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A Study on Detection of Carbapenem Resistant Klebsiella pneumoniae from clinical samples & their Molecular characterization in a tertiary care hospital.

[1]Dr. N. Ram Murugan, M.D, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.
[2]Dr. M. Uma Maheswari, M.D, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.
[3]Dr. S. Mahesh Prabhu, M.D, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.
[4]Dr. S. Rajeswari, M.D, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.

Corresponding Author: Dr. S. Rajeswari, M.D, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.

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Abstract

Introduction: Carbapenem Resistant Klebsiella pneumoniae (CRKp) is a global burden nowadays because of their higher prevalence and wide range of clinical infections. Furthermore, there is limited treatment options available for CRKp. Hence it is important to detect carbapenem resistance from clinical samples to initiate appropriate treatment and prevent further transmission of resistant strains to the community. The aim of the present study, is to detect the prevalence of Carbapenem Resistant Klebsiella pneumoniae in our Institution.

Materials And Methods: A period prevalence study was performed at Madurai Medical College & Government Rajaji Hospital, Madurai. A total of 100 clinical isolates of Klebsiella pneumoniae were studied. All these isolates were subjected to antibiotic susceptibility testing by Kirby–Bauer disc diffusion method to detect Carbapenem resistance. The resistant strains were then subjected to PCR (Polymerase Chain Reaction) to detect Carbapenemase genes (KPC, NDM, OXA 48, VIM, IMP)

Results: Among 100 Klebsiella pneumoniae isolates 12 were carbapenem resistant (12%) , of which 4 isolates harboured NDM-1 gene , 6 isolates carried both NDM -1 & OXA-48 genes and 2 isolates had both OXA-48 & IMP genes. None of the isolates having VIM or IMP genes.

Conclusion: Carbapenem resistant Klebsiella pneumoniae isolates in the present study was 12%. Our study highlights the urgent need of proper monitoring, judicious use of antibiotics, and implementation of strict infection control practices in our clinical settings.

Keywords: Carbapenemase, Carbapenem Resistant Klebsiella pneumoniae, CRKp.

Introduction

Klebsiella pneumoniae, one of the members of Enterobacteriaceae is ubiquitous in nature. It is found in environmental niches and mucosal surfaces of human and

animals. They are the normal colonizers of the human gastrointestinal tract, skin, and nasopharynx.

Klebsiella pneumoniae is an opportunistic pathogen and an important causative agent of both community-acquired and hospital-acquired infections like pneumonia, urinary tract infections, soft tissue infections, meningitis, and septicemia.

Nowadays there is emergence of multi-drug resistance (MDR) among Klebsiella pneumoniae isolates which is quite problematic, especially in immunocompromised patients and in New borns. Increased use of broad spectrum antibiotics in hospitalized patients leads to increased carriage of Klebsiella pneumoniae due to removal of normal flora and thereby development of multidrug resistance. The multidrug resistant strains are highly virulent and have an extraordinary ability to spread.

The Multidrug resistance in Klebsiella pneumoniae is due to several factors, including its innate efflux pump mechanisms to a number of antimicrobial agents, and the increased use of broad-spectrum antibiotics, which promotes the selection of resistant clones and production of newer beta-lactamases with high hydrolytic activity.

Production of β-lactamase enzymes is an important mechanism which is responsible for Multidrug resistance. β-lactamase enzymes can be divided into four classes (A, B, C, and D) according to Ambler classification. It includes Extended-spectrum beta-lactamases (ESBLs), Amp C and carbapenem-hydrolyzing enzymes. It has been reported that the incidence of β-lactamase-producing Klebsiella pneumoniae ranges from 6 to 88% in different health care facilities. These β-lactamase enzymes are encoded by plasmids, that are capable of transferring MDR genes (blaTEM, blaCTX-M, blaSHV, blaVEB, blaPER, blaGES, blaVIM, blaIMP, blaOXA, and blaKPC) by vertical or horizontal transmission. The detection of different β-lactamase genes in resistant bacteria and characterization of their antimicrobial susceptibility profiles is essential for appropriate antimicrobial therapy and for Infection control. Thus, the aim of the present study is to determine the prevalence of Carbapenem Resistant Klebsiella pneumoniae (CR Kp) strains in our Institution and genes that are responsible for the resistance (blaVIM, blaIMP, blaOXA,and blaKPC,blaNDM) using Polymerase chain reaction (PCR).[1-5]

Materials and Methods

It is a prospective study conducted at Institute of Microbiology, Madurai Medical College & Government Rajaji Hospital, Madurai. Ethical approval has been obtained from the Institutional Ethical Committee, Madurai Medical College.

