase Classification Compared to the Ambler Molecular Class System[6]

ESBLs:

ESBLs belongs to class A or D βlactams which

1. Have an active site serine
2. Can hydrolyze oxyimino cephalosporins
3. Inhibited by BLIs such as clavulanic acid or sulbactam

ESBL production is either chromosomally mediated or plasmid-mediated [18]

The spectrum extends mutation in amino acid sequence position, providing space for enzyme interaction.

ESBLs can hydrolyze β lactam antibiotics containing oxyimino group (Ceftazidime, Cefotaxime, Ceftriaxone, Cefuroxime, Aztreonam).[27]

Carbapenems:

They are a broad-spectrum group of antibiotics structurally similar to penicillin but have a Sulphur group in the C1 position. Resistance to this group of antibiotics by Gram-negative organisms is by acquiring:

Structural alterations in drug targets like penicillin-binding proteins (PBPs), or loss of porins or with upregulation of efflux pumps or expression of Carbapenemase enzymes. [6]

Production of Carbapenemase enzyme by the organisms is considered to be of utmost importance.

Carbapenemases are enzymes that hydrolyze the βlactam groups with alteration at the target site of the antibiotics, which in turn reduces its binding capacity.

These enzymes are generally classified into two types based on the amino acid sequences:

1. Metallo beta –lactamases
2. Serine beta -lactamases

Class A Carbapenemase is an imperative mechanism of resistance across the world.[20]

The current dissemination of Carbapenemase enzyme-producing organisms across the world cause great concern. [28]

MBL genes are mobile genetic elements that can be transferred quickly, possess a more significant threat to the spread of drug-resistant strains.[29]

Epidemiology

Resistant strains are isolated from hospitals and communities worldwide, along with the increase in the incidence of organisms producing Carbapenemase enzymes; [30] this leads to limited treatment options. Hence it is of the highest priority to know the prevalence of these pathogens to formulate treatment policies. [31]

Some of the risk factors for infection with Carbapenemase-producing organisms are:

1. Long term antibiotic therapy
2. Prolonged ICU admissions
3. Severe illness.
4. Instrumentation or Catheterization.

Global scenario

Carbapenemases belonging to the KPC family have the most extensive global distribution. The first KPC-producing organisms in the US were isolated from a patient from North Carolina. [32]

KPC enzymes have also been reported in Asia, mainly from China

In a study conducted in Shangai, it was observed that Carbapenemase resistant Escherichia coli were more predominant, producing KPC.[33]

In another study from 2011, bla KPC-2 was observed to be 71% of 109 ertapenem-resistant Klebsiella pneumoniae isolates. [34]

In India

New Delhi Metallo-beta-lactamase (NDM) is found to be higher in India.

A study conducted by Swaminathan et al. in Kerala observed that 11% of organisms isolated were resistant to Meropenem and Imipenem.[35]

They were subjected to PCR, confirming NDM in the isolates.

In another study done at a tertiary care referral hospital, Guwahati, northeast India, by Bora et al. reported that among 219 K. pneumonia isolates, 19 were screened for the Carbapenemase production, and all were found to be finally positive for MHT. [36]

In a retrospective study conducted by Modi et al., the prevalence of CRE was found to be 29.07% in Klebsiella species at a tertiary hospital in Gujarat[37]

Healthcare-associated infection

They are infections that occur when in a health care facility or developed in a hospital or other health care facility, appearing 48 hours or more after admission or within a month of discharge. [38, 16] also includes infections in a neonate as a result while passing through the birth canal(congenital infections are an exception) and occupational infections among the health care facility staff. [22]

The burden of HAI

Healthcare-associated infections are common adverse events of the Healthcare system. The endemic and epidemic occurrence of HAIs has become a significant public health problem related to mortality, morbidity, quality of life, and economic stability. [38]

The CDC identifies nearly 1.7 million hospitalized patients acquire HCAIs yearly while being in treatment for other health issues, and approximately more than 98,000(one in 17) die due to these. [39]

Causative organisms in HAI's

Most of the organisms causing HAIs are Multidrug-resistant, one of the reasons being irrational and unethical use of antibiotics, which leads to an increase in the number of resistant organisms in the environment around health care facilities under the influence of selective antibiotic pressure. [22]

As a result of these drug-resistant organisms replace the susceptible organisms in the environment, which contribute to increasing resistance patterns in community-acquired infections.

Common organisms responsible for HAI have been given a new acronym by the Infectious Diseases Society of America (IDSA) of ESKAPE pathogens, indicating the capability of these organisms to 'Escape' the biocidal actions of antibiotics mutually represents newer paradigms in pathogenesis, transmission, and resistance. (22)

Enterococcus faecium

Staphylococcus aureus

Klebsiella pneumoniae

Acinetobacter baumanii

Pseudomonas aeruginosa

Enterobacter species

Compilation of these organisms has been expanded as ESKAPES to include Stenotrophomonas maltophilia; it is not enlisted in IDSA. In comparison with the Indian setting, multidrug-resistant Escherichia coli was a significant pathogen causing HAIs.[22]

Types of Healthcare-associated infections[13]

The common types which are often monitored to estimate the burden of HAIs are :

1. Catheter-associated urinary tract infection(CAUTI)
2. Central line-associated bloodstream infection(CLABSI)
3. Ventilator-associated pneumoniae(VAP)
4. Surgical site infection(SSI)

HAI Prevention

The prevention of HAI can be classified into

1. Standard precautions(Routine)
2. Transmission or specific precautions

Standard precautions are routine infection control practices implemented to prevent diseases, which can be easily acquired by contact with body fluids or mucous membrane.

The measures for precaution include hand hygiene, use of PPEs, sharps handling, biomedical waste management, environmental disinfection practices, etc

Specific precautionary measures mainly include additional precautions which are taken where standard precautions are insufficient.

Transmission based precautions are mainly of three forms

1. Droplet precautions
2. Airborne precautions
3. Contact precautions

WHO has proposed specific components for infection control.

They recently set up the core components for infection prevention and control (IPC) in 2016. It recommends eight evidence-based core components that must be implemented in healthcare facility and at the national level.

All the components are crucial for the implementation and proper functioning of IPC.[19]

1. Infection prevention control programmes, consisting of dedicated and trained personals.
2. Infection prevention and control guidelines for reducing HAI and antimicrobial resistance(AMR)
3. Infection prevention and control education with training and facilities available for healthcare workers reduce HAI and AMR.
4. Surveillance, timely AMR surveillance, and feedback of results HCWs, so that outbreaks won't be missed and promptly notified at the national level.
5. Multimodal strategies: WHO recommends five components as a part of multimodal strategies
   i. System change
   ii. Education and training of healthcare workers
   iii. Monitoring infrastructures, practices, outcome, providing da ta
   iv. Workplace visual reminders
   v. Cultural change with establishments and strengthening, safety climate
6. Monitoring/audit of IPC practices
7. Workload, staffing, and occupancy of bed
8. Environment, materials, and equipment for IPC should be built at the facility

Screening for multi-drug resistant organisms in a healthcare facility is important to reduce the mortality, morbidity and burden of the healthcare-associated infections and prevent the dissemination of these organisms into the community.

METHODOLOGY

Study center: Department of Microbiology, KAHER's J.N.Medical Coleege, Belagavi

Source of data: All Klebsiella pneumoniae isolated from various clinical samples in the microbiology laboratory.

Study design: Cross-sectional study

Study period: One year- 1st January -March 31st 2020

Study population: Klebsiella pneumoniae isolated from various clinical samples received from a tertiary care center

Inclusion criteria: All Klebsiella pneumoniae isolated in the lab

Exclusion criteria: All Commensals (Klebsiella pneumoniae isolated from stool samples)

Sample size :123 Klebsiella pneumoniae isolated over one year

Sampling procedure: Universal sampling

Statistical analysis: Prevalence was calculated and expressed in percentage.

Instruments used for data collection: Proforma

Methodology overview

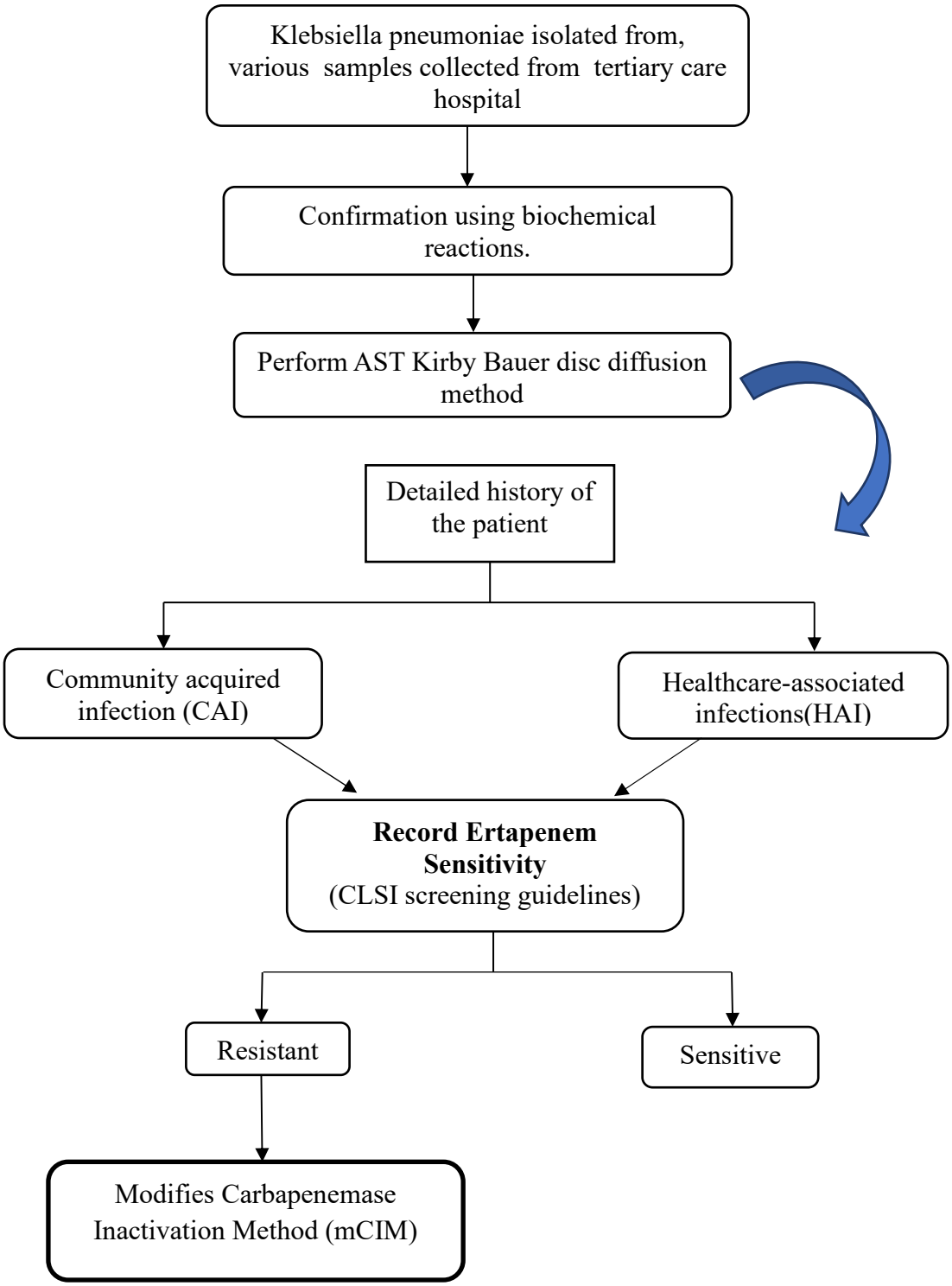


Figure 5: Overview of the methodology

IDENTIFICATION OF KLEBSIELLA SPECIES

According to gram stain, colony morphology, and specific biochemical reactions, the organism was identified and confirmed to be Klebsiella pneumoniae.

Gram stain: shows short, stumpy straight rods about 1-2 μm long and 0.5-0.8μm in diameter, with parallel sides and rounded ends

Colony morphology:

1. On MacConkey agar: circular colonies measuring 2-3mm in diameter, with regular edge, convex, mucoid, lactose fermenting colonies were observed.
2. On blood agar: greyish circular colonies measuring 2-3mm in diameter, regular edge, convex, nonhemolytic, mucoid colonies were observed.
3. On chocolate agar: circular greyish, large with a regular edge, convex, mucoid colonies were observed

The isolated colonies were inoculated in sterile peptone water for further identification with biochemical reactions and antibiotic susceptibility testing. The inoculum was adjusted to 0.5 Mc Ferlands standard and was kept for incubation for 2 hrs. at 37°C

The battery of biochemical tests put for identification of Klebsiella pneumoniae was

1. Catalase test
2. Indole production test (Kovac's reagent)
3. Urease production test on Christensen's media
4. Citrate utilization test on Simmon's citrate utilization media
5. Mannitol fermentation test

6. Triple sugar iron media
7. Hugh Leifson's Oxidative – Fermentative test
8. Nitrate reduction test
9. M R test
10. V P test
11. Glucose fermentation test
12. Ornithine decarboxylase test

After overnight incubation, the results were interpreted according to the following biochemical reactions table

Table 2: Biochemical reaction of important Klebsiella species

Biochemical tests	K.pneumoniae	K.oxytoca	K.ozaenae	K.rhonoscleromatis
1. Catalase Test	+	+	+	+
2. Indole Test	-	+	-	-
3. Urea Test	+	+	-	-
4. Citrate test	+	+	+	-
5.Mannitol Fermentation Test	F	F	F	F
6. TSI Test	A /A ,GAS	A /A ,GAS	A /A ,GAS	A /A ,GA S
7. Hugh-Leifson's O/F test	F	F	F	F
8. Nitrate Reduction Test	+	+	+	+
9. Methyl Red	-	-	+	+
10. Voges Proskauer Test	+	+	-	-
11. Glucose Fermentation Test	+	+	+	+
12. Ornithine Decarboxylase Test	-	-	-	-

Antibiotic sensitivity testing was done on Mullen Hinton Agar by Kirby Bauer disc diffusion method; Each isolated organism was tested against AMP(30µg), GEN(30µg), TOB(10µg), AMC(20/10µg), IMP(10µg), MRP(10µg), CTX(30µg), PIT(100/10µg), also NIT(300µg) and FO(300µg) were used for only urine samples

Screening of Carbapenemase producers

All Klebsiella pneumoniae isolates were screened using Ertapenem (10µg) on Muller Hinton agar. Whether the strain was sensitive or resistant to ertapenem, it was confirmed for Carbapenemase production using a confirmatory test. [4]

Interpretation

According to CLSI guidelines 2019, Ertapenem sensitivity pattern was recorded.

