International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

Original Research Article.

Prevalence of Carbapenem Resistance in Klebsiella Pneumoniae Isolates And Their Antibiogram

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ABSTRACT

Aim: The objective of study is to evaluate the prevalence of carbapenem resistance in Klebsiella pneumoniae isolates and their antibiogram.

Method: All the samples received in the microbiology laboratory from the various departments were cultured on Blood and MacConkey's agar and blood was on Brain heart infusion broth. Antibiogram was done by Modified Kirby Bauer disk diffusion method. Detection of MBL cases were done by double disc diffusion test and confirmed by E-test of Imipenem.

Results: Total 1580 samples were tested in this study. Out of 1580, 207 Klebsiella pneumoniae were reported. Out of 207, 62 (29.95%) samples were found carbapenem resistant klebsiella pneumoniae by double disc diffusion rest 70.05% was sensitive to carbapenems. In 62 carbapenem resistant Klebsiella pneumoniae samples 67.74% were obtained from males and 32.25% from females. Maximum 19 cases were in between the age of 41-50 years & mean of the age was 44.9 ±20.53. All carbapenem resistant Klebsiella pneumoniae were sensitive to *Colistin*.

Conclusion: Carbapenem resistant Klebsiella pneumoniae is rapidly and becoming major problem for society. To control such organisms in a hospital/health care setup, strategies such as strict infection control measures, antibiotics resistance surveillance programs & restrict to clinicians, prescribe last resort drugs only where primary & secondary drugs are resistant and antibiotic cycling must be followed. Colistin could be a drug of choice in carbapenem resistant Klebsiella pneumoniae infections.

Keywords: Carbapenem Resistance, Klebsiella Pneumoniae, Antibiogram.

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Article History:

Received: 04-10-2021, **Revised:** 29-10-2021, **Accepted:** 26-11-2021

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2021.7.6.018	

INTRODUCTION

Antibiotic resistance is now becoming a global threat. The burden is more in developing countries where infectious diseases are building up. In the developing countries, there is rampant use of antibiotics mainly because, due to limited resources, most clinicians choose symptomatic treatment that's why very few microbiological samples are taken for culture and antibiotic sensitivity testing.[1,2] Increasingly, governments around the world are beginning to pay attention to a problem so serious, that it threatens the achievements of modern medicine. AMR is a complex global public health challenge, and no single or simple strategy will suffice to fully contain the emergence and spread of infectious organisms that become resistant to the available antimicrobial drugs.

Carbapenem are a group of β-lactam anti-microbial agents with an exceptionally broad spectrum of activity.[1] They are used as a last resort drug against many MDR micro-organisms such as ESBL, MBL and Amp C enzyme producing gram negative bacilli.[3] The emergence and transmission of carbapenem resistant bacteria in recent time represents a serious threat to public health. Resistance has been observed in the family of enterobacteriaceae specially to Klebsiella pneumoniae. This organism is associated with high mortality rates and has the potential to spread widely.[4]

Resistance to carbapenems can be brought about by various mechanisms, the most common being the production of carbapenemases, a class of enzymes capable of hydrolyzing Carbapenem and other b-lactam.[5] Resistance to Carbapenem can also be due to the poor binding of Carbapenem to penicillin-binding proteins present in the bacteria, the over-expression of multidrug efflux pumps by the bacteria or lack of porins present in the bacterial cell membrane. However, for significant resistance to emerge, it is the thought that a combination of resistance mechanisms is required.[6]

MATERIALS AND METHODS

Samples were collected from various wards, Indoor & outdoor patient departments of PBM Hospital from Aug 2018 to Jan 2021. Clinical samples such as blood, CSF, urine, respiratory secretions, swabs from non-healing ulcers, pus/wound swab & other samples from sterile body fluids were collected by taking aseptic precautions. All the samples except blood were cultured on blood agar & MacConkey's agar. Blood culture was done on Brain heart infusion broth. A culture plates were incubated overnight at 37ºC. Isolated gram-negative organisms were further identified by standard set of biochemical tests.[8]

Klebsiella pneumoniae isolates from the various samples; which were having less sensitivity zone size of Imipenem on modified Kirby Bauer disk diffusion method were suspected for carbapenem resistance and tested for MBL detection by Imipenem & Imipenem EDTA combined 'E-test'.