Study Period: January 2022 to June 2022

Sample size: 100 consecutive non repetitive, clinically significant isolates of Klebsiella pneumoniae from various clinical specimens were included in the study. The specimens like blood, urine, pus swab, sputum, CSF and other sterile body fluids like pleural fluid, peritoneal fluid, synovial fluid were collected aseptically from the patients depending on the clinical symptoms, after getting the consent. The specimens were transported immediately to the microbiology laboratory, Madurai medical college.

Inclusion criteria: Patients admitted in various wards (ICU, Surgery, Medicine, Pediatrics, O.G, Urology) with signs and symptoms suggestive of infections such as post operative wound infection, pyrexia of unknown origin, urinary tract infection, Pneumonia, meningitis, endocarditis, intra-abdominal abscesses and septicemia were included in this study.

Exclusion criteria: Patients with age group of less than 1 years and more than 65 years are not included in this study.

Study method: The specimens were inoculated into Nutrient agar, Blood agar and MacConkey agar media & incubated at 37º c for 24 hours. After 24 hrs of incubation at 37°C, plates were examined for the presence of growth and the organisms were identified by colony morphology, Gram staining and biochemical reactions.

Antimicrobial susceptibility testing was performed by Kirby Bauer Disc diffusion method according to CLSI (Clinical and Laboratory Standards Institute) guidelines [6]. The antibiotics tested are - Ampicillin (10µg),Gentamicin (10 µg), Piperacillin- Tazobactam (100/10µg), Cefoperazone –sulbactam(75/30 µg) Amoxicillin/clavulanate (20/10µg),Cefotaxime(30µg),Cefepime(30µg), Cefoxitin (30µg), Ceftazidime(30µg), Ciprofloxacin (5µg),Meropenem (10 µg), Amikacin (30 µg),Trimethoprim- Sulfamethoxazole (1.25 /23.75 µg), Aztreonam (30µg), imipenem (10µg). Klebsiella pneumoniae isolates that are resistant to Carbapenems were subjected to PCR to detect β-lactamase genes.

Molecular identification of Antibiotic Resistance Gene

PureFast® Bacterial DNA minispin purification kit was used . [Kit contains Lysozyme, Lysozyme digestion buffer, Proteinase-K, Binding buffer, Wash Buffer-1, Wash Buffer-2, Spin columns with collection tube and elution buffer.) HELINI 2XReDdyePCR Master Mix, Agarose gel electrophoresis consumables and Primers are from HELINI Biomolecules, Chennai, India.

The primers used are HELINI Ready to use blaKPC gene Primer mix – 2.5µl/reaction, PCR Product: 100bp HELINI Ready to use blaOXA48 gene Primer mix – 5µl/reaction, PCR Product: 380bp

HELINI Ready to use blaNDM gene Primer mix – 5µl/reaction, PCR Product: 230bp

HELINI Ready to use blaIMP gene Primer mix – 2.5µl/reaction, PCR Product: 210bp

HELINI Ready to use blaVIM gene Primer mix – 2.5µl/reaction, PCR Product: 390bp

Bacterial DNA extraction was done by the following method

1ml of overnight culture centrifuged at 6000rpm for 5min. Supernatant discarded, and the Pellet was suspended in 0.2ml PBS. Then 180µl of Lysozyme digestion buffer and 20µl of Lysozyme [10mg/ml] added. Incubated at 37ºC for 15min after that 400µl of Binding buffer, 5µl of internal control template and 20µl of Proteinase K added, mixed well by inverting several times and incubated at 56ºC for 15min; Added 300µl of Ethanol and mixed well. Then the entire sample transferred into the PureFast® spin column and Centrifuged for 1 min. Discarded the flow-through and placed the column back into the same collection tube. Added 500µl Wash buffer-1 to the PureFast® spin column and Centrifuged for 30-60 seconds ; the flow-through was discarded; The column placed back into the same collection tube. Added 500µl Wash buffer-2 to the PureFast® spin column and Centrifuged for 30-60 seconds. The flow-through discarded and the column placed back into the same collection tube. This step was repeated for an additional 1 min. This step is essential to avoid residual ethanol. Transferred the PureFast® spin column into a fresh 1.5 ml micro-centrifuge tube.Added 100µl of Elution Buffer to the center of PureFast® spin column membrane and Incubated for 1 min at room temperature and centrifuged for 2 min. The column was discarded .and purified DNA was extracted . PCR Procedure was carried out by by mixing 7.5µl of purified bacterial DNA with10µlof HELINI RedDye PCR Master

mix and 2.5µl of HELINI Ready to use - Primer Mix to make total volume of 20µl. The master mix was placed into PCR machine and programmed it as follows; Initial Denaturation: 95ºC for 5 min; Denaturation: 94ºC for 30sec, Annealing: 58ºC for 30sec, Extension: 72ºC for 30sec (35 cycles) ; Final extension: 72º C for 5 min.