- <18 mm– Resistant
- 19-21 mm- Intermediate
- >22 mm- Sensitive

Modified Carbapenemase Inactivation Test (mCIM) [40]

As per CLSI 2019 guidelines, the mCIM test is recommended for the Carbapenemase detection replacing MHT

For the mCIM test, each isolate which was resistant to Ertapenem was emulsified (1µl) in two ml of TSB, a Meropenem disc(10µg) was placed in the broth and incubated at 35 °C,4 hours ± 15 minutes.

After the required time, the meropenem disc was taken out from TSB and placed on a Muller Hinton agar, freshly inoculated with 0.5 McFarland suspension of

Carbapenemase sensitive E.coli ATCC 25922 strain. The inoculated plates were incubated at 35°C for 18- 24hrs, interpretation of the result was made according to CLSI guidelines 2019. [5]

Interpretation

- Carbapenemase positive : zone size 6 – 15mm
- Carbapenemase negative : zone size >19mm
- Carbapenemase intermediate: 16 – 18mm.

Classification of isolates into a community-acquired infection (CAI), Healthcare-associated infections(HAI) was done based on taking a detailed history of the patient regarding previous hospital stay, any antibiotic regimen taken during the stay, and any recurrent hospital admissions, with the help of proforma

Criteria for HAIs[22]

Patients who satisfy the following criteria were included in this category

1. Infection acquired in a facility by a patient admitted for reasons other than the infection in the context
2. The infection should not be in the incubation period, or no symptoms should be present when getting admitted.
3. Symptoms appearing after 48hrs of admission or after within 30 days of leaving the facility

Criteria for CAI

They are contracted outside of a health care facility or diagnosed within 48 hours of admission without any previous encounter.[41]

COLOUR PLATES

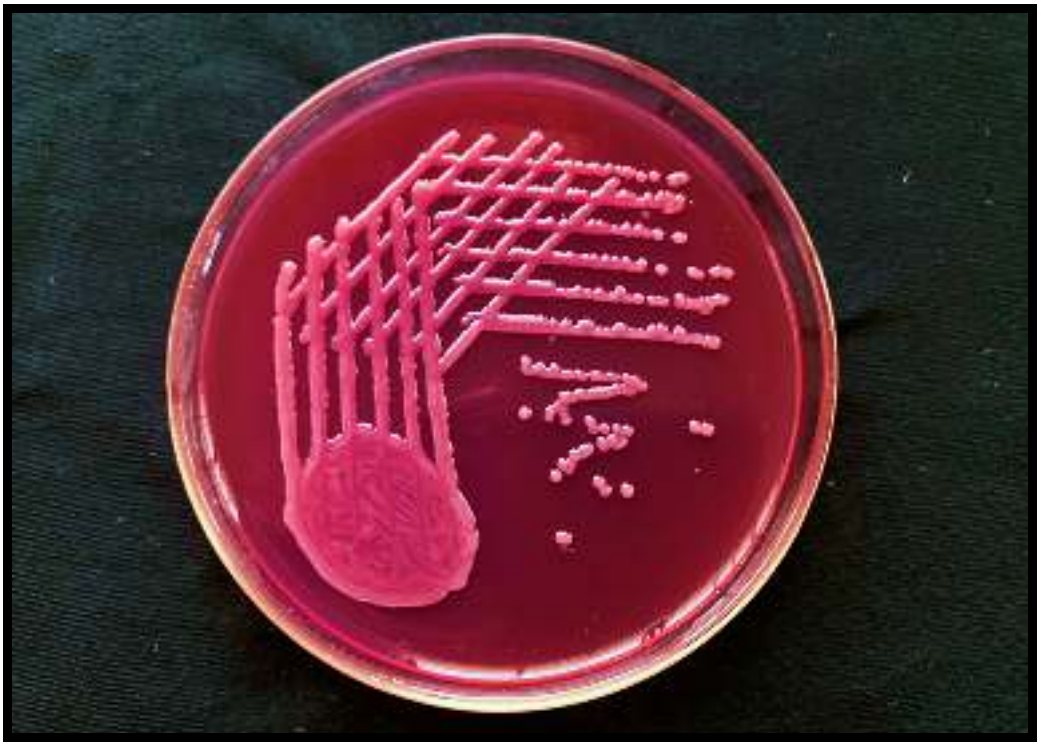


Figure 6: Showing lactose fermenting, mucoid colonies of Klebsiella pneumoniae on MacConkey agar medium

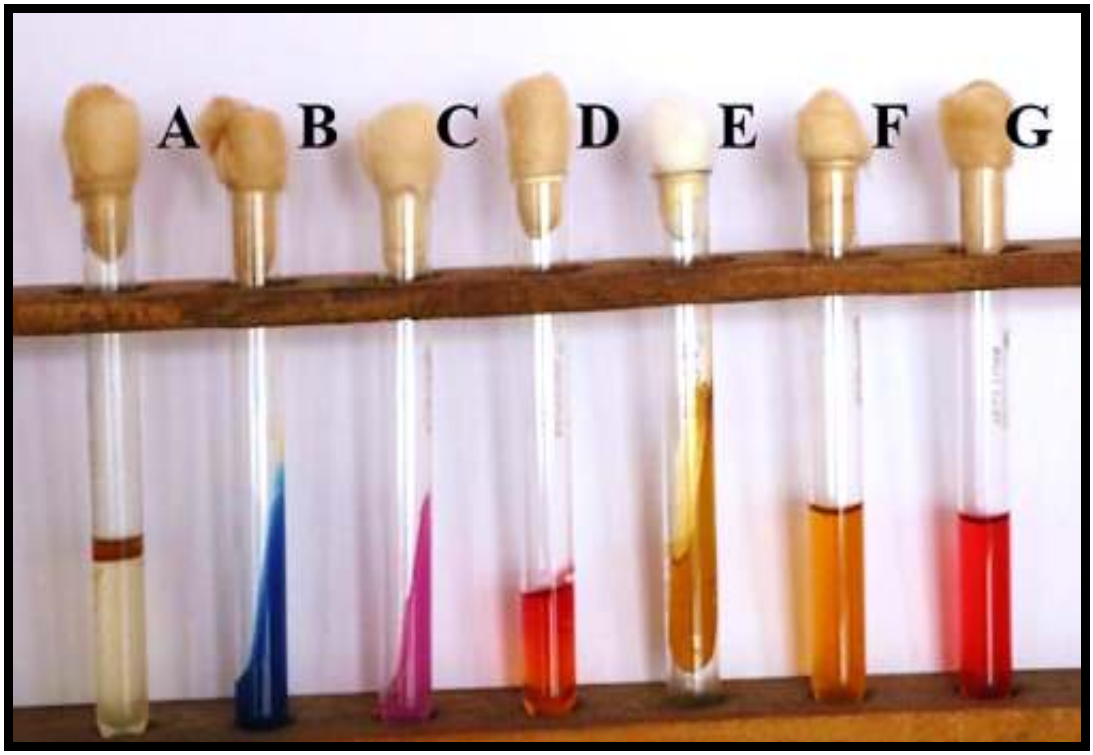


Figure 7 a:Biochemical reactions of Klebsiella pneumoniae

Table 3a: Biochemical reactions of Klebsiella pneumoniae

Tubes	Biochemical reactions	Result
A	Indole	Negative
B	Simmon's Citrate agar	Utilized
C	Christensen urea agar	Hydrolyzed
D	Mannitol motility medium	Fermented and non-motile
E	TSI	Acid /acid with gas; no H2S production
F	MR	Negative
G	VP	Positive

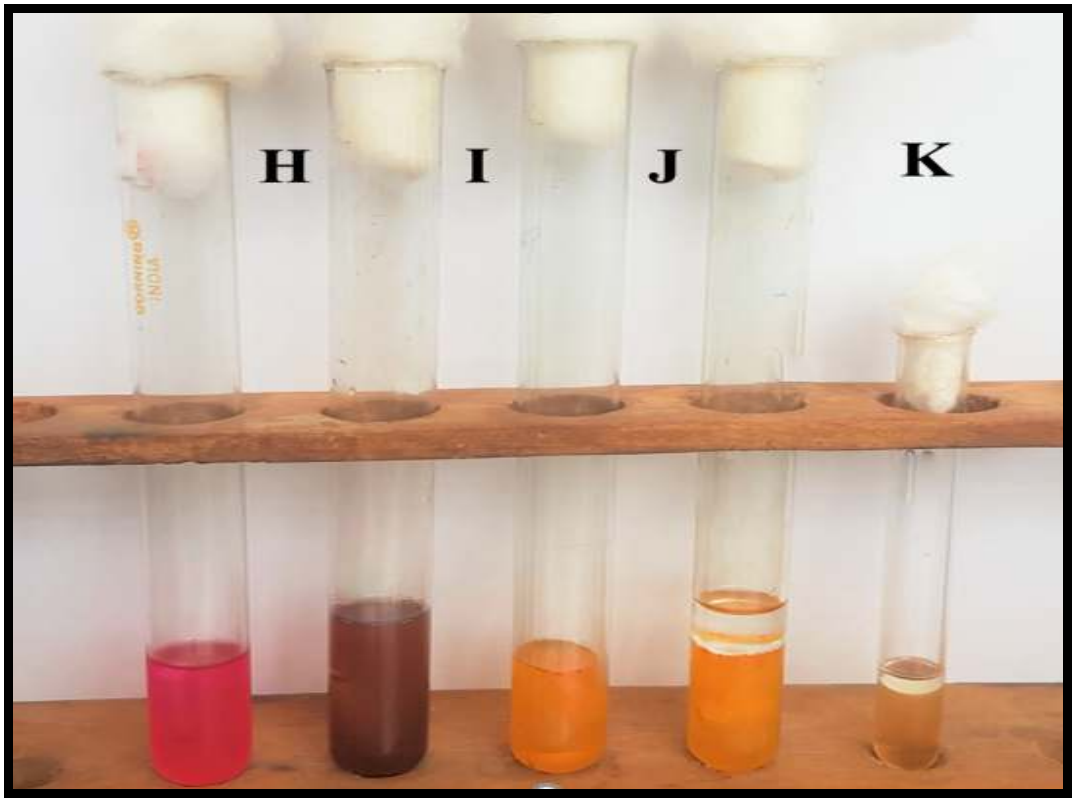


Figure 7b: Biochemical reactions of Klebsiella pneumoniae

Table 3b: Biochemical reactions of Klebsiella pneumoniae

Test tube	Biochemical Test	Result
H	Glucose fermentation test	Fermented with gas
I	Nitrate reduction test:	Reduced to nitrite
J	Hugh and Leifson's Oxidative-fermentative Test	Fermentative with gas production
K	Ornithine decarboxylase test	Negative

Figure 8: Screening for CP-KPN using Ertapenem disc by disc diffusion method.

Result interpretation

According to CLSI guidelines 2019, Ertapenem sensitivity pattern was recorded.

- <18 mm– Resistant
- 19-21 mm- Intermediate
- >22 mm- Sensitive

Figure 9: Showing Meropenem(10µg) in Trypticase soy broth inoculated with the test isolate

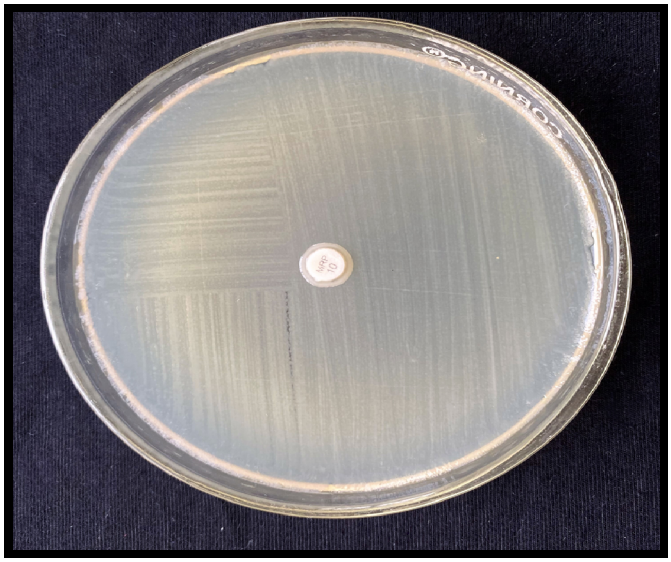


Figure 10: Meropenem disc plated on MHA plate inoculated with E.coli ATCC25922

Result interpretation

- Carbapenemase positive : zone size 6 – 15mm
- Carbapenemase negative : zone size >19mm
- Carbapenemase intermediate: 16 – 18mm.