1. Antibiotic Susceptibility Testing (Anti-biogram): Anti-microbial sensitivity testing was performed on Mueller Hinton agar (Hi-Media, Mumbai) plates by disk diffusion method according to CLSI guidelines[9]. The diameter of the zones of inhibition on MHA was interpreted as sensitive, intermediate and resistant. *Escherichia coli* ATCC 25922 (β-lactamase negative) *Pseudomonas aeruginosa* ATCC 27853 (β- lactamase negative) and *Klebsiella pneumonia*e ATCC 700603 (ESBL positive) strains were used as control organisms. *Klebsiella pneumonia*e with intermediate levels of resistance to the antibiotics Imipenem was included in percentage of resistant organisms for final analysis by Imipenem & EDTA combined disc Imipenem test.

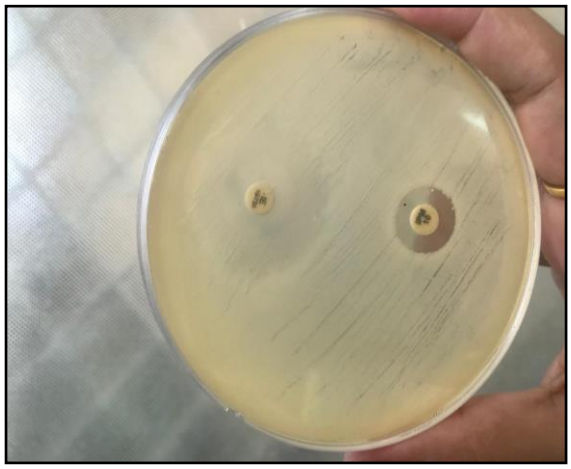

Fig 1: *K. pneumoniae* (DDST)

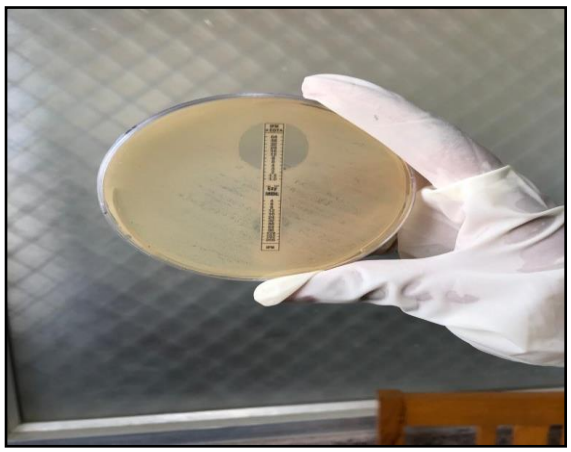

Fig 2: *K. pneumoniae* (E Test)

2. Double Disc Synergy Test (DDST) Using Imipenem and Imipenem Plus EDTA - Test organism was inoculated on to plates with Mueller Hinton agar as recommended by the CLSI.[9] An Imipenem (10 µg) disc was placed 20mm centre to centre from a disc containing 10µg Imipenem plus 0.5 M EDTA. After overnight incubation, a zone diameter difference of ≥ 7 mm between Imipenem disc & Imipenem plus EDTA disc were interpreted as Metallo-β-Lactamse positive.[9] [Fig 1]

3. MBL E-Test:

For MIC detection of Meropenem, the E-test strip method is used. The E-test MBL strip (Hi-Media) containing a double sided even dilution range of Imipenem (MRP 4 to 256 µg /ml) gradient at one end & Imipenem + EDTA (1 to 64 µg/ml) in incubation with a fixed concentration of EDTA at these other end. 100-mm-diameter Mueller-Hinton Agar plates are inoculated with swabs saturated with suspensions of the study isolates equivalent to a 0.5 McFarland standard. The results were read after 24 hrs of the incubation. The ratio of the MIC of Imipenem/(Imipenem+EDTA) > 8 dilution indicate MBL production[10]. [Fig 2]