PCR Samples are loaded after mixed with gel loading dye along with 10µl HELINI100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp,1000bp and 1500bp]. Subjected to electrophoresis at 50V till the dye reaches three fourth distance of the gel. Gel viewed in UV Transilluminator and observed the bands pattern.

Results & Discussion

Table 1: Sex wise distribution of Klebsiella pneumoniae isolates (n=100)

Gender	Number of Patients	Percentage
Male	55	55%
Female	45	45%

Table 2: Department wise isolation of Klebsiella pneumoniae (n=100)

Department	Number of Isolates	Percentage
ICUs (IMCU, IRCU, PICU)	22	22%
Surgery	18	18%
Medicine	15	15%
Orthopaedics	20	20%
Obstetrics & Gynaecology (OG)	17	17%
Paediatrics	8	8%

Table 2 & Fig.1 reveals that most of the Klebsiella pneumoniae strains were isolated from ICUs (22%) and from Orthopaedic wards (20%).

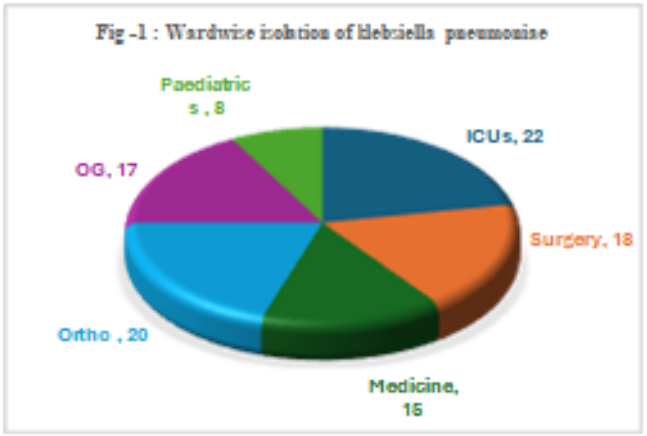


Table 3: Specimen wise isolation of Klebsiella pneumoniae species (n=100)

Specimen	Number of isolates	Percentage
Urine	38	38%
Pus	30	30%
Wound swab	5	5%
Blood	17	17%
Sputum	8	8%
Tracheal aspirate	2	2%
Total	100	100 %

Out of 100 Klebsiella pneumoniae isolates 38 were from urine, 30 from pus , 17 from blood , 8 from sputum, 5 from wound swab, and 2 from tracheal aspirate. Maximum number of Klebsiella pneumoniae were isolated from urine samples (38%) and least from tracheal aspirate (2%).

Table 4: Age wise distribution of Klebsiella pneumoniae (n=100)

Age in years	Number of patients	Percentage
1-5 yrs	7	7%
16 - 30 yrs	8	8%
31- 45 yrs	23	23%
46- 60 yrs	32	32%
61- 65 yrs	30	30%
Total	100	100 %

Table 4 shows the age wise distribution of Klebsiella pneumoniae which indicates, about 32% were isolated from patients in the age group of 45- 60 years, followed by 30 % were from patients in the age group of 61- 65 years, and 23% were from 31 -45 yrs of age group. This shows the Klebsiella pneumoniae infections are common in elderly.

Table 5: Antimicrobial Susceptibility Pattern of isolated Klebsiella pneumoniae (n=100)

Antibiotic	Susceptible		Resistant	
	Number of isolates (n)	Percentage (%)	Number of isolates (n)	Percentage (%)
Ampicillin	0	0	100	100
Gentamicin	70	70	30	30
Amikacin	92	92	8	8
Amoxicillin/clavulanate	82	82	18	18
Cefepime	80	80	20	20
Cefoxitin	82	82	18	18
Cefotaxime	78	78	22	22
Ceftazidime	82	82	18	18
Piperacillin-Tazobactum	86	86	13	13
Cefoperazone-sulbactum	89	89	11	11
Meropenem	90	90	10	10
Imipenem	88	88	12	12
Trimethoprim-Sulfamethoxazole	80	80	20	20
Ciprofloxacin	78	78	22	22
Aztreonam	95	95	5	5

