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Original Research Article

Prevalence of MDR and Colistin resistant *Klebsiella Pneumoniae* clinical isolates- A study from western India

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Abstract

Introduction: *Klebsiella pneumoniae* is an important cause of nosocomial and community-acquired Gram-negative bacteraemia; it causes pneumonia, urinary tract infections and surgical site infections worldwide. Multidrug resistant (MDR) *K. pneumoniae* has become a major increasing serious public health threat around the world due to the dearth of alternative existing antibiotics.

Aim and Objectives: The present study was undertaken to find out the prevalence of multi drug resistant (MDR) *K. pneumoniae*, with objectives to isolate, identify the *Klebsiella pneumoniae* from clinical samples, to determine antibiotic resistance pattern of *Klebsiella pneumoniae* isolates and to study colistin susceptibility of *Klebsiella pneumoniae* isolates by broth micro dilution method.

Materials and Methods: Sputum, broncho alveolar lavage (BAL), blood, urine, pus and cerebrospinal fluid (CSF) samples were processed by standard microbiological techniques. *Klebsiella pneumoniae* was identified by manual as well as by automated method (VITEK-2). Antibiotic susceptibility was tested by automated system (VITEK 2, bioMérieux). Colistin minimum inhibitory concentration (MIC) detection was done by broth micro dilution method.

Results: Out of 263 *K. pneumoniae* isolates, 119(45.24%) were carbapenem resistant(CR) and 55(20.91%) were only extended spectrum beta lactamase (ESBL) producer. 16(6.08%)isolates were colistin resistant.

Conclusion: Increasing prevalence of MDR *Klebsiella pneumoniae* could pose a real problem in a patient care and management. Emergence of colistin resistant strains of *K. pneumoniae* is alarming. Therefore, early detection and prompt implementation of infection control measures is important to prevent further spread of MDR *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae,* MDRO, Colistin resistant, Broth micro dilution

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1. Introduction

In 1882, Carl Friedlander first described *Klebsiella pneumoniae* as a bacteria isolated from the lungs of patients who had died from pneumonia. *Klebsiella* species are found in nature including water, soil, they can colonise medical devices and the healthcare environment.[1]

K. pneumoniae colonizes the mucosal surfaces of oropharynx and gastrointestinal tract. From these sites it can easily spread to other tissues and also enters in circulation causing infections such as bacteraemia, septicaemia, urinary tract infection, hospital acquired pneumonia, ventilator-associated pneumonia and surgical site infection.[1]

Klebsiella pneumoniae is an important cause of community and hospital acquired Gram-negative bacterial infections worldwide. It is the predominant cause of community-onset pyogenic infection.[2]

Klebsiella pneumoniae belongs to *Enterobacteriaceae* family. It is non-motile and Voges Proskauer (VP) test positive. It hydrolyses urea and utilises citrate. In addition to various virulence factors like cell wall lipopolysaccharide, siderophores, fimbriae it produces immense amount of capsular polysaccharides.[3]

K. pneumoniae strains are showing a high degree of resistance to broad spectrum of antibiotics from beta-lactams, fluoroquinolones, and aminoglycosides groups.[4]

Some of *K. pneumoniae* strains show resistance to about 95% of antimicrobials available in pharmaceutical market all over the world.[5]

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Multidrug resistant (MDR) *K. pneumoniae* predominantly develop antimicrobial resistance, due to production of enzymes like Extended spectrum beta lactamase (ESBL), Metallo Beta-Lactamase (MBL), AmpC and *Klebsiella Pneumoniae* Carbapenemase (KPC).[3]

MDR and carbapenem resistant *K. pneumoniae* has become a major therapeutic challenge for clinicians worldwide due to the limitation of alternative effective treatment options.[3] In addition there is lack of new antibiotics in the pipe line of process of development. At the same time, occurrence of carbapenem and colistin resistance is increasing.[3]

In view of this the present study was aimed to detect the prevalence of MDR *Klebsiella pneumoniae*, with objectives to isolate and identify the *K. pneumoniae* from clinical samples, to determine antibiotic susceptibility pattern of *K. pneumoniae* isolates and to study colistin susceptibility of *K. pneumoniae* isolates by broth micro dilution method.