RESULTS

Total 1580 non-repetitive variable samples from various wards were collected and processed in the Department of Microbiology, Sardar Patel Medical College and PBM Hospital, Bikaner. Out of 1580, 207 (13.1%) Klebsiella pneumoniae were isolated. These 207 Klebsiella pneumoniae were further processed for the detection of carbapenemase resistance & other antibiotic sensitivity test by disk diffusion method on Muller Hinton Agar as per CLSI guideline. Out of 207, 62 (29.95%) samples were found carbapenem resistant Klebsiella pneumoniae by double disc diffusion rest 70.05% was sensitive to carbapenems. In 62 carbapenem resistant Klebsiella pneumoniae samples 67.74% were obtained from males and 32.25% from females. Maximum 19 cases were in between the age of 41-50 years & mean of the age was 44.9 ±20.53.

90.82% carbapenem resistant Klebsiella pneumoniae were isolated from various wards & ICUs while only 9.18 % were found in OPD [Fig.1, Fig.2]. Out of 100 carbapenem resistant Klebsiella pneumoniae cases, 32.85% were reported from Surgery ward infections while18% from wound infections, 13% of Urinary tract infections and 2% each from meningitis & bacteremia were found. [Fig. 5]

Table 1: *Antibiotic sensitivity pattern of* carbapenem resistant Klebsiella pneumoniae *clinical isolates.*

Antibiotic	Klebsiella pneumoniae (n=207)	%
Amikacin	83	40.0 %
Aztreonam	50	24.15 %
Ceftaxime	4	1.93 %
Cefoparazone-sulbactum	90	43.47 %
Ciprofloxacin	25	12.07 %
Colistin	201	97.10 %
Meropenem	94	45.41 %
Imipenem	134	64.73 %
Piperacillin-Tazobactum	68	32.85 %
Levo floxacin	47	22.7 %
Tigecycline	173	83.57 %

Various antibiotics included in the study were sourced from commercial batches belonging to β-lactam, aminoglycoside, quinolone, and tetracycline classes as per the CLSI guideline. Carbapenem resistant Klebsiella pneumoniae were not only resistant to carbapenem group but also resist to most of antibiotics.

Carbapenem resistant klebsiella pneumoniae isolated from UTI were sensitive to fosfomycin & nitrofurantion. Colistin is only drug which showed 97.10% sensitivity in all carbapenem resistant klebsiella pneumoniae isolates. [Table 1] Out of total 207 cases of Klebsiella pneumoniae 65 carbapenem resistant klebsiella pneumoniae by Imipenem with & without EDTA Ezy MIC™ Strips. 34 klebsiella pneumoniae were resistant with MIC level >256 mcg/ml. [Fig. 6]