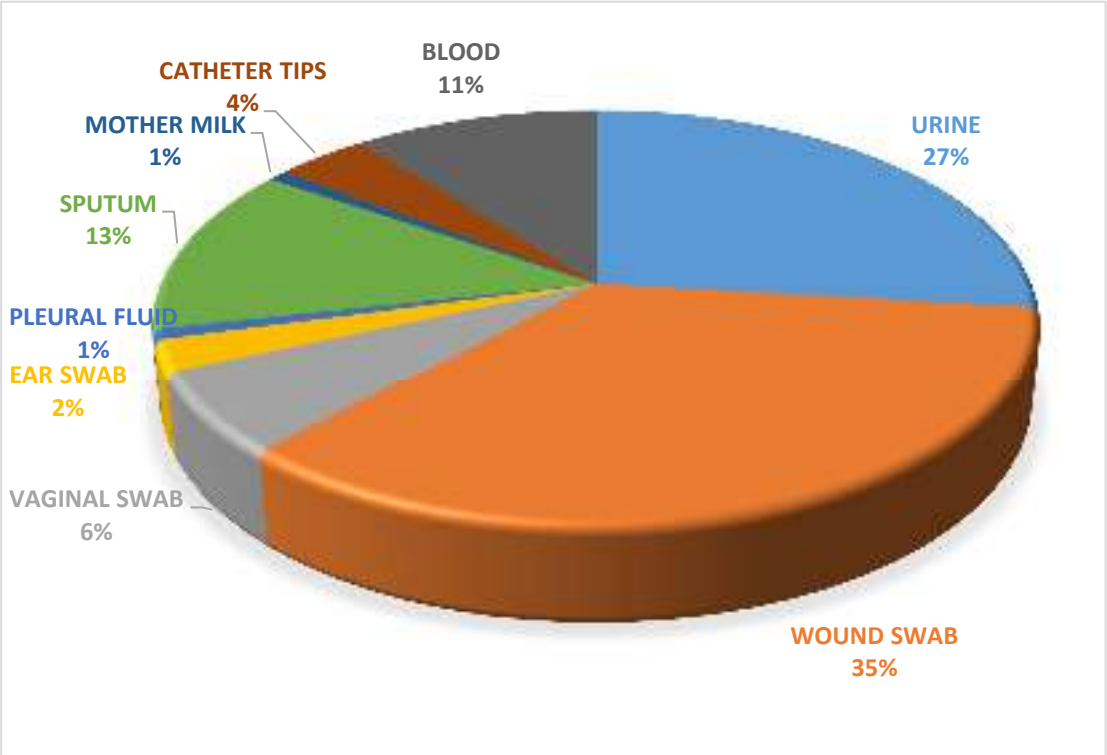
RESULTS

Various samples received in the department of microbiology, were processed for isolation, antibiotic sensitivity, and Carbapenemase production.

Table No 4: Distribution of Klebsiella pneumoniae in various clinical samples

Types of samples	Number of samples	Percentage
WOUND SWAB	43	35%
URINE	33	27%
SPUTUM	16	13%
BLOOD	13	10.5%
VAGINAL SWAB	8	6.5%
CATHETER TIPS	5	4%
EAR SWAB	3	2.4%
PLEURAL FLUID	1	0.8%
MOTHER MILK	1	0.8%
TOTAL	123	100%

Graph No.1: Distribution of Klebsiella pneumoniae in various clinical samples



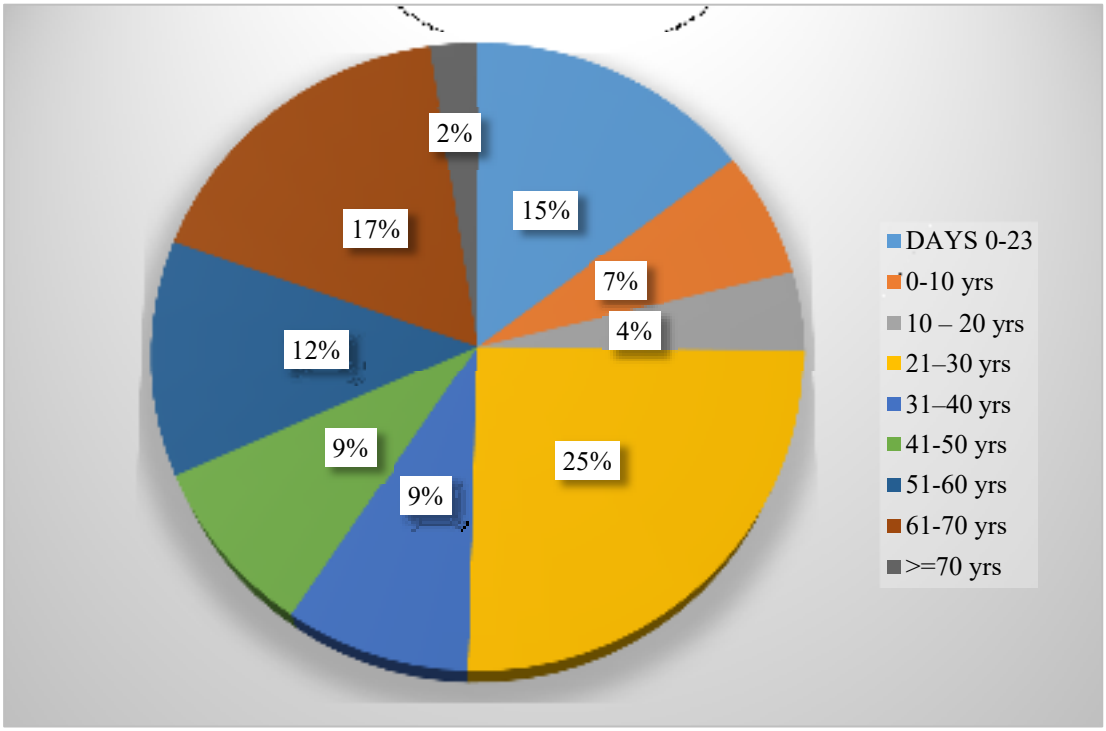
During this study period, 123 confirmed KPN species, isolated from diverse samples for the study.

Among them, it was commonly isolated from Wounds swabs(35%), followed by Urine sample n=(27%)

Table No.5: Age-wise distribution of cases (n= 123)

Age group	Number of patients	Percentage
DAYS 0-23	18	15%
0-10 yrs	8	6.5%
10 – 20 yrs	5	4%
21–30 yrs	31	25%
31–40 yrs	11	9%
41-50 yrs	11	9%
51-60 yrs	15	12%
61-70 yrs	21	17%
>=70 yrs	3	2.5%
TOTAL	123	100%

Graph No.2: Age-wise distribution of cases (n=123)

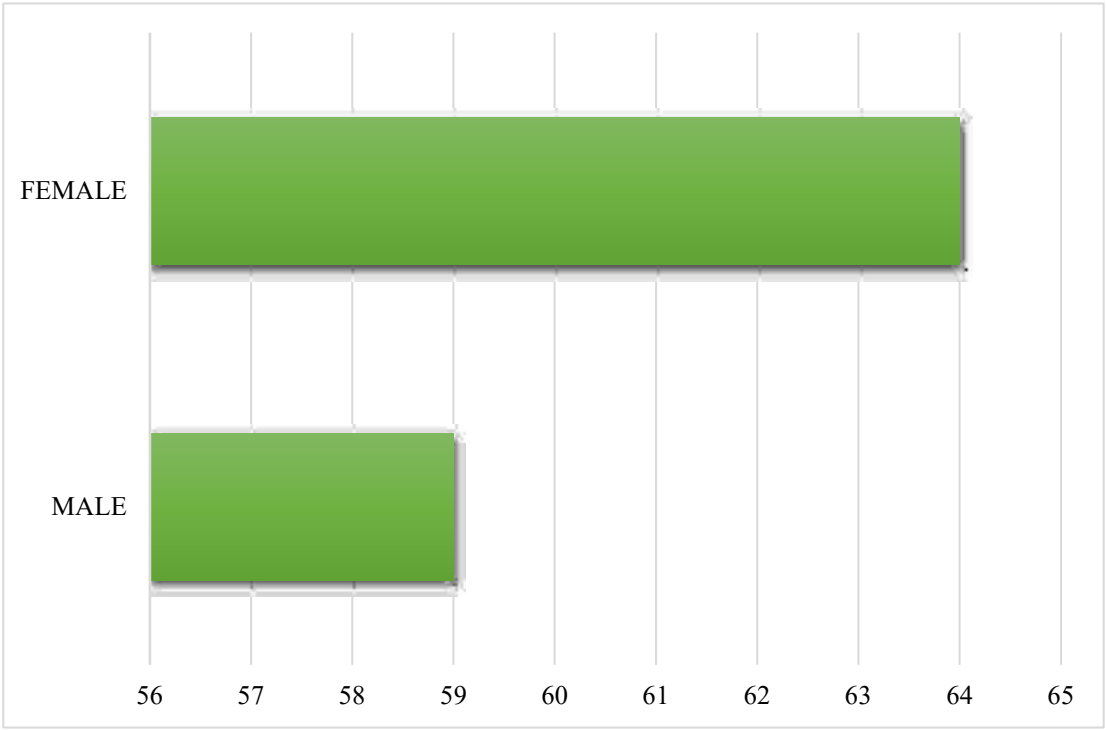


This study among the age groups included, Klebsiella pneumoniae commonly isolated between 21yrs -30yrs (n= 31, 25%), followed by age group of 61yrs - 70yrs (n= 21, 17%). It was also observed that the neonatal age group 0- 23days(n=18, 15%) was also highly affected by Klebsiella pneumoniae.

Table No.6: Gender wise distribution of isolates

Gender	No.of patients	Percentage
Male	59	48%
Female	64	52%
Total	123	100

Graph 3: Gender wise distribution of isolates

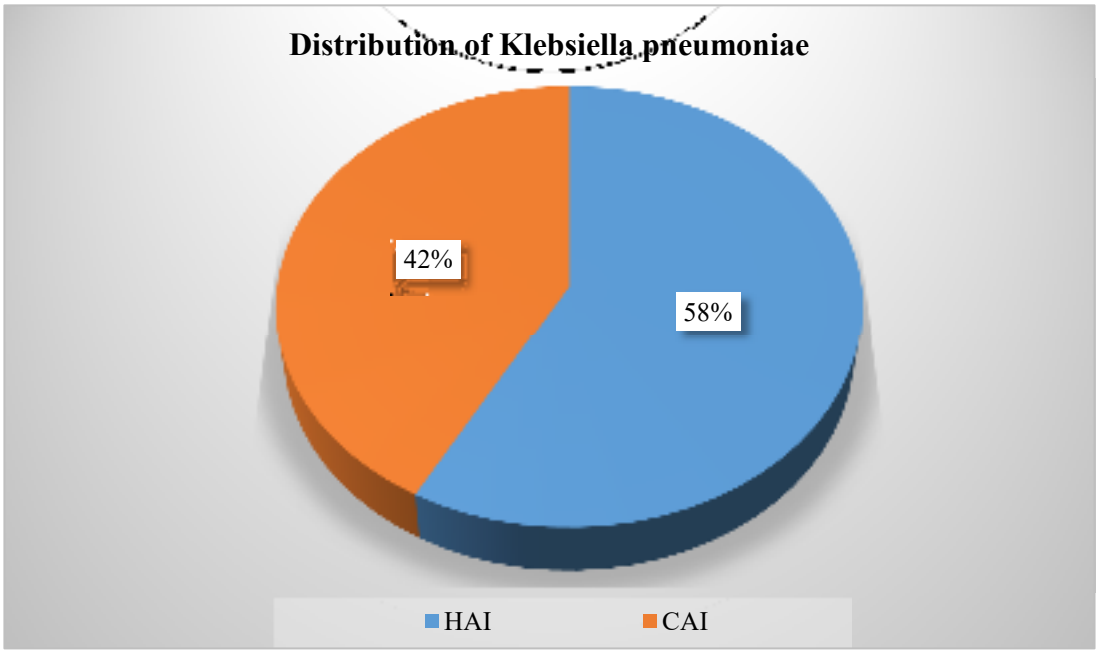


In our study, the maximum number showed a predominance of females, 58%, compared to males, 48%.