Table 5 shows the antimicrobial susceptibility pattern of the Klebsiella pneumoniae strains isolated from clinical samples. It shows maximum (95%) sensitivity to Aztreonam followed by Amikacin (92%), Meropenem (90 %), Cefoperazone – sulbactum (89%) . The isolates were least sensitive to Ampicillin (0 %)

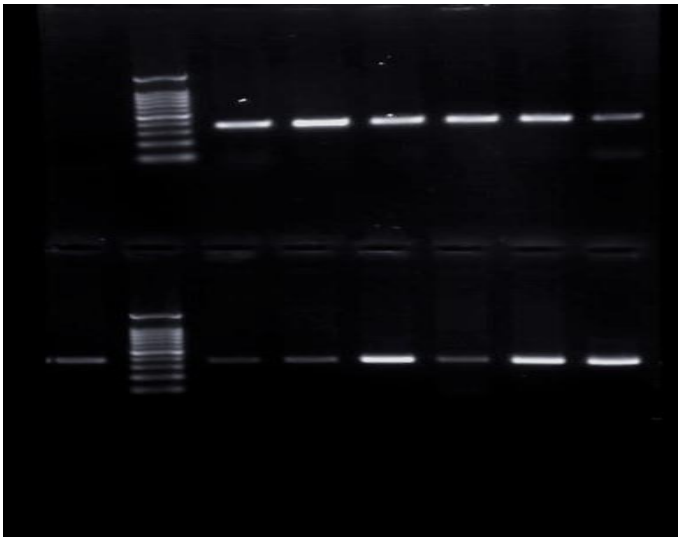
Out of 100 isolates, 12 isolates were resistant to Carbapenems . They are subjected to PCR to detect resistant genes.

Table 6: Genotypic Detection of carbapenemases in Klebsiella pneumoniae(n=12)

Resistant genes	Number of Isolates	Percentage
KPC	0	0
NDM-1	4	33.33%
NDM-1 + OXA-48	6	50%
VIM	0	0
OXA-48 + IMP	2	16.67%

Among 12 Klebsiella pneumoniae isolates , NDM-1 gene alone present in 4 isolates (33.33%), NDM -1 and OXA 48 genes seen in 6 isolates (50%) and 2 isolates (16.67%) contain IMP gene with OXA 48 gene. None of the isolates contain KPC and VIM genes

Figure 1 OXA 48 gene s1- s5 ,s9,s11,s12



Discussion

The prevalence of Carbapenem Resistant Klebsiella pneumoniae in our study is 12%. This is similar to a study done by Pravin et al [7] where the MDR in Klebsiella pneumoniae has been reported as 7.5% . In Contrast to our study, Farhadi M. Ahanjan M. Goli H.R. et al [8]had reported 58% and i Li, Hui Shen et al [9] had

reported 48.1%, MDR Klebsiella pneumoniae in their study.

Among 12 Carbapenem Resistant Klebsiella pneumoniae, NDM -1 gene present in 4 isolates, (4 %). 6 isolates (6%) harbor both NDM & OXA -48 genes and 2 isolates (2%) harbor both OXA 48 and IMP genes. The most prevalent gene among the 12 CRKP isolates genes was oxa 48 , which is similar to a previous study done by S.S.Morsi[10] in Egypt, where blaOXA-48-like types were the most predominant at 28.6% and blaKPC accounted for only for 19% . Whereas Dalia Moemen et al[11] in their study ,they had detected only 4.3% blaNDM-1 genes.

None of the isolates were positive for Metallobetalactamases (NDM-1). This is similar to a study done by Mohamudha parveen et al[12] at Pondicherry, India . In our study Most of the CRKp were isolated from Urine and Blood samples when compared to other specimens.

Conclusion

The spread of CRKP isolates represents a serious threat to hospitals. Infections caused by CRKP are associated with higher mortality [13, 14]. By doing PCR we can identify the resistant genes that are responsible for multidrug resistance. Multiplex PCR is an effective method for detection of carbapenemase genes which overcomes the limitations of the phenotypic tests. By monitoring the resistance patterns, we can contain the spread of such resistant strains in the community by developing new antimicrobials for CRKP, together with strict infection control measures including hand hygiene promotion, patients' isolation, contact precautions, environmental cleaning, active surveillance and antibiotics stewardship programs.

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