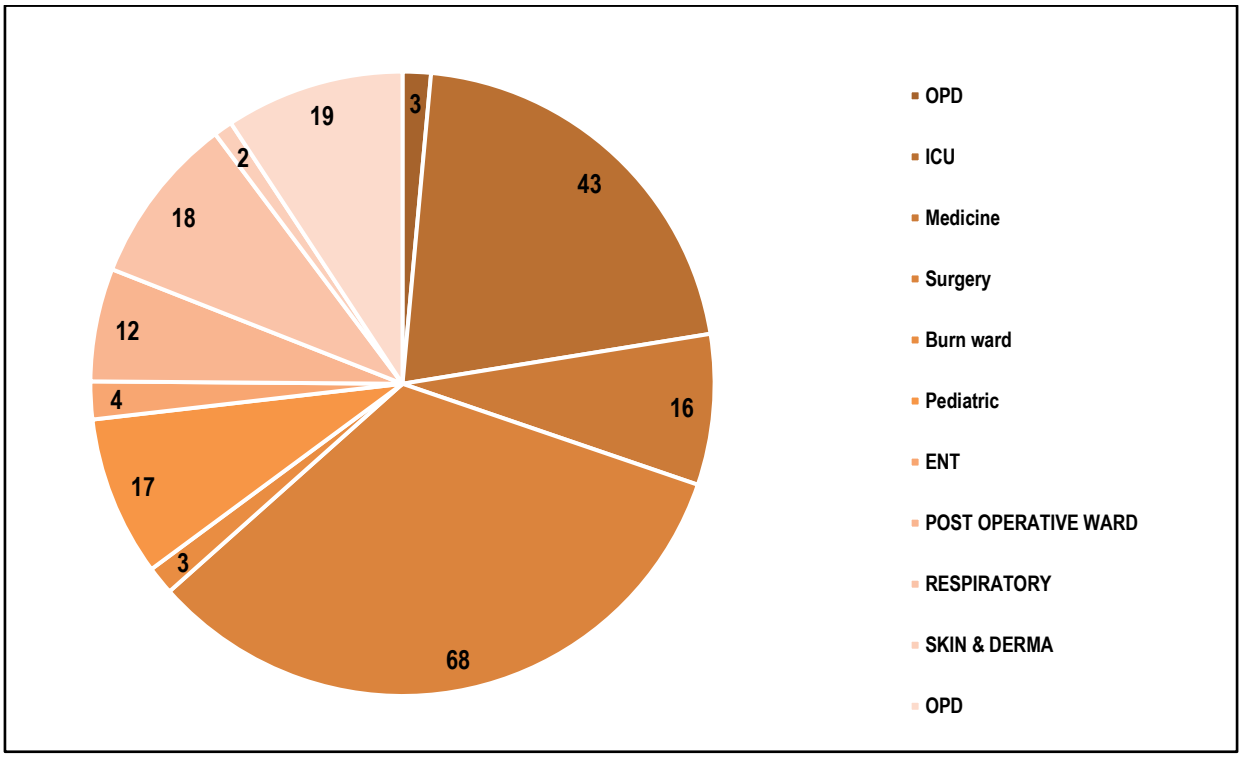

Fig 3: CR Klebsiella pneumoniae ward wise

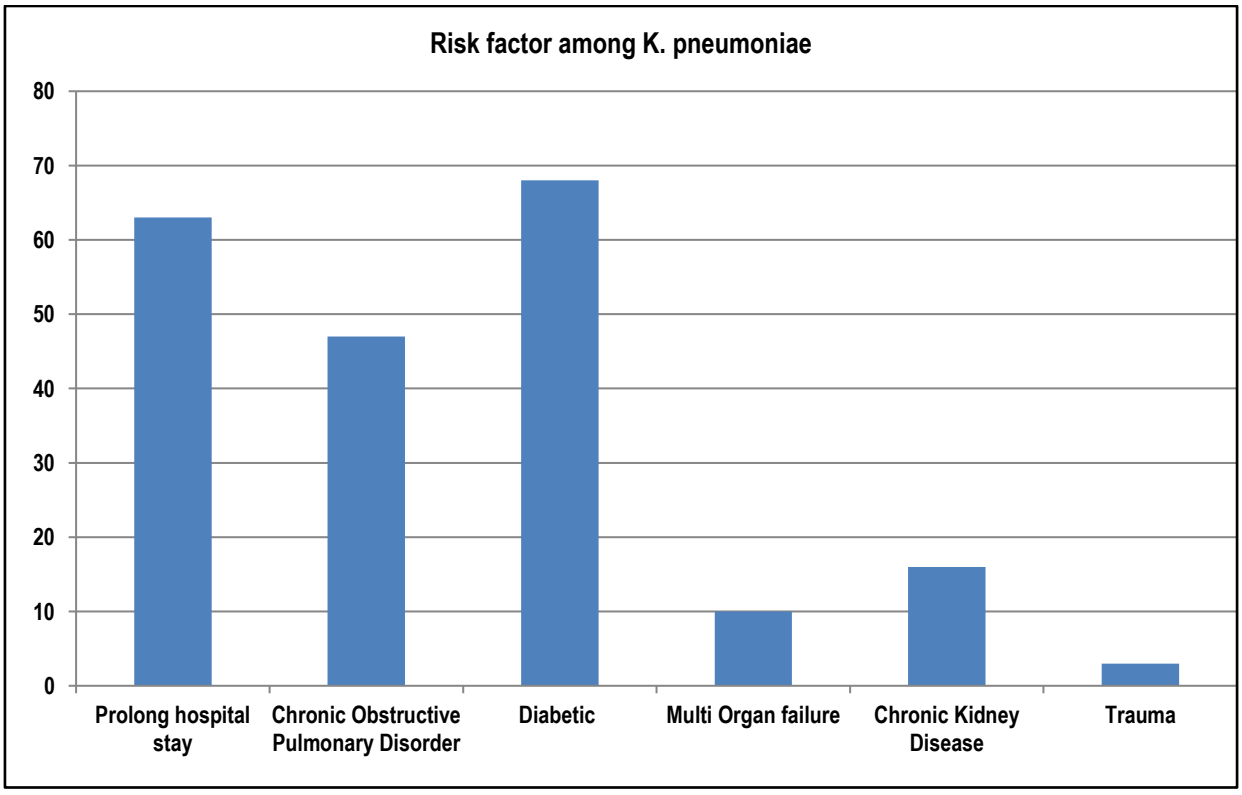