Table No7 : Distribution of Klebsiella pneumoniae isolated in the study

Klebsiella pneumoniae isolates	No. of isolates
HAI	71(58%)
CAI	52(42%)
TOTAL	123

Graph No.4: Distribution of Klebsiella pneumoniae in the study



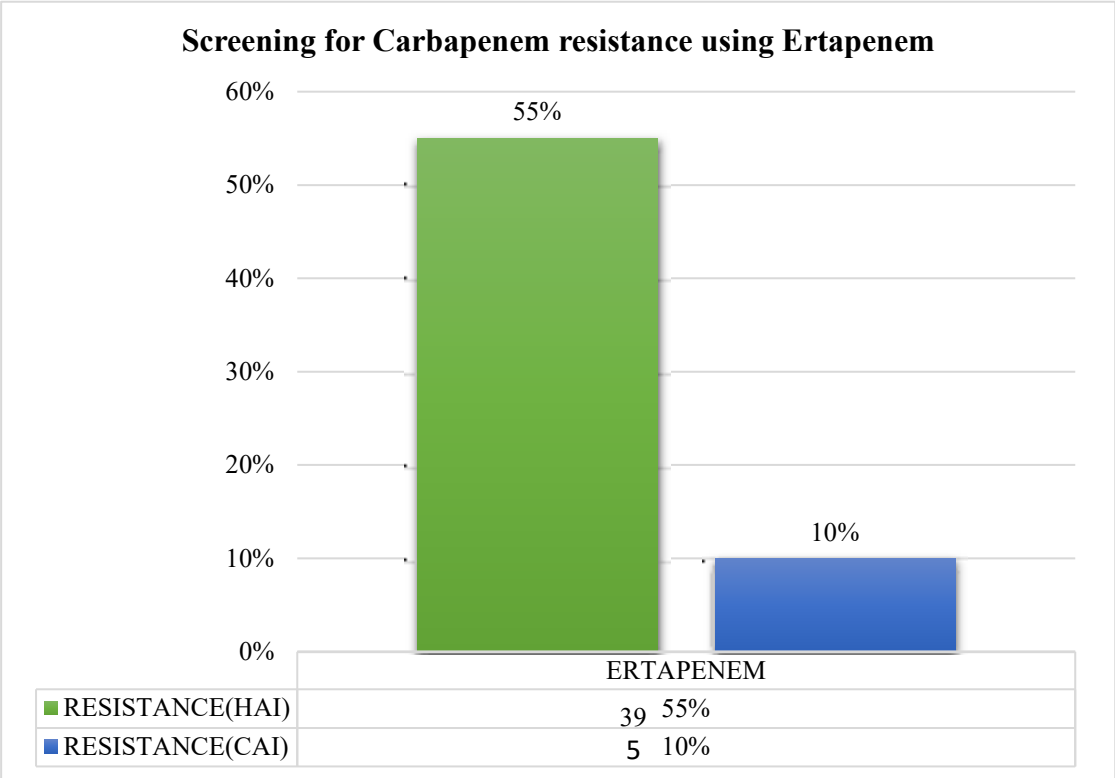
In this study, the isolates were categorized as 1) HAIs and 2) CAIs, after taking a proper patient history and according to the definition.

Among the 123 isolates of Klebsiella pneumoniae, it was observed that 71(58%) was categorized under Hospital-acquired infection, and 52(42%) was classified under Community-acquired infection.

Table No 8: Screening of Carbapenemase production using Ertapenem

Antibiotic	RESISTANCE	
	Healthcare Associated Infections	Community Acquired Infections
ERTAPENEM	39(55%)	5(10%)

Graph No.5: Screening for Carbapenem resistance in Klebsiella pneumoniae

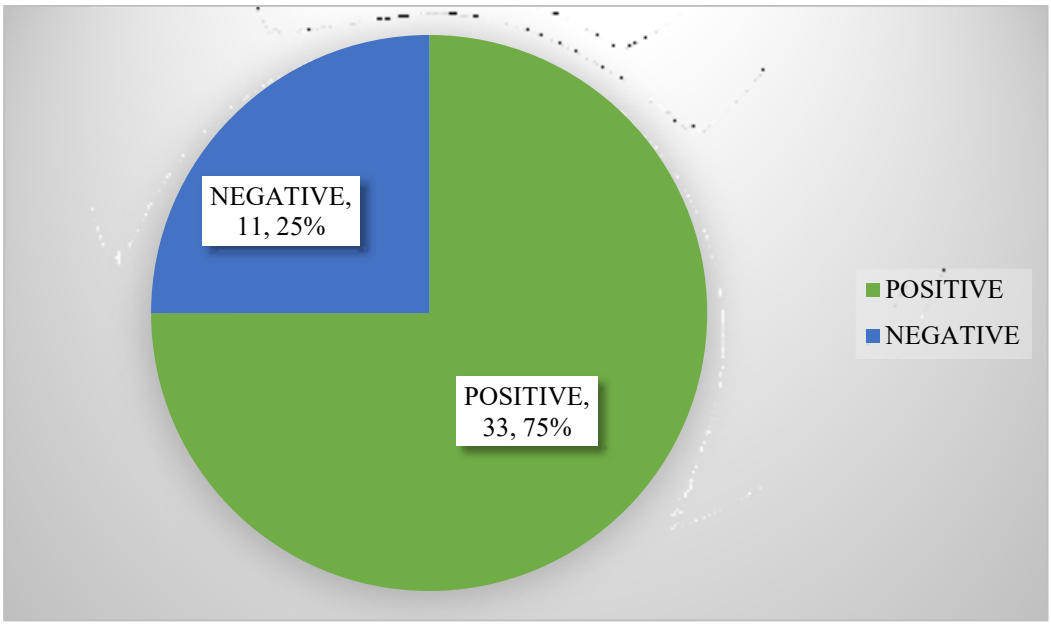


All the 123 samples were screened for Carbapenemase production using Ertapenem (10µg) antibiotic disc. Among them, total of 44 (36%) isolates were found to be resistant to Ertapenem, out of which 39(55%) belonged to the healthcare-associated infection, and 5(10%) belonged to the Community-acquired infection category.

Table No 9: Modified Carbapenemase Inactivation test(mCIM) on Ertapenem resistant Klebsiella pneumoniae isolates.

Modified Carbapenemase inactivation method (mCIM)				
	HA I	**CA I**	**TOTA L**	
Positive	33	0	33	75%
Negative	6	5	11	25%

Graph No.6: Modified Carbapenemase Inactivation test (mCIM) on Ertapenem resistant Klebsiella pneumoniae isolates.



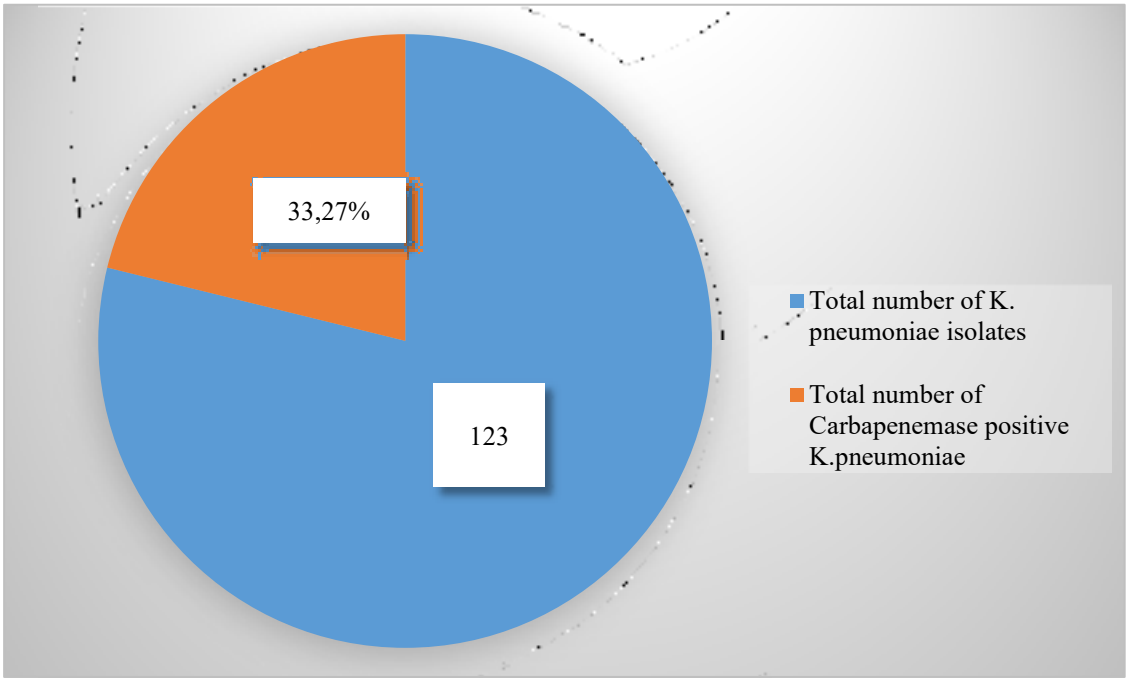
A total of 44 isolates resistant to Ertapenem were tested with the Modified Carbapenemase Inactivation test (mCIM).

Out of which 33(75%) were found to be positive with the test, indicating confirmed Carbapenemase enzyme production by the organism, and 11 (25%) were found to be negative.

Table No.10: Prevalence of Carbapenemase-producing Klebsiella pneumoniae (CP-KPN)

Klebsiella pneumoniae isolated	Cp-KPN isolated
123	33 (27%)