2. Materials and Methods

2.1. Study design

This study was prospective observational study which was conducted at Tertiary care hospital from western India in the Period 1years, from December 2020 to November 2021. In this study total No of isolates were 263. Considering prevalence rate of 20.8% by- *Kalaivani Ramakrishnan et al*, (2019) sample size was calculated.[3] In this study Clinical Samples was taken are Blood, urine, sputum, BAL, pus and CSF

Institutional ethics committee approval was taken and the study was conducted in accordance with the ethical principles.

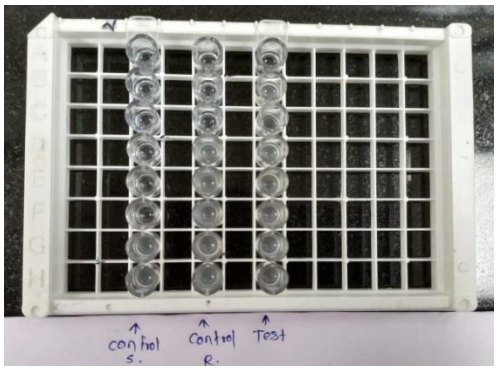
All clinical specimens from OPD and IPD patients received, were processed in the laboratory according to the standard operating procedures. Total 263 *Klebsiella pneumoniae* isolates were obtained. *Klebsiella pneumoniae* was identified by manual tests like fermentation of glucose, lactose, mannitol and sucrose, Indole formation, MR, citrate utilisation, urea hydrolysis and nitrate reduction; as well as by automated method (VITEK2 compact). Antibiotic susceptibility was tested by automated system. VITEK AST card used were- AST-N235 (urine) for OPD patients and AST-N280, AST-N40 for IPD patients.

Colistin MIC determination was done by broth micro dilution method-
(Reference- Cat. No.: MLT00057, Kit details - Company name- Erba Lachema
Kit name- MIKROLATEST)
Following control strains were used for internal testing of the antibiotics in the laboratory.

1. CCM3954 (ATCC25922) *Escherichia coli* [MIC (mg/l) – COL 0.25 to 2
2. CCM39545(ATCC27853) *Pseudomonas aeruginosa* [MIC (mg/l) - COL 0.25 to 2

The tested isolates were categorized as susceptible and resistant to colistin on the basis of MIC determination according to EUCAST (2021) interpretation or according to CLSI (2021) document M100-S27.

According to CLSI- (MIC Breakpoint (mg/l) for colistin.
For *Enterobacteriaceae* – Susceptible- (≤2)
Resistance – (≥4)
MIC is determined based on growth of isolates in wells of Micro titre plate.
(MIC 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0.)



Colistin MIC by broth micro dilution

3. Results

Total 263 K. *pneumoniae* isolates were isolated from clinical samples collected from patients suffering from variety of infections. Out of which 177 strains of *K. pneumoniae* were recovered from male and 86 from female patients.

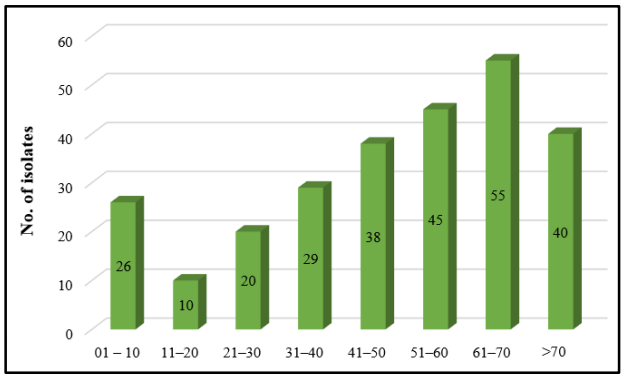


Figure 1: Age wise distribution of *Klebseilla pneumonaie* isolates

55(20.91%) *K. pneumoniae* strains were isolated from age group 61-70 yrs. followed by 51-60yrs (17.11%).

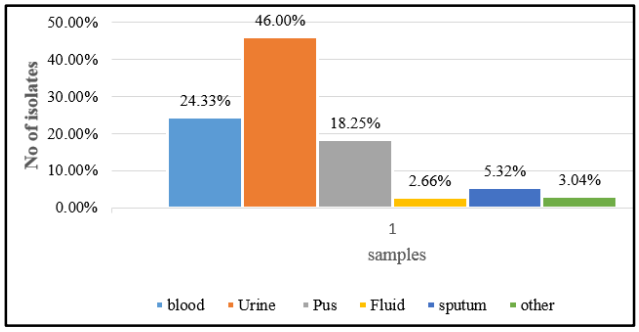


Figure 2: Sample wise distribution of *Klebsiella pneumoniae* isolates

Isolation rate of *Klebsiella pneumoniae* was highest from urine specimens 121(46.00%) followed by blood (24.33%), pus (18.25%) and sputum (5.32%).