Fig 4: Risk Factor among CR Klebsiella pneumoniae

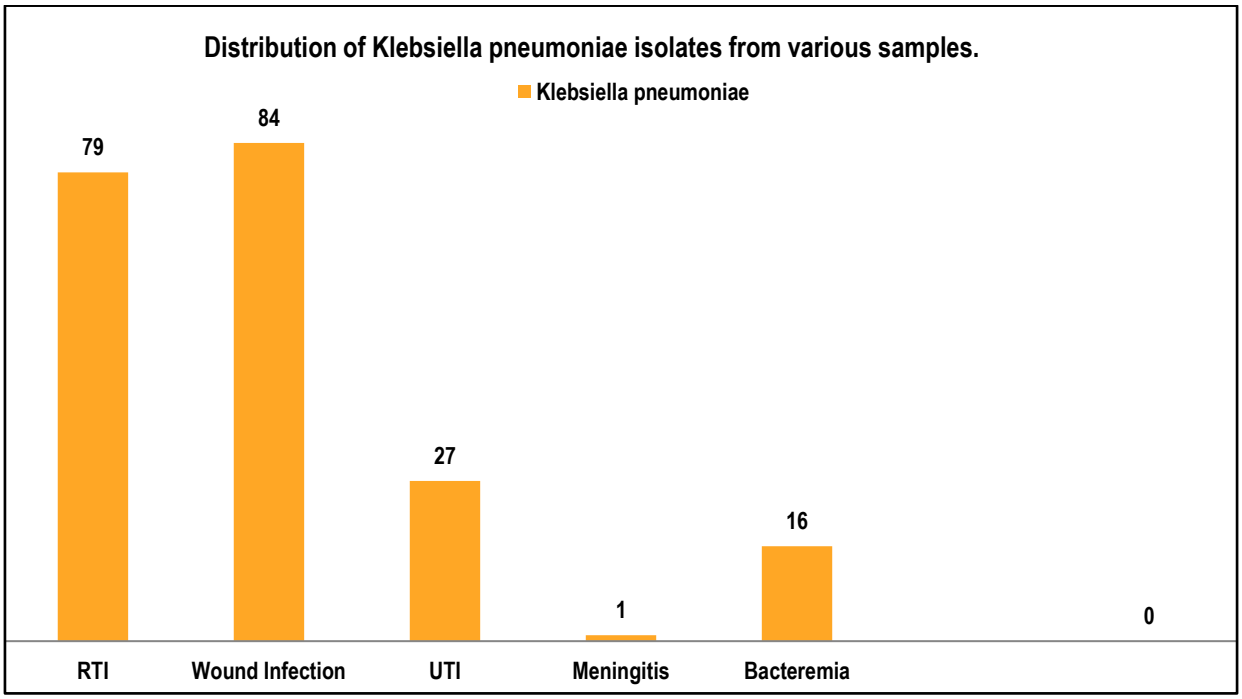

Fig 5: Klebsiella pneumoniae isolates from various samples.