Graph No.7: Prevalence of CP-KPN in the study

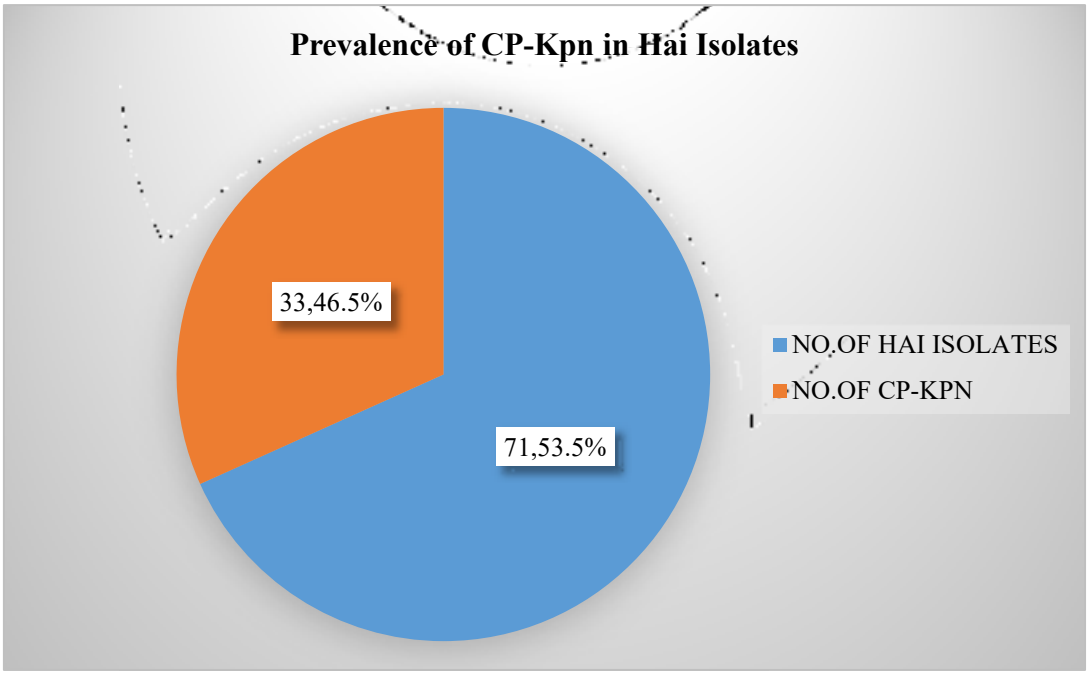


The prevalence of CP-KPN was found to be 27%

Table No.11: Prevalence CP. KPN in HAI &CAI

Klebsiella pneumoniae isolated	No.of CP.KPN
HAI	33(46.5%)
CAI	0

Graph No.8: Prevalence of Cp- KPN from HAI



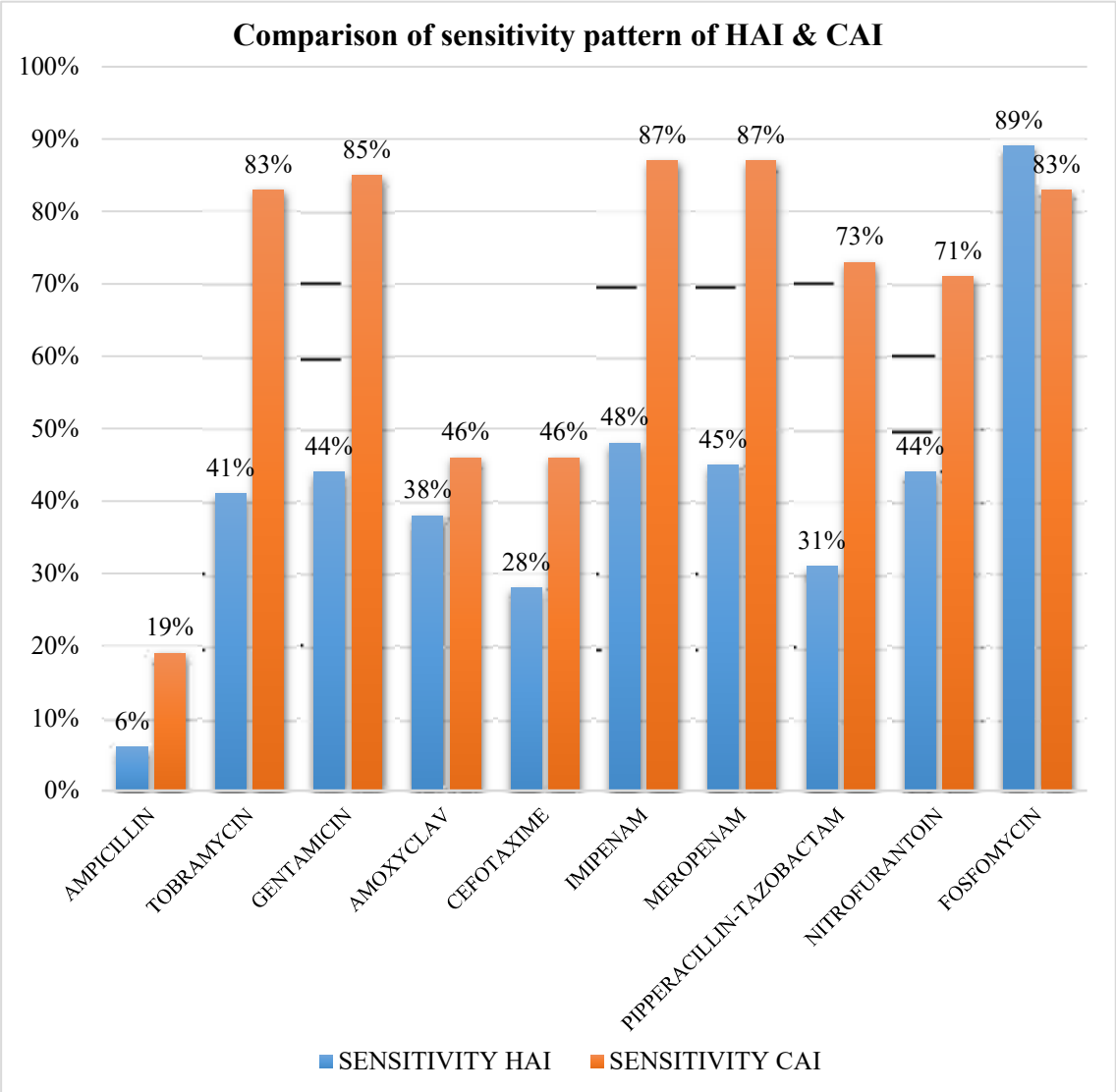
This study also observed that Klebsiella pneumoniae producing Carbapenemase enzyme was seen more in the case of organisms isolated from patients categorized under healthcare-associated infections.

As mentioned in Table, No 4, among the 39 organisms isolated with the screening method, 33 isolates confirmed with the mCIM test to be positive for Carbapenemase production belonged to healthcare-associated infection category.

Table No.12: Comparison of antibiotic sensitivity pattern of HAI & CAI

Antibiotics	Sensitivity HAI	Sensitivity CAI	Chi-sq(χ2)	P value
AMPICILLIN	4(6%)	10(19%)	5.505	**0.019**
TOBRAMYCIN	29(41%)	43(83%)	21.659	**<0.001**
GENTAMICIN	31(44%)	44(85%)	21.157	**<0.001**
AMOXYCLAV	27(38%)	24(46%)	0.817	**0.366**
CEFOTAXIME	20(28%)	29(46%)	9.54	**0.002**
IMIPENAM	34(48%)	45(87%)	19.517	**<0.001**
MEROPENAM	32(45%)	45(87%)	22.047	**<0.001**
PIPPERACILLIN-TAZOBACTAM	22(31%)	38(73%)	21.284	**<0.001**
NITROFURANTOIN	4(44%)	17(71%)	18.715	**<0.001**
FOSFOMYCIN	8(89%)	20(83%)	17.995	**<0.001**

Graph No.9: Comparison of antibiotic sensitivity pattern HAI & CAI



The antibiogram study of Klebsiella pneumoniae isolated from patients categorized under community-acquired infection showed more sensitivity towards antibiotics when compared with those isolated healthcare-associated infections.

The maximum sensitivity of isolates from community-acquired infection was seen with Imipenem (87%) and Meropenem(87%) followed by Gentamicin(85%) and Tobramycin(83%), and maximum resistance was Ampicillin(19%).

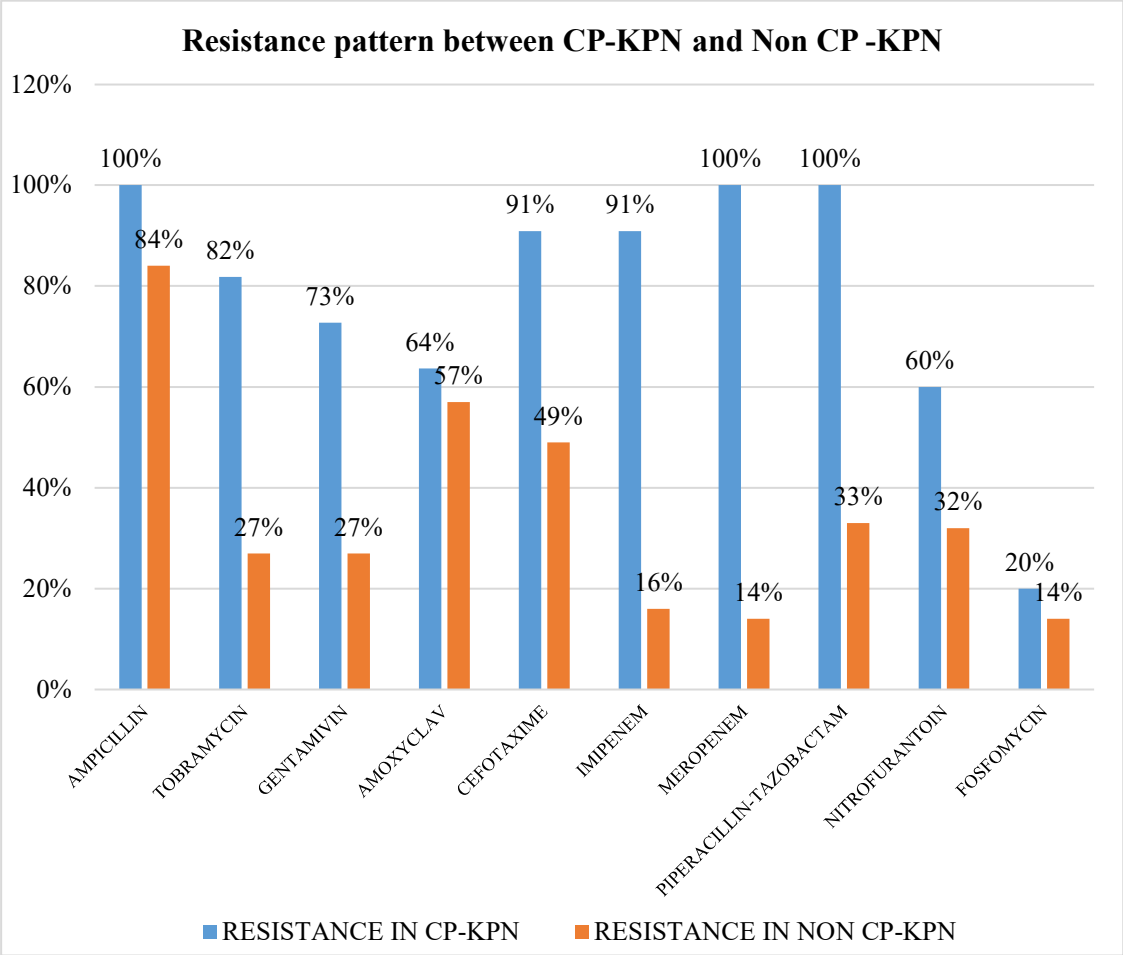
In the case of isolates from healthcare-associated infections, it was observed that maximum sensitivity was seen with Imipenem (48%) followed by Meropenem (45%) and Gentamicin (44%), and maximum resistance was seen with Ampicillin (6%) followed by Cefotaxime(28%).

Following statistical analysis, there was a statistically significant difference between the sensitivity pattern of both groups for all antibiotics used in this study, except for amoxicillin-clavulanic acid.

Table No.13 Antibiotic resistance between CP-KPN and Non-CP-KPN.

Antibiotics	Resistance in CP-KPN	Resistance in Non CP-KPN
AMPICILLIN	100%	84%
TOBR MYCIN	82%	27%
GENTAMICIN	73%	27%
AMOXYCLAV	64%	57%
CEFOTAXIME	91%	49%
IMIPENEM	91%	16%
MEROPENEM	100%	14%
PIPERACILLIN-TAZOBACTAM	100%	33%
NITROFURANTOIN	60%	32%
FOSFOMYCIN	20%	14%

Graph 10 Antibiotic resistance pattern between CP-KPN and Non-CP-KPN.



The antibiotic resistance pattern of Carbapenemase-producing (CP-KPN) and Non-Carbapenemase Klebsiella pneumoniae was observed that resistance was higher in CP-KPN isolates when compared with the Non-CP-KPN isolates.

Maximum resistance was shown towards Piperacillin-Tazobactam(100%), Meropenem(100%) and Ampicillin(100%) in CP-KPN isolates when compared to Non- CP-KPN isolates which showed maximum resistance to Ampicillin (84%), Amoxiclav(57%) and Cefotaxime(49%).

Maximum sensitivity was observed with Amoxyclav(36%) followed by Gentamicin(27%) in CP-KPN isolates and Meropenem(86%) and Imipenem(84%) in Non-CP KPN isolates.

DISCUSSION

Klebsiella pneumoniae has been causing life-threatening diseases even before the golden era of antibiotics. Since then, variations in infections caused by these organisms have been moving towards the higher side concerning an increase in virulence, prevalence, and antibiotic resistance pattern.

As years went by, there was an increase in Klebsiella pneumoniae infections in hospitalized patients or those who had prolonged stays in the health care system.[16] Increased emergence of the resistant organism, newer antibiotics were also introduced, the latest being the carbapenems, considered to be higher-end antibiotics, the final drug for infections caused by Klebsiella pneumoniae.[42]

They were highly effective initially for organisms that were thought to be ESBL producers. Later on, it was observed that the organism acquired resistance against this carbapenem group of antibiotics, leading to a new and more virulent group of organisms known as Carbapenemase Resistant Enterobacterales (CRE), among them, Escherichia coli and Klebsiella were predominant and are now considered under urgent threat category according to CDC 2019.

The factors contributing to an increase in infections with multidrug-resistant organisms include arterial or urinary catheterization, prolonged hospital stay or in the intensive care unit, and inappropriate use of various broad-spectrum antibiotics.

The prevalence of Carbapenemase producers is found to differ significantly according to geographical areas and in various institutes.

Therefore this research project was intended to identify the prevalence and their antibiogram.

Distribution of Carbapenemase-producing Klebsiella pneumonia

Among the 123 isolates of KPN isolated in this study, 33(27%) were found to be confirmed CP-KP. This was similar to the observations made by Nair et al. in Mumbai, the prevalence of Cp KP was 33%, which correlates with our results. [43]

On the contrary to the study conducted in Tumkur, Karnataka, the prevalence of Carbapenemase-resistant Klebsiella pneumoniae was found to be 42.5%, but in that study, all organisms of the Enterobacteriaceae family were included, and only Modified Hodge test was done for Carbapenemase detection[44], which has been replaced by mCIM test according to CLSI 2019 guidelines[5]

Among the 123 samples, the most commonly affected age group was found between 21yrs-30yrs(31,25%), followed by 61-70yrs(21,17%), this was close to another study conducted by Thomas et al. [45]

Among the 123 isolates, 71(58%) isolates were defined under HAI, and 52(42%) were defined under CAI; it was observed that all the 33(46.5) isolates which were CP-KP, belonged to the HAI category, which was almost identical to the study conducted by Nair et al. in Mumbai, and in a study done in Egypt by Koteb et.al[46], unlike the study done by Veeraraghavan et al., which reported a prevalence rate of 95% in nosocomial infections.[47]

It was also observed that among the 33 isolates, most of the isolates were isolated from wound swabs(15,45%) followed by blood samples (9,27%), this was similar to a study conducted by TV Parimala [44] and Pawar et al. [48]

Our study observed that the neonatal age groups were affected more by Klebsiella pneumoniae(18,15%), out of which (14,78%) were infected with Carbapenemase resistant Klebsiella pneumoniae. The results were concordant with the observations made by Mukherjee et al. [49]

Comparison of antibiotic sensitivity pattern in Healthcare-associated infection (HAI) and community-acquired infection (CAI).

In our study, it was observed that maximum sensitivity was seen with Imipenem 34(48%) for HAI, and 45(87%) for CAI respectively, followed by Meropenem 32,(45%);45(87%), and Gentamicin 32 (44%); 44(85%), these results were comparable observations by Veeraraghavan .et.al.[47] and Trojan .et.al.[50]

Statistical analysis was done, and statistically, significant differences were observed between both groups' sensitivity patterns.

In the resistance pattern between the two groups, the highest resistance was seen with Ampicillin 67(94%) in HAI and 42(81%) in CAI, followed by Cefotaxime 51(72%) in HAI and Amoxicillin- Clavulanic acid 28 (54%) in CAI, these results were similar to the observations made by Jitendra et al. [51] and Veeraraghavan et al. [47]

When comparing the resistance pattern between CP-KPN & Non CP-KPN, The antibiotic resistance pattern of Carbapenemase-producing(CP-KPN) and Non-Carbapenemase Klebsiella pneumoniae was observed that resistance was higher in CP-KPN isolates when compared with the Non-CP-KPN isolates.