Table 1: Resistant isolates of *Klebsiella Pneumoniae*

Resistant isolates	No of isolates-263
Only Extended Spectrum Beta Lactamase (ESBL)	55 (20.91%)
Carbapenem resistant	119(45.24%)
Colistin resistant	16(6.08%)

Out of 263 isolates of *Klebsiella pneumoniae*, 119(45.24%) were carbapenem resistant (CR) and 55(20.91%) were only extended spectrum beta lactamase (ESBL) producers. Out of 119 carbapenem resistant isolates 16(6.08%) were colistin resistant.

The Maximum carbapenems resistant (CR) *K. pneumoniae* strains were isolated from urine sample 56(21.29%) followed by blood (13.30%) and pus (6.84%) samples.

31(11.78%) Extended -Spectrum beta lactamase (ESBL) producing *K. pneumoniae* strains were isolated from urine sample and 12(4.56%) from blood specimen followed by pus 8(3.04%), sputum and other specimens.

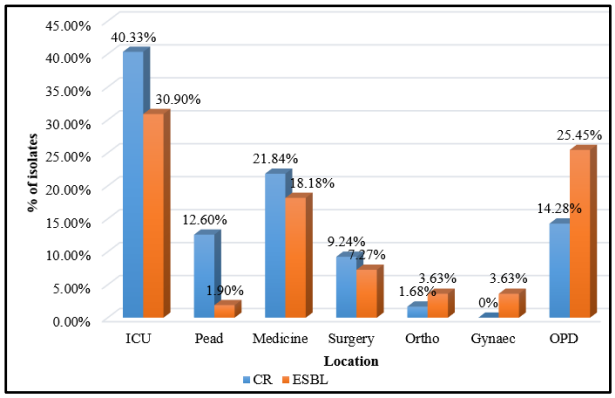


Figure 3: Location wise distribution of resistant isolates of *Klebsiella pneumoniae*

The highest number of carbapenem resistant isolates *K. pneumoniae* were from patients in ICU 48(40.33%) followed by medicine ward 26 (21.84%).17(30.90%) ESBL isolates were from ICU and 14(25.45%) ESBL isolates were from OPD patients

Table 2: Sample wise distribution of resistant isolates of *Klebsiella pneumonia*

Samples	Total Isolates 263	Extended-spectrum beta lactamase 55	Carbapenem Resistant 119	Colistin resistant 16
Blood	65(24.33%)	12(4.56%)	36(13.30%)	01(0.38%)
Urine	121(46.00%)	31(11.78%)	56(21.29%)	11(4.18%)
Pus	48(18.25%)	08(3.04%)	18(6.84%)	04(1.52%)
F Fluid	07(2.66%)	01(0.38%)	03(1.14%)	00
Sputum	14(5.32%)	02((0.76%)	03((1.14%)	00
Other	08(3.04%)	01(0.38%)	03(1.14%)	00

Table 3: Antimicrobial susceptibility pattern of of *Klebsiella pneumoniae* isolates (N-263)

Group of antibiotic	Sensitive	Resistant
Cephalosporins		
2nd generation	108 (41.07%)	155(58.93%)
3rd generation	89(33.84%)	174 (66.16%)
4th generation	99 (37.64%)	164(62.35%)
B Lactam (BL+BI)		
Piperacillin/ tazobactam, Cefoperazone/ sulbactam	144 (54.75%)	119(45.24%)
Carbapenems		
Ertapenem, Imipenem, Meropenem	144(54.75%)	119(45.24%)
Aminoglycosides, Amikacin, Gentamicin	138(52.47%)	125(47.52%)

Fluroquinolones- Ciprofloxacin, Norfloxacin	84(31.93%)	179(68.07%)
Trimethoprim/sulfamethoxazole	109(41.44%)	154(58.55%)
Colistin	247(93.92%)	16(6.08%)

Total 162 *Klebsiella pneumoniae* isolates were resistant to three or more different classes of antibiotics. Prevalence of MDR *Klebsiella pneumoniae* was 61.59%

5 (31.25%) colistin resistant strains of *K. pneumoniae* were isolated from ICU followed by 4(25%) from paediatric ward, 3(18.75%) from medicine ward and 3(18.75%) from surgery ward.