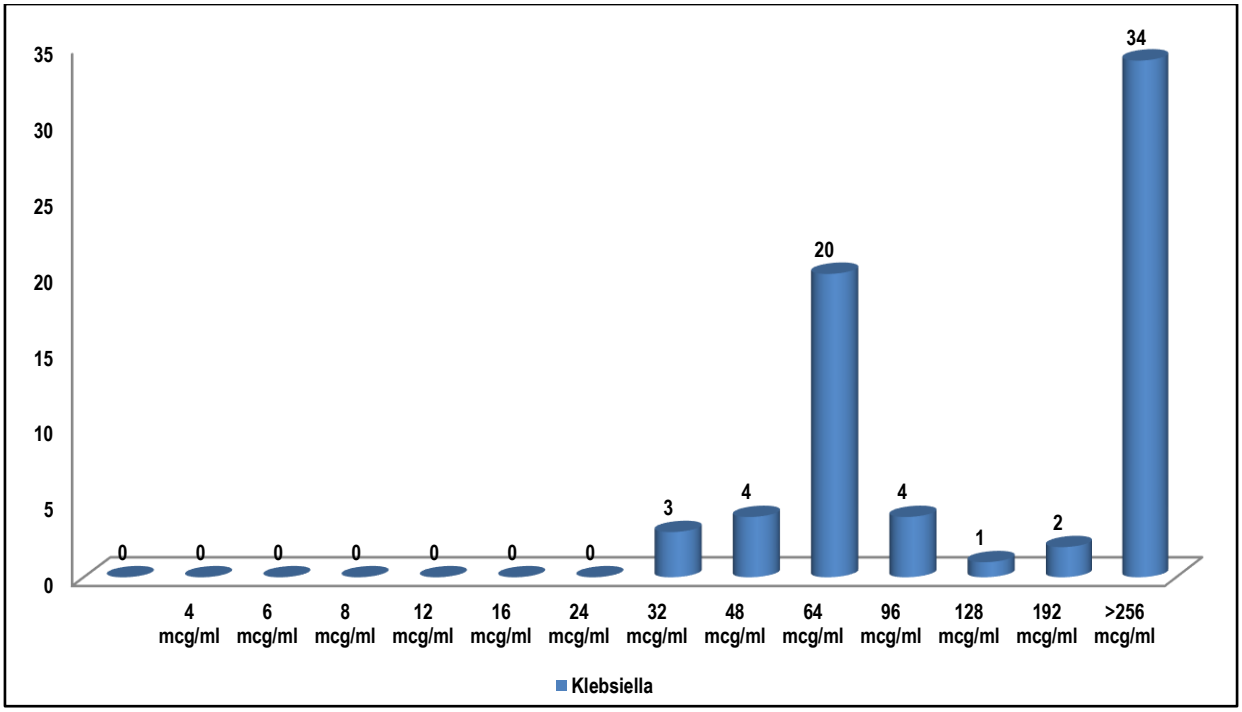

Fig 6: MIC level of Imipenem in Klebsiella pneumoniae

DISCUSSION

This study was conducted in the department of Microbiology, SPMC Medical College, Bikaner during the period of Aug 2018 to Jan, 2021. The present study includes 207 clinically significant, consecutive, non-duplicate *Klebsiella isolates.* In the present study, Out of total 207 klebsiella isolates, there was a male predominance (63.28%) among the isolates obtained from the patients. The male to female ratio 1.72:1 which is in an agreement with Sunil kumar Biradar et al where he reported male to female ratio of 1.7:1.[11] In the present study, out of 207 isolates maximum number of isolates (56) were from the patients in the age group of 21-40 yrs (27.05%) followed by (28) 13.52% from 51-60 years of age and (27) 13.04% were belongs to age group of 41-50 years.

In the present study, the distribution of *Klebsiella* species in various clinical specimens was in the following order, pus specimen 79 (38.16%), sputum 36 (17.4%), Tracheal aspirates 28 (13.5%), urine 26 (12.6%), (1.5%), and blood 16 (7.7%).This is very similar to the study conducted by Sunil kumar Biradar et al. where 50% of isolates were from pus specimens followed by urine (21%) whereas Shalley Dahiya et al, obtained Maximum isolates were from pus samples (21.55%) followed by throat swabs (18.42%), urine (15.59%), stool (9.37%), blood (9.18%). Whereas Amit kumar singh et al, in his study reported that *Klebsiella* isolates were common from blood (65%) followed by sputum (58%) urine (53%) and pus (44%).[11]

In this study, the Multidrug resistance *Klebsiella* isolates were isolated predominantly from patients in Male surgery ward-49(23.67%) followed by ICUs including NICU & PICU 43 (20.77%), Female Surgery ward & OPD (9.17%), Male Respiratory ward (8.62%) and Peadiatric ward (8.21%) etc. This is similar to the study conducted by Vemula Sarojamma et al where most of isolates were obtained from ICU (19%) followed by surgery and trauma (13%).[12]