Maximum resistance was shown towards Piperacillin-Tazobactam(100%), Meropenem(100%) and Ampicillin(100%) in CP-KPN isolates when compared to

Non- CP-KPN isolates which showed maximum resistance to Ampicillin (84%),Amoxiclav(57%) and Cefotaxime(49%)

Maximum sensitivity was observed with Amoxyclav(36%) followed by Gentamicin(27%) in CP-KPN isolates and Meropenem(86%) and Imipenem(84%) in Non-CP KPN isolates

This study emphasizes that there is an increase in the resistance pattern of CP.KPN in this area, compared to other studies done by Veeraraghavan et al. [47] and Pandey et al. [52]

In our study, Ertapenem disc(10µg) was used to screen the isolates, and modified Carbapenemase inactivation (mCIM) test was done to confirm the production of a carbapenemase enzyme, CLSI approves both for the phenotypic identification of Carbapenemase resistant Klebsiella pneumoniae.[5]

This test is also simple, cost-effective, easy to interpret; the sensitivity and specificity are also above 97% compared to molecular methods, according to a study done by Pierce et al. [40]. Another vital point is that this test can be done in any microbiology laboratory with basic facilities, where molecular confirmation cannot be done.

With the findings of our study, it raises the importance of microbiology laboratories to set up screening and confirmation of Carbapenemase production in organisms as a part of Multi-Drug Resistant Organisms(MDRO) surveillance since it is a growing threat worldwide

The study emphasizes the importance of knowing the prevalence and antibiotic sensitivity pattern of the organisms in a geographical area to prevent further increase in AMR and reduce the spread of MDR organisms in the community and healthcare facility.

CONCLUSION

The prevalence of Klebsiella pneumoniae causing life-threatening conditions are increasing day by day around the world. Due to the wide range of enzymes produced by the organism, they tend to show more resistance to higher-end antibiotics.

Detecting carbapenem and other enzymes like ESBL and AmpC production is essential in hospital and community isolates.

This is because,

- These strains are probably more prevalent than expected.
- These enzymes pose a challenge to currently available antibiotics.

Timely identification of infection with MDRO and appropriate antibiotic therapy is now given topmost priority.

Prevalence CP-KPN was found to be at 27 % in the current study.

These isolates showed a high resistance pattern when compared to the non-carbapenem-producing Klebsiella pneumoniae(CP KPN).

This study also observed the antibiogram of healthcare-associated and community-acquired infections isolates, which showed a significant difference between the sensitivity patterns; this might be due to the indiscriminate use of antibiotics.

To conclude, the increasing prevalence of Carbapenem-producing Klebsiella pneumoniae in our clinical strains is alarmingly high, and it reflects the excessive use of carbapenems empirically. Therefore, thoughtful use of antibiotics, strict hand hygiene etiquettes and implementation of apt infection–control measures and antibiotic stewardship programs in the hospital are, need of the hour for preventing the spread of these multidrug-resistant organisms.

Screening procedures must be implemented worldwide for 'at risk' patients, including neonates. Along with implementing antimicrobial stewardship programmes for controlling the spread of multidrug-resistant organisms, it is also recommended to implement active surveillance mainly for MRSA, VRE, and CREs to reduce the burden of healthcare-associated infection

Limitations of the study

- Limited sample size; only Klebsiella pneumonia was included.
- Genotypic confirmation of the isolates was not done

SUMMARY

A total of 123 isolates of Klebsiella pneumoniae isolated were evaluated in the study. Among that, a total of 44 samples were identified as positive for Carbapenemase-producing Klebsiella pneumoniae by using Ertapenem disc as a screening method. Of these, 33 samples were found to be confirmed positive with the mCIM test.

Among the 123 isolates, 71(58%) isolates were defined under HAI, and 52(42%) were defined under CAI, and their antibiotic sensitivity pattern was recorded.

The present study reports a 27% prevalence of CP-KPN infection in and around Belagavi, with a slightly higher prevalence rate(46.5%) with healthcare-associated infections(HAI).

The most common age group affected by Klebsiella pneumoniae, in general, was between 21yrs-30yrs, 31(25%), followed by 61-70yrs,21(17%).

The neonatal age group also showed alarmingly high CR-KP, 11.3%, which indicates a need for CRE screening in neonates.

Carbapenemase-producing Klebsiella pneumoniae was most commonly isolated from wound samples(SSIs, pressure ulcers,non-healing diabetic ulcer), 15(47%), followed by blood samples 9(27%).

Maximum sensitivity was seen with Imipenem 48% for HAI, 87% for CA I, respectively, followed by Meropenem 87% and Gentamicin 44% for HAI & 85% for CAI.

The highest resistance was seen with Ampicillin 94% in HAI and 81% in CAI, followed by Cefotaxime,72% in HA I, and Amoxicillin- Clavulanic acid 54% in CAI.

This study concludes that antimicrobial resistance in Klebsiella pneumoniae is an emerging threat to both community and health care facilities. Robust surveillance is required to curb this current menace.

Although a low resistance rate to Meropenem and Imipenem was recorded in this study, more cautious efforts should be made to control and prevent the spread of these multidrug-resistant organisms(MDRO).

Control measures must include a multi-faceted strategy coordinated by the health care officials in fields, regular monitoring of antibiotic resistance patterns, which will help in timely modification of the antibiotic usage policies and aid in using them judiciously.

Therefore, including a simple, inexpensive method for screening and confirmation to detect Carbapenemase production in microbiology laboratories is essential for infection control, prevention, and epidemiological surveillance.

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ANNEXURES

ANNEXURE- I (ETHICAL CLEARANCE LETTER)

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed - to-be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
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Ref: MDC/DOME/ 270 **Date: 24/12/2019**

To,

REG NO: BI0119001
PG student in Microbiology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PREVELANCE OF CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIA ISOLATED FROM VARIOUS CLINICAL SAMPLES – A ONE YEAR CROSS SECTIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE II – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

TITLE: Prevalence Of Carbapenemase Producing Klebsiella Pneumoniae Isolated From Various Clinical Samples-A One Year Cross Sectional Study.

PURPOSE OF THE STUDY:

The purpose of the study is to isolate and identify Carbapenemase resistant Klebsiella pneumoniae and to determine the antibiotic susceptibility of the isolated organisms.

PROCEDURE:

You are requested to participate in this study which will provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge. The principal investigator of this study is _____,under the guidance of _____.

If you agree to enroll yourself in the study ,you will be interviewed regarding your present, past and family history and your clinical manifestations.

RISK AND BENEFITS:

There are no risks involved and benefit is to know about the causative bacteria and antibiotic susceptibility of the same, so that appropriate treatment can be given.

ALTERNATIVES:

Your participation in research is voluntary. Your decision whether or not to participate in the study ,will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate, you are free to withdraw at any time.

PRIVACTY AND CONFIDENTIALITY:

All the information collected during the course of this study will be kept confidentially to the extent permitted by law. The code numbers will identify you in this research record.

Information from this study will be published but you or the information provided by you during research will remain confidential. No information about you or information provided by you during this research will be disclosed to others without your written permission except :

1. In an emergency to protect your rights and welfare.
2. If required by law.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not be paid/offered any gifts/incentives for participating in this study.

You will not be reimbursed for expenses.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed in a a conference, no information will be displaced that would disclose your identity. Any information that is

obtained in connection with this study and that can be identified with you will remain confidential.

Question: In case you have any questions related to the study, you can contact:

1. In case you have any question about your rights as a participant, you can contact Dr.Roopa M Bellad, Professor of Paedeatrics and Chairman of Ethical Committee, JNMC. (PH.NO. 9448113403, OFFICE NUMBER 0831-2471350,EXT. 4052)

CONSENT STATEMENT

I, the undersigned_____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also, I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name: Signature/Thumbprint:

Name of the witness: Signature/Thumbprint:

Name of the investigator Signature:

Date:

Place:

ANNEXURE III

PROFORMA

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING DATA

NAME: **IP.NO:**

AGE: **DOA:**

SEX: **DOC:**

ADDRESS: **NATURE OF SAMPLE:**

DIAGNOSIS:

Presenting complaints:

H/0 presenting complaints

Current antibiotic therapy :

H/0 any interventions: **If yes,**

Type of intervention:

Type and course of antibiotic therapy taken:

H/O previous hospital admission: **If yes,**

Details of previous treatment:

Course and duration of antibiotic treatment:

Associated illness, if any:

LAB FINDINGS

1. **Gram stain**

	MacConkey agar	**Blood agar/ chocolate agar**
Colony Morphology		

2. **Culture on**

3. **Biochemical reactions:**

- **Catalase**
- **Indole**
- **Urease**
- **Citrate utilization**
- **Mannitol fermentation**
- **TSI**
- **Oxidative – Fermentative test**
- **Nitrate reduction test**
- **Methyl red test**
- **Voges Proskauer test**
- **Glucose fermentation test**
- **Ornithine decarboxylase test**

4. Antibiotic sensitivity testing:

ANTIBIOTICS	SENSITIVE	RESISTANT
1. Ampicillin		
2. Gentamycin		
3. Tobramycin		
4. Amoxillin-Clavulanic Acid		
5. Imipenem		
6. Meropenem		
7. Cefotaxime		
8. Piperacillin and Tazobactam		
9. Nitrofurantoin (if urine sample)		
10. Fosfomycin (If urine sample)		

5.Modified Carbapenemase inactivation method (mCIM):

ANNEXURE – IV

PROCEDURES

A) Stains used

Gram stain: Hucker's modification[53]

Principle: The Gram positive bacteria retain para- rosaniline dyes even after treatment with decolorizing agents, appear violet color while gram negative bacteria lose the dye and take up counter stain and appear pink in color.

Procedure:

a) Label a clean grease free glass slide and prepare thin smear with colony and allowed to air dry.

b) The smear was fixed by passing the slide two to three times through the flame of a Bunsen burner.

c) Place the slide on the staining slide rack and the smear was covered with crystal violet solution.

d) After 20 seconds, the slide was washed thoroughly under running tap water.

e) Cover the slide with Gram iodine solution for 20 seconds and washed with running tap water.

f) The hold the smear between the thumb and fore finger and the surface is flooded with a few drops of acetone-alcohol decolorizer, until no color washed off.

g) Wash the smear with running tap water. Cover the Surface of the smear with safranin (counter stain) for 10 seconds and wash with running tap water.

h) The slide was placed in an upright position in a rack, allowing excess water to drain off.

i) Observe under oil immersion objective (100X) of the microscope

Controls:

1) Gram positive – ATCC Staphylococcus aureus 25923

2) Gram negative – ATCC Escherichia coli 25922

B) MEDIA AND REAGENTS USED

1) MacConkey Agar

Peptone – 20g

Sodium taurocholate – 5g

Water – 1 L

Agar – 20 g

Neutral red solution, 2% in 50% ethanol – 3.5 ml

Lactose, 10% aqueous solution – 100 ml

Dissolve peptone and taurocholate in the water by heating. Add agar and dissolve it in the autoclave and filter. Adjust the pH to 7.5. Add the lactose and neutral red and mix.

Sterilize in the autoclave at 121° C for 15 minutes and pour as plates.

2) Blood agar medium(5% sheep blood agar)

Composition

Ingredient's gram/liter

Peptone 10.00

Distilled water 1 ltr.

Sodium chloride 5.00

Agar 15.00

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml cold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 1210C and 15 lbs pressure for 15 minutes.

The autoclaved materials were allowed to cool to a temperature of 450C in a water bath. Defibrinated 5-10% sheep blood was then added to the medium aseptically and distributed to sterile petri dishes. Sterile media was stored in refrigerator at 40C for future use.

3) Mueller – Hinton agar (hi-media)

   Beef Infusion – 300g

   Peptone - 17.5g

   Starch - 1.5g

   Agar - 17g

   Dissolve 38 grams of dehydrated media in 1-liter distilled water and heat to dissolve. Autoclave at 121° C for 15 minutes. Adjust pH to 7.1 ± 0.2 and pour on plates.

4) Hugh Leifson's oxidation fermentation medium:

   Peptone – 2

   Sodium chloride – 5 g

   Dipotassium hydrogen phosphate – 0.3g

   Bromothymol Blue (1%) – 3 ml

   Agar – 3 g

   Distilled water – 1 L

   PH-7.1

   Sterilize by autoclaving at 121° C for 15 minutes in a flask. The carbohydrate to be added is sterilized by separately and added to give final concentration of 1%. The medium is then tubed to a depth of about 4 cm.

5) Methyl Red / Voges - Proskauer Broth

   Polypeptone – 7g

Glucose – 5g

Dipotassium phosphate – 5g

Distilled water – 1 L

pH - 6.9

Dispense into tubes and sterilize by autoclaving at 15 lbs. pressure (121° C) for 15 minutes.

6) Christensen's Urease Medium

Peptone – 1 g

Sodium chloride – 5 g

Dipotassium hydrogen phosphate – 2g

Phenol red (1 in 500 aqueous solution) - 6g

Agar – 20 ml

Distilled water - 1 L

Glucose 10% solution (sterile) – 10 ml

Urea 20% solution (sterile) – 100 ml

Sterilize the glucose and urea solution by filtration. Prepare the basal medium without glucose or urea. Adjust the pH to 6.8 – 6.9 and sterilize by autoclaving in a flask at 121°C for 15 minutes. Cool to about 50° C. Add glucose and urea solution and pour as deep slopes in tubes.

7) Simmon's Citrate Medium

Sodium chloride – 5g

Magnesium sulphate – 0.2g

Ammonium dihydrogen phosphate – 1g

Potassium dihydrogen phosphate – 1g

Sodium citrate Bromothymol blue (0.2%) – 40 ml

Distilled water - 1L

Sterilize by autoclaving at 121° C for 15 minutes in a flask and distribute as slopes in tubes.