Out of 16 colistin resistant *Klebsiella pneumoniae* isolates, 11(68.75%) were from urine sample followed by pus (4) and blood (1) samples.

Out of 263 isolates of *K. pneumoniae* 30 (11.40%) were recovered from COVID positive patients.

4. Discussion

Klebsiella pneumoniae is one of the most important causes of MDR infections in the world. This bacterium is often associated with HAIs and highly contagious outbreaks in hospitals with increased mortality rate and longer stays in the hospitals, all of which results in inflated healthcare costs.[1]

The widespread non-judicious use of antibiotics in hospitals has led to emergence of multidrug resistant organisms of low virulence like *K. pneumoniae.* Over the years numerous outbreaks of infections with multi drug resistant (MDR) *K. pneumoniae* have been observed worldwide. MDR *K. pneumoniae* infections lead to poor outcome and is a great threat.

In this study 177 (67.30%) strains of *Klebsiella pneumoniae* were isolated from male patient and 86 (32.69%) from female patient. Similar observation was also made by Nirwati *et. al*.2019.[1] Akter *et al*. reported that male patients had a higher risk to get *Klebsiella* infection than females.[6]

In our study, 55(20.91%) strains of *K. pneumoniae* were isolated from 61-70 yrs. followed by 51-60yrs (17.11%). Nirwati *et al*. (2019), reported64 (38.10%) *Klebsiella pneumoniae* isolated from patients aged more than 60years old.[1]

In particular, elderly patients admitted to hospitals are generally multi-morbid and have reduced immune competence. They are increasingly susceptible to antibiotic-resistant bacteria.

In this study the isolation rate of *K. pneumoniae* was highest from urine specimens 121(46.00%) followed by blood (24.33%), pus (18.25%) and sputum (5.32%).

Sonia, *et al.,* reported out 75 isolates of *Klebsiella pneumoniae*, 28(37.33%) were isolated from wound swab, followed by sputum 20(26.67%).[7]

A study by Das *et al* (2015), reported that highest *K. pneumoniae* (46.70%) were found from sputum and other lower respiratory tract secretions followed by blood (31.30%) and wound swab (24.20%).[8]

A study by Chakraborty *et al*. (2020) reported that highest prevalence of *K. pneumoniae* was observed in urine sample followed by wound swab.[9]

Kanwalpreet Sodhi *et al* (2020), reported that among all the *Klebsiella* spp. identified (n = 533), 30.01% (n = 160) were in blood, 30.76% (n = 164) in urine, 33.77% (n = 180) in respiratory specimen and 5.44% (n = 29) in pus sample.[10]

In our study, out of 263 isolates of *Klebsiella pneumoniae*; 119(45.24%) were carbapenem resistant and 55(20.91%) were only extended spectrum beta lactamase (ESBL) producers.

Resistance to Beta lactam agents like penicillin, cephalosporins, and carbapenems is mainly due to the production of hydrolysing enzymes called beta lactamases.

High prevalence of Extended spectrum beta lactam producing *Klebsiella peumoniae* has been reported by various authors, Ferreria *et al*., (2019) & S. Rath *et al*., (2014).[4,11]

In our study 119(45.24%) *K. pneumoniae* isolates were carbapenem resistant.

30-50% carbapenem resistant *Klebsiella pneumoniae* were isolated by Shakti Rath and R.N. Padhy in (2014); a study from Bhuvaneswar.[11] Apondi *et al*. (2016), documented 23.2% carbapenem resistance rates for *K. pneumonia*.[12]

Carbapenem resistant *Klebsiella* are superbugs. Carbapenems are the drug used for treatment of ESBL producing *Klebsiella spp***.** Carbapenem resistance has been shown to result from changes in membrane permeability, high β lactamase and cephalosporinase levels and production of carbapenemases.[5]

Carbapenem resistance has emerged as a outcome of the selection pressure of using carbapenems to treat ESBL infections, and *K. pneumoniae* is the most common carbapenem-resistant *Enterobacteriaceae* (CRE).[6]

The maximum carbapenem resistant *Klebsiella pneumoniae* 56(21.29%) were isolated from urine sample followed by blood (13.30%) and pus (6.84%) sample.