In this study, the high-risk factors for infection with Multidrug resistance *Klebsiella* isolates were predominantly from patients in Surgery ward followed by Intensive care units -, followed by other high risk associated factors like Diabetes mellitus-68 (32.85%) prolong hospital stay 63 (30.43%) and COPD 47 (22.7%). This is very similar to Sunil kumar Biradar et al (2015), but Several studies done suggest these as the most common risk factors in patients (Namratha *et al.,* 2015; Abhilash et al., 2010). most common risk factor associated with *Klebsiella* infections was found to be diabetes followed by alcoholism and previous surgeries.[13-16]

The Antimicrobial sensitivity pattern of *Klebsiella* species were studied. In this study, the *Klebsiella pneumoniae* isolates were 97% Colistin, 83.57% Tigecycline 64% sensitive to Imipenem, Meropenem (45%), Piperacillin/tazobactum (32.85%), Amikacin (40%), Ciprofloxacin (12.07%). The results were interpreted as per CLSI guidelines. It correlates with the study done by Sasirekha et al. (2010) and Singh and Goyal (2003) in India.[17,18]

The increasing and rapid spread of carbapenem producing *Enterobacteriaceae*, particularly *in K. pneumoniae* constitutes a serious threat to public health worldwide. The present study indicated a high incidence of MBL producing *K. pneumoniae* (29.95%) in different clinical samples. this was similar to Aqsa humatun et al, 2018[155] reported 37%, but our resistance rate was higher to the study done by Loveena Oberoi et al (2012) it was 10.98% and Bandekar et.al. who reported 15.7% MBL producers? A previous study from another tertiary care hospital in Nepal reported comparatively lower incidence of carbapenem producing gram negative bacteria (1.3%) in lower respiratory tract specimens.

However, a recent study from Nepal addressed the issue of increasing incidence of carbapenem producing *K. pneumoniae* (18.2%) in tracheal aspirate samples. Several recent studies from other parts of Asia also demonstrated increasing incidence of carbapenem production in Enterobacteriaceae isolates.[19-21] In general, production of carbapenem resistance in Enterobacteriaceae isolates currently follows an increasing prevalence pattern and the prevalence rate may vary greatly in different geographical areas and from institute to institute. In our hospital setting, extended spectrum beta-lactamases are prevalent in *Klebsiella* pneumoniae and there is a gradual rise in the use of carbapenems, which could be a major cause of MBL mediated resistance.

Although molecular techniques are regarded as the most appropriate method for the detection of carbapenem resistance, it becomes impractical in a routine diagnostic laboratory setup up due to cost factors, availability of molecular set up. Carbapenem resistant Klebsiella pneumoniae infected patients serve as reservoirs for spreading infection and contaminating the environment. That's why, identified carbapenem resistant Klebsiella pneumoniae colonized patients must be contact isolated.

CONCLUSION

This study shows a clearer spectrum of the current carbapenem resistant Klebsiella pneumoniae scenario in the hospital setup. MDR Klebsiella pneumoniae are accelerating and becoming major problem in the area of infectious diseases. In order to control carbapenem producing Klebsiella pneumoniae in a hospital/health care setup, strategies such as strict infection control measures, antibiotics resistance surveillance programs & restrict to clinicians, prescribe last resort drugs only where primary & secondary drugs are resistant and antibiotic cycling must be followed. Regular monitoring and documentation of carbapenem resistance should be done. Colistin could be a drug of choice in carbapenem resistant Klebsiella pneumoniae infections. It should be used when no other drug is effective.

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Source of Support: Nil.

Conflict of Interest: None Declared.

Cite this article as: Puneet Sharma, Anjli Gupta, Geeta Tinna, Sanju Pannu, Supriya Rajvi. Prevalence of Carbapenem Resistance in Klebsiella Pneumoniae Isolates And Their Antibiogram. Int J Med Res Prof. 2021 Nov; 7(6): 87-92.
DOI:10.21276/ijmrp.2021.7.6.018