8) Triple Sugar Iron Agar

Beef extract – 3g

Yeast extract – 3g

Peptone – 15g

Proteose peptone – 5g

Lactose – 10g

Glucose – 1g

Sucrose – 10g

Ferrous sulfate – 0.2g

Sodium chloride – 5g

Agar – 12g

Phenol red – 0.024g

Distilled water – 1L

pH - 7.4

Sterilize by autoclaving in a flask at 121° C for 15 minutes and distribute evenly in tubes as slopes.

Biochemical tests used for identification

1) Catalase Test

One ml of 3% hydrogen peroxide solution is taken in a small tube. One test colony is picked up using a sterile wooden stick and inserted into the H2O2 solution. The occurrence of vigorous effervescence is indicative of catalase activity and was taken as positive.

Positive control - ATCC Staphylococcus aureus 25923

Negative control - ATCC Enterococcus faecalis 29212

2) Oxidase Test

Principle: The cytochromes are iron containing hemoproteins that acts as last link in the chain of aerobic respiration by transferring electrons to oxygen with the formation of water.

Procedure: Method 1-Loopful of the colony to be tested was taken and smeared over the wet filter paper strip containing 1% tetramethyl-para-phenylene diamine dihydrochloride.

Method 2- Disks impregnated with N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) was used(redox indicator).

Results:

Positive: Development of dark purple color within 10 seconds.

Negative: Absence of any color or development of blue color in 10 seconds

Control:

Positive Control- Pseudomonas aeruginosa 27853

Negative control - Escherichia coli ATCC25922.

3) Indole Test

Inoculate the peptone water with test organism and incubate at 37°C for 24 hours. Add 0.5 ml of Kovac's reagent and shake gently. A red color in the alcohol layer indicates a positive reaction.

4) Citrate Utilization

Principle-Some bacteria can obtain energy by utilizing citrate as the sole source of carbon. The utilization of citrate was detected in Simmon's citrate medium by the production of alkaline products.

Procedure - The entire surface of the slant was inoculated lightly from a young culture and incubated at 37°C for 24-48 hours. The test was considered positive when the medium turned deep blue in color along with growth on the surface.

Results

Positive-Blue color and streak of growth

Negative- Original green color and no growth

5) Triple Sugar Iron Agar Test

Triple sugar iron (TSI) agar medium contains 10 parts lactose; 10 parts sucrose; 1 part glucose and peptone. Phenol red and ferrous sulphate serve as indicators of acidification and H2S production respectively. With a straight inoculation needle, touch the top of a well- isolated colony. Inoculate TSI by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. Incubate the tube at 35deg C in ambient air for18 to 24 hours. The results are interpreted as follows.

Alkaline slant/No change in butt - glucose, lactose and sucrose (K/No change) non-utilizers.

Alkaline slant/Acid butt (K/A) -glucose fermentation only Acid slant/Acid butt (A/A) -glucose, sucrose and/ or lactose fermenter.

A black precipitate in the butt indicates production of ferrous sulphide and H_2Sgas

(H_2S+). Bubbles or cracks in the tube indicate the production of CO_2 or H2.

6) Urease Test

Principle -Bacteria possessing urease enzyme, hydrolyse urea, producing ammonium bicarbonate resulting in alkalinization and increase in the pH of the medium.

Medium -Christensen's urease medium

Procedure - The medium was inoculated with the test organism and incubated at 37°C for 24-48 hours. Development of pink color throughout the medium was taken as a positive test and yellow color as a negative test.

Results:

Positive-Red color throughout the medium.

Negative-Medium remains original yellow color.

7) Nitrate reduction test

Principle: This test is based on the ability of an organism to reduce nitrates to nitrites in the presence of an electron donor which can be tested by a colorimetric reagent. The presence of nitrites in the test medium is detected by the addition of alpha naphthylamine and sulfanilic acid

Procedure: Organism was inoculated into 2 test tube of Nitrate broth medium and incubated for 96 hours at 37° C. 0.1 ml of the test reagent is added to the test culture.

Results:

Positive - Development of red color within 30 seconds after adding the reagents indicates the presence of nitrites.

Negative - If no color develops after adding the reagents indicates either that nitrates have not been reduced (true negative reaction) or they are reduced to other products like ammonia, nitric oxide, or nitrous oxide and hydroxylamine. Development of red color after adding zinc indicates the presence of residual nitrates and confirms the true negative reaction.

Controls

Positive control: Escherichia coli

Negative control: Salmonella typhimurium

8) Hugh's Leifsons Oxidation –Fermentation test

Medium-Hugh's Leifsons Oxidation –Fermentation Medium

Procedure- Organism was inoculated into 2 test tube of Hugh's Leifsons Oxidation –

Fermentation Medium. One tube is promptly covered with a layer of sterile liquid paraffin to a depth of 1cm for anaerobic environment. Both the tubes are incubated for 30 days.

Results

Fermenting organisms produce an acid reaction throughout the medium in both tubes.

Oxidizing organisms produce an acid reaction only in the open tube (oxidative pattern).

9) Methyl Red Test

Inoculate the glucose phosphate peptone water medium with a young culture and incubate at 37 deg C for 48 hours. Add about five drops of the methyl red reagent. Mix and read immediately. Positive tests are bright red and negative tests are yellow.

10) Voges-Proskauer Test

Medium - MR / VP broth

Reagents -1) 5% α-Naphthol: 5 g α- Naphthol in 100 ml absolute alcohol

2) 40% Potassium hydroxide: 40 g KOH in 100 ml distilled water

Procedure - A tube of MR / VP broth is inoculated with a pure culture of the test organism and incubated for 24 hours at 37° C. At the end of this time, I ml of the aliquot is taken into a clean test tube and 0.6 ml of 5% α-naphthol is added, followed by 0.2 ml of 40% KOH in that order and the tube shaken gently after

adding each reagent. Following this the tube is allowed to remain undisturbed for 10-15 minutes.

Results: Development of a red color indicates a positive reaction.

11) Sugar Fermentation Test

Sugar fermentation medium

Peptone 15 gm Andrade's indicator 10 ml Sugar to be tested 20 gm Water 1 liter Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1mol/lit. sodium hydroxide has been added to turn the color of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 liter of water and add 20 gm of the sugar; sugar to be tested generally includes glucose, sucrose, lactose and maltose.

Distribute 3 ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100°C for 30 min on 3 consecutive days.

Procedure: Inoculate each tube with 1 drop of 18-24 hours broth culture. Incubate at 35°C for up to 7 days in ambient air. Examine the tubes for acid (indicated by a pink color) and gas production. Positive test is indicated by change in color to pink with or without gas formation in Durham tube. Negative test is indicated by growth, but no change in the color

Turbidity Standard Equivalent to Mc Farland 0.5[53]

- 1% solution of Sulphuric acid was prepared by adding 1 ml of concentrated Sulphuric acid to 99 ml 0f water and mixed well.
- 1% solution of barium chloride was prepared by dissolving 0.5g of dehydrate barium chloride in 50 ml of distilled water.

- 0.6 ml of barium chloride solution was added to 99.4 ml of sulphuric acid solution and mixed well.
- Stored in a well-sealed container in the dark at room temperature {20 ˚C-28˚C}

Antibiotic Susceptibility Testing:[5]

Medium - Mueller Hinton agar (MHA)

Method- Kirby Bauer's Disc Diffusion Method

The antibiotic discs are placed on the inoculated plates using a pair of sterile forceps (evenly distributed so that they are no closer than 24mm from center to center). Alternatively, a needle tip may be used for the same. Each disc is gently pressed down to ensure even contact with the medium. Within 30 minutes of preparation, the plates are placed in the incubator at 35˚C in ambient air for 16 – 18 hours. The results are interpreted according to critical diameters given in recent CLSI guidelines.

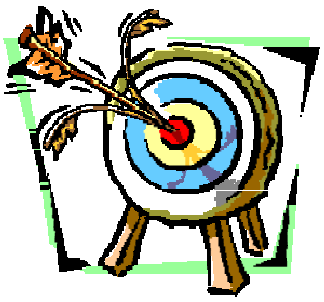
ANNEXURE V: MASTERCHARTS

Sl no	Name	Age	Sex	IP/OP no	Sample	Diagnosis	h/o of prev adm	Sensitivity pattern											Mcim	HAI
								AMP	TOB	GEN	AMC	CTX	IMP	MRP	PTZ	NIT	FO	EMP		
1	MALLAPPA	60	M	977785	Sputum	ptb	YES	S	S	S	R	R	S	S	R			S		YES
2	SUVARNA	54	F	978115	Urine	R LL cellulitis	YES	R	S	R	R	R	S	S	R	R	S	S		YES
3	PRABHAVATI	45	F	5376926	Ear swab	L CSOM	OPD	R	S	S	R	R	S	S	S			S		NO
4	SOMANING	30	F	980882	Sputum	Pleural effusiion	NO	R	S	S	S	R	S	S	S			S		NO
5	SHAMEL	28	F	983313	Urine	PV Leak	NO	R	S	S	R	R	S	S	R	S	S	S		NO
6	SHANTA	60	F	989114	Sputum	Bronchial asthma	YES	S	S	S	S	R	S	S	R			S		NO
7	SANAFAROOKH	23	F	5484659	Urine	WDPV	OPD	R	S	S	R	R	S	S	S	S	S	S		NO
8	SHANKAR	57	M	982882	Tracheostomy tip	Pneumonia	Yes	R	R	R	R	R	S	S	S			S		YES
9	SHIVAGOUDA	70	M	986590	Sputum	AGE	YES	S	S	S	R	R	S	S	R			S		YES
10	POOJA	24	F	5494532	LSCS wound swab	LSCS wound infection	NO	R	R	R	S	R	S	S	R			S		YES
11	B/0 SWETA	Day3	F	989446	Blood	Failure to thrive	no	R	R	R	R	S	S	S	R			S		YES
12	MALLESHAPPA	54	M	984785	Sputum	Cervical radiculopathy	yes	R	S	S	S	R	S	S	S			S		YES
13	MUBINA	22	F	5484922	Urine	ANC	OPD	S	S	S	R	S	S	S	S	S	S	S		NO
14	DEVAPPA	65	M	990799	Wound swab	Venous ulcer	yes	R	R	S	S	R	S	S	R			S		NO
15	GOURAKKA	69	F	989763	Wound swab	wound sepsis	yes	R	R	R	R	R	R	R	R			R	POSITIVE	YES
16	SHARADA	40	F	5498006	URINE	uti	OPD	R	S	R	R	S	S	S	S	S	R	S		NO
17	SHOUDHIKA	23	F	995075	Urine	ANC	NO	R	S	S	R	R	S	S	R	R	R	S		NO
18	SHIVANAND	24	M	993046	Wound swab	Pressure ulcer	YES	R	S	S	R	R	R	S	S			R	NEGATIVE	YES
19	ABHISHEK	16	M	992877	Wound swab	Lap cholecystectomy	no	R	S	R	R	S	S	S	R			S		YES
20	FAKIRAVVA	26	F	1002149	Urine	ANC	OPD	R	S	S	R	S	S	S	S	S	S	S		NO
21	TUKARAM	80	M	1002554	sputum	COPD	OPD	R	S	S	R	S	S	S	S	S	S	S		NO
22	PRANITA	47	F	1002379	Urine	Nephrotic syndrome	OPD	R	S	S	R	S	S	S	S	S	S	S		NO
23	MAYA PATIL	23	F	5520923	Urine	PROM	NO	R	S	S	R	S	S	S	S	S	S	S		NO
24	SAVITRI	24	F	3751751	urine	ANC	YES,prev delivery	R	S	R	R	S	S	S	S	S	R	S		NO
25	RESHMA	25	F	5625235	urine	ANC	OPD	R	R	S	R	R	S	S	R	S	S	S		NO
26	AKSHATA	24	F	1001725	vaginal swab	pre term labour	NIL	R	S	S	R	R	S	S	S			S		NO
27	NINGAPPA	68	M	1001675	pleural fluid	Hydropneumothorax	yes	R	S	S	R	S	S	S	S			S		NO
28	RABIYA	60	F	1002199	Wound swab	Diabetic foot ulcer	yes	R	S	S	R	S	S	S	S			S		YES
29	MAHADEVI	20	F	1004001	urine	ANC	OPD	S	S	R	R	S	S	S	S	S	S	S		NO
30	KENCHAPPA	63	m	1002707	Wound swab	Diabetic foot ulcer	YES	R	S	S	R	R	S	S	S			S		YES
31	SANTHOSH	36	M	1011969	sputum	bronchiectasis	no	R	S	S	R	R	S	S	S			S		NO
32	SONAM	3	F	1012849	Urine	Nephrotic syndrome	yes	R	S	S	R	R	S	S	S			S		NO
33	PRIYANKA	20	F	1016491	Vaginal swab	PROM	NO	R	S	S	R	S	S	S	S			S		NO