K. Sreeja Wasmi *et al* (2021) isolated 223 *Klebsiella spp* .Out of these 67 Carbapenem resistant *Klebsiella spp* were isolated from ETT, 64 from Blood followed by sputum (36), pus (30) and urine (9).[13]

In this study out of 263 isolates, highest number of carbapenem resistant *Klebsiella pneumoniae* isolates were from ICU patients 48(40.33%), and medicine ward 26 (21.84%) .

Kanwalpreet Sodhi *et al* (2020) have isolated carbapenem resistant *Klebsiella pneumoniae* mainly from ICU patients.[10]

One of the risk factors for getting CR *Klebsiella pneumoniae* infection is admission of patient in ICU.[15] The morbidity of CR *Klebsiella pneumoniae* infection in ICU patients is higher as compared to other patients.[16] Hand hygiene and environmental cleaning both are important, because CR *Klebsiella pneumoniae* can be cross-transmitted through the hands of medical staff and patients' surroundings.[16]

Prevalence of CR *Klebsiella pneumoniae* in patients with coronavirus disease (COVID-19), ranged from 0.35–53%.[17,18] Pulmonary and bloodstream infection were the main types. This might be because of mechanical ventilation and central line catheters in ICUs. In the present study out of 263 isolates of *Klebsiella pneumoniae;* 30(11.40%) were isolated from COVID positive patients.

In this study 162 *Klebsiella pneumoniae* isolates were resistant to three or more different classes of antimicrobials. This accounts 61.59% prevalence rate of MDR *Klebsiella pneumoniae.*

Similar observation was reported by Ferreira *et al.*2019, majority (84%, 21/25) of *K. pneumoniae* isolates showed MDR patterns.[4] Very high MDR *Klebsiella pneumoniae* prevalence (98.5%) was reported by Tewachew Awoke *et al* (2021), from Ethiopia.[5]

In our study out of 119 carbapenem resistant isolates; 16(6.08%) were colistin resistant. Maximum colistin resistant *Klebsiella pneumoniae* strains were isolated from ICU (31.25%) (Critical care units) followed by paediatric ward (25%), medicine ward (18.75%) and surgery ward (18.75%).

Out of 16 colistin resistant isolates of *Klebsiella pneumoniae* 11(68.75%) were from urine sample, 4 from pus sample and 1 from blood sample.

Kanwalpreet Sodhi *et al*. (2020), reported 5.6% prevalence of colistin resistant *Klebsiella* species in ICU patients during 2015-2017.[8] The prevalence of colistin resistant *Klebsiella* isolates in blood, urine, respiratory and pus specimen was 8.75%, 4.26%, 4.4%3.44% respectively.[10]

Azam *et al*. (2021) reported, out of 335 isolates of *Klebsiella pneumoniae*, total 11 (3.2%) *Klebsiella* isolates from the clinical samples viz. urine (5), pus (3), tissue (1), wound swab (1), and blood (1) were Colistin-resistant.[19]

The results of one meta-analysis showed, 6.9% prevalence of colistin resistance among the clinical isolates of *K. pneumoniae* in Iran.[20]

The usage of colistin as a life-saving treatment for carbapenem-resistant and MDR *K. pneumoniae*, has increased over the years which has slowly led to emergence of colistin resistance across the globe. The emerging colistin resistance is a serious cause of concern for both clinicians and patients, particularly in countries with high rates of carbapenem resistant *Enterobacteriaceae (CRE)* such as China and India. Hence, it is necessary to perform surveillance studies for colistin resistant organisms in Indian hospitals.

Though colistin is the last resort medication, it is associated with incidence of nephrotoxicity. This questions the safety in use of colistin. Colistin has a narrow antibacterial spectrum. Lack of uniform availability of accurate minimum inhibitory concentrations it becomes very difficult to balance efficacy and toxicity. Combination of antibiotics CAZ-AVI (with or without ATM) can be a better choice for infections caused by CRE.[21] Other valuable therapeutic options for CRE are meropenem-vaborbactam.[22] and fosfomycin.[23]

5. Conclusion

In the present study, we came to the conclusion that, in our hospital, the prevalence of MDR *Klebsiella pneumoniae* is high and could be a real threat in a patient care and management. Emergence of colistin resistant strains of *Klebsiella pneumoniae* in our hospital is alarming. Therefore, early detection, prompt and adequate infection control measures are important to prevent further spread of MDR *Klebsiella pneumoniae.*

6. Ethical Approval

This study was conducted after taking approval from Institutional Ethical approval committee

7. Conflict of Interest

No conflict of interest or any affiliation or involvement in any organization.

8. Source of Funding

None.

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