34	DYAMAPPA	68	M	1014092	sputum	koch sequale	Yes	R	S	S	R	R	S	S	S			S		NO
35	VEERABHADRAYA	60	M	1015097	Wound swab	R foot abscess	Yes	R	S	S	R	S	S	S	R			S		YES
36	AKASH	16	M	1014810	PUS SWAB	Appendiccitis	Yes	R	S	S	R	R	S	S	S			S		NO
37	DEVAMMA	25	F	1012784	Wound swab	Post op fistula in ano	NO	R	R	R	R	R	R	R	R			R	POSITIVE	YES
38	VIJAYALAKSHMI	28	F	1015465	vaginal swab	PV Leak	YES	R	S	S	R	S	S	S	S			S		NO
39	GURUPUTRAPPA	61	M	1015567	Wound swab	R palm cellulitis	Yes	R	S	S	R	R	S	S	S			S		NO
40	YAMANAPPA	42	M	1015064	WOUND SWAB	post op renal calculi	Yes	R	S	S	R	R	S	S	S			S		YES
41	NAGARATNA	27	F	1014502	Wound swab	Breast abscess	NO	R	S	S	R	R	S	S	R			S		YES
42	JYOTI GOUDA	27	F	1013300	LSCS wound swab	LSCS wound infection	NO	R	R	R	R	R	R	R	R			R	NEGATIVE	YES
43	JAYAWANTH CHAVAN 50	50	M	1025411	WOUND swab	Diabetic foot ulcer	yes	R	R	R	R	R	R	R	R			R	NEGATIVE	YES
44	RUPESH ASHTEKAR	44	M	1024703	Wound swab	necrotizing fascitis	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
45	KIRTI MANE	20	F	1026598	Vaginal swab	PV Leak	no	S	S	S	S	S	R	R	R			S		NO
46	PRAKASH PATIL	8	M	586730	URINE	UTI	NO	S	S	S	S	R	R	R	R	R	S	S		NO
47	BASAVRAJ	7	M	1027207	URINE	UTI	NO	S	S	S	S	S	S	S	S	S	S	S		NO
48	GIRIAPPA JANAGOUDA	55	M	1025525	WOUND SWAB	DN FOOT	YES	R	S	R	R	R	S	S	S			S		YES
49	PREMA	28	F	1028089	VAGINAL SWAB	PV Leak	YES	S	S	S	S	S	S	S	S			S		NO
50	CHANDRU NAIK	56	M	1026992	SPUTUM	PV Leak	YES	R	R	S	R	R	R	R	R			S		YES
51	VINAYAR	4	M	1029167	URINE	NS	YES	R	S	S	S	S	S	S	S	S	S	S		YES
52	TRISHA SALE	5	F	1029970	URINE	Fever	NO	R	S	S	S	S	S	S	S	S	S	S		NO
53	ROOHI	1	F	1030758	Wound swab	burns	NIL	R	R	S	R	R	R	R	R			R	POSITIVE	YES
54	POONAM PATIL	21	F	1031286	URINE	Primi&PPROM	NIL	R	S	S	S	S	S	S	S	S	S	S		NO
55	BANUMA	65	F	1037171	sputum	PTB	YES	S	S	S	S	S	S	S	S			S		NO
56	SIDDALINGAVVA	55	F	1037680	URINE	UTI	YES	R	S	S	S	S	S	S	S	R	S	S		NO
57	KAKUSAB	32	M	1038488	Wound swab	DVT	YES	R	R	S	S	S	R	R	R			R	POSITIVE	YES
58	PARASHURAM	60	M	1038988	sputum	PTB	YES	R	S	S	S	S	S	S	R			S		YES
59	PRAKASH KHOT	51	M	1039656	WOUND SWAB	GLUTEAL ABSCESS	NO	R	S	S	S	S	S	S	S			S		NO
60	GANGAVVA	72	F	1034026	Wound swab	SSI	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
61	AMIT HONGEKAR	26	M	1036558	WOUND SWAB	R ch.OM	no	R	S	S	S	S	S	S	S			S		YES
62	ANIL	29	M	1039932	PUS SWAB	popliteal abscess	NIL	R	R	R	R	R	S	S	R			S		NO
63	VRUSHALI RANE	40	F	1040906	BLOOD	CA CERVIX	YES	R	S	S	S	S	S	S	S			S		YES
64	ARHA	30	F	1044682	WOUND SWAB	SSI	YES	R	R	S	R	S	S	S	S			S		YES
65	LAXMI	34	F	1043085	WOUND SWAB	GLUTEAL ABSCESS	NIL	R	S	S	R	R	S	S	S			S		NO
66	PARASHURAM.H	53	M	1043005	URINE	Gef,induced p.v	yes	R	S	S	R	S	R	R	R	R	S	R	POSITIVE	YES
67	JAYAPRAKASH	19	M	103991	URINE	ENTERIC FEVER	NIL	R	S	S	S	S	S	S	R	R	S	S		NO
68	MALIK MULLA	63	M	1042559	WOUND SWAB	Hollow Viscous Perforation	YES	R	R	R	S	R	R	R	R			R	POSITIVE	YES
69	B/O DEEPALI	D14	M	1041263	BLOOD	SEPSIS	NO	R	S	S	R	R	R	R	R			R	POSITIVE	YES
70	KHATALSAH	62	M	1044906	WOUND SWAB	PVD	YES	R	S	S	S	S	S	S	S			S		YES
71	RUPA.R	35	F	5982194	URINE	UTI	NIL	S	S	S	S	S	S	S	S	S	S	S		NO
72	NANDU	29	M	1040829	PUS SWAB	GLUTEAL ABSCESS	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
73	SANDYA.A	24	F	1045305	URINE	UTI	NIL	R	S	S	S	S	S	S	S	R	S	S		NO
74	MARUTI.B	65	M	1039193	PUS SWAB	PVD	YES	R	R	S	S	R	R	R	R			R	POSITIVE	YES
75	FAKIRAPPA	47	M	1046133	sputum	COPD	YES	R	S	S	S	S	S	S	S			S		NO
76	MALAPRABHA	56	F	1044316	URINE	UTI	YES	R	R	R	R	R	R	R	R	R	S	R	POSITIVE	YES
77	BAJIRAO	63	M	1046151	WOUND SWAB	Diabetic foot ulcer	YES	R	S	S	S	S	S	S	R			S		YES
78	DAYANAND	31	M	1047061	WOUND SWAB	PERI-ANAL ABSCESS	NIL	R	R	R	R	R	R	R	R			R	NEGATIVE	NO
79	IBRAHIM	54	M	1046055	Wound swab	Diabetic foot ulcer	YES	R	R	R	R	R	S	S	S			S		YES
80	B/O RUSKAN	D 23	F	1043669	BLOOD	SEPSIS	NIL	R	R	R	S	S	S	S	S			S		YES
81	JYOTI BHARAT	25	F	595875	vaginal swab	PRETERM	NIL	R	R	R	R	R	R	R	R			R	NEGATIVE	NO
82	RAMAJAN	1	M	6057130	EAR SWAB	CSOM	NIL	R	R	R	S	S	S	S	S			S		YES

83	RESHMA SUDHEER	30	F	1048108	WOUND SWAB		YES	R	S	S	R	R	R	R	R			R	POSITIVE	YES
84	MARUTI	62	M	1048937	WOUND SWAB	GANGRENE	YES	R	R	R	S	S	S	S	S			S		YES
85	BEBILAI	55	F	1043548	WOUND SWAB	Diabetic foot ulcer	YES	R	R	R	S	S	S	S	S			S		YES
86	RATNAKAR	67	M	1047558	sputum	Pneumonia	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
87	B/O SANA	D14	F	1050028	URINE	SEPSIS	NIL	R	R	R	S	R	R	R	R	S	S	R	POSITIVE	YES
88	B/O RENUKA	D22	F	1051368	E.T.TIP	SEPSIS	NIL	R	S	S	S	S	S	R	R			R	POSITIVE	YES
89	RALEIGN.Y	47	M	1050835	SPUTUM	PTB	YES	R	S	S	S	S	S	S	S			S		YES
90	SHOBHA	20	F	1051904	vaginal swab	PV Leak	NIL	R	S	S	S	S	S	S	S			S		NO
91	SAVITHA.S	19	F	M	EAR SWAB	CSOM	NIL	R	S	S	S	S	S	S	S			S		NO
92	VIDYASHREE	20	F	1052666	MOTHER MILK	POST LSCS	NIL	R	S	S	S	R	S	S	S			S		YES
93	KIRAN	15	M	1052202	PUS SWAB	SEC.SUTURING	NIL	R	S	S	R	R	R	R	R			R	POSITIVE	YES
94	RENUKA	23	F	6089065	VAGINAL SWAB	PROM	NIL	S	S	S	S	S	S	S	S			S		NO
95	FALAK BANU	27	F	4832342	URINE	SCAR TENDERNESS	NIL	R	S	S	R	R	S	S	S	S	S	S		YES
96	SURENDRA	43	M	1052615	PUS SWAB	PYOPNEUMOTHORAX	YES	R	S	S	S	S	S	S	S			S		NO
97	B/O RENUKA TWIN 2	D 22	F	1051368	I.C TIP	PT&LBW	NIL	R	S	S	S	R	S	R	R			R	POSITIVE	YES
98	BASAVRAJ	47	M	1053320	PUS SWAB	Diabetic foot ulcer	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
99	JANAKIBAI	65	F	1052991	SPUTUM	COPD	YES	R	R	S	R	R	R	R	R			R	NEGATIVE	YES
100	RAJESH	38	M	1053651	PUS SWAB	PERI-ANAL ABSCESS	NIL	R	R	R	R	R	R	R	R			R	POSITIVE	YES
101	B/O JYOTI	D4	M	1053554	BLOOD	SEPSIS	NIL	R	R	R	S	R	R	R	R			R	POSITIVE	YES
102	B/OAKSHATA	D14	F	1053417	BLOOD	SEPSIS	NIL	S	S	R	R	S	S	S	S			S		YES
103	B/O MANISHA	D10	F	1053918	BLOOD	LBW/SEPSIS	NIL	R	R	R	S	R	R	R	R			R	POSITIVE	YES
104	B/O POOJA	D4	F	1053562	BLOOD	PT/IUGR/RDS	NIL	R	R	R	S	R	R	R	R			R	POSITIVE	YES
105	B/0 ASHWINI	D4	M	1053901	U.V.C TIP	VLBW/RDS	NIL	R	R	R	R	R	R	R	R			R	POSITIVE	YES
106	B/O DEEPA	D3	F	1054181	BLOOD	PT/LBW	NIL	R	R	R	R	R	R	R	R			R	POSITIVE	YES
107	B/O SUMAN	D3	F	1054507	BLOOD	SEPSIS	NIL	R	R	R	S	R	S	R	R			R	POSITIVE	YES
108	B/0 SHILPA	D22	M	1054697	BLOOD	RDS	NIL	R	R	R	S	R	R	R	R			R	POSITIVE	YES
109	CHINAVVA	62	F	6068636	URINE	UTI	YES	R	R	R	R	R	R	R	R	R	S	R	POSITIVE	YES
110	B/O TANU	D1	F	1054019	BLOOD	RDS/PT	NIL	S	S	S	S	R	S	S	S			S		YES
111	SAMRUDHI	3	F	1055230	URINE	Nephrotic syndrome	NIL	R	R	R	S	R	S	S	R	S	S	S		NO
112	SHILA	23	F	6071415	URINE	UTI	NIL	R	S	S	S	S	S	S	S	R	S	S		NO
113	B/O ROSHINI	D6	M	1054923	U.V.C TIP	SEPSIS/RDS	NIL	R	R	R	R	R	R	R	R			R	POSITIVE	YES
114	B/O GUFRANA	D4	M	1054177	URINE	PT/LBW	NIL	R	R	R	S	R	R	R	R	S	S	R	POSITIVE	YES
115	FAHEED MULLA	30	M	1054217	PUS SWAB	NEC.PANCREATITIS	YES	R	S	S	S	R	R	R	R			R	NEGATIVE	NO
116	B/O VEENA PATIL	D1	M	1055496	BLOOD	PT/LBW	NIL	R	R	R	R	R	R	R	R			R	POSITIVE	YES
117	AJITH M	51	M	3917720	URINE	UTI	NIL	R	R	S	S	S	S	S	S	S	R	S		NO
118	BASAVARAJ,M	47	M	1053320	WOUND SWAB	Diabetic foot ulcer	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES
119	INDUMATI	57	F	1055942	sputum	COPD	YES	R	R	S	R	R	R	R	S			R	NEGATIVE	NO
120	REKHA PRABHAKAR	34	F	612059YS7	URINE	UTI	YES	R	R	R	R	R	R	R	R	R	S	R	NEGATIVE	NO
121	MALLAPPA	68	M	1055637	PUS SWAB	Diabetic foot ulcer	YES	R	R	R	R	R	R	R	R			R	NEGATIVE	YES
122	BHAVAKESH	24	M	1057012	URINE	CKD	NIL	R	R	R	R	R	R	R	R	R	S	R	NEGATIVE	YES
123	NINGAPPA	58	M	1054883	PUS SWAB	Diabetic foot ulcer	YES	R	R	R	R	R	R	R	R			R	POSITIVE	YES

Introduction



Objectives of the Study

Review of Literature

Methodology

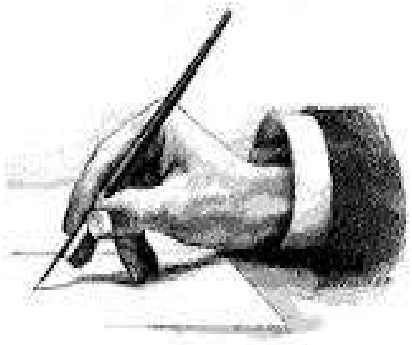
Results

Discussion

Conclusion

Summary

Bibliography



Annexure-I

Annexure-II

Annexure-III

Annexure-IV

Annexure-V