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DISTRIBUTION AND CHARACTERIZATION ESBL AND CARBAPENEMASE GENES IN KLEBSIELLA PNEUMONIAE IN A TERTIARY CARE CENTER-SOUTH INDIA

Microbiology

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ABSTRACT

Introduction: MDR K. pneumoniae was responsible for 8.7%- 10% global prevalence of nosocomial infections; the burden is increased on Immuno compromised patients. A comprehensive understanding of the genes associated with MDR resistance mechanisms is essential for enhancing patient management **Materials & Methods:** A total of 150 non-duplicate multidrug resistant Klebsiella pneumoniae strains isolated from various clinical samples such as Blood, Urine, Pus, and Sputum was included. β-Lactamase genes were amplified in multiplex RTPCRs using a panel of primers for detection ESBL (blaSHV, blaTEM, blaCTX-M) and carbapenemase (blaKPC, blaVIM, blaNDM, blaIMP, and blaOXA-48like) genes. **Results:** Out of 150 isolates, 142 (95%) isolates harboring ESBL and carbapenemase genes. blaCTX-M (15.5%), blaSHV (7.7%), blaTEM (1.4%), blaCTX+SHV(21.1%) and blaNDM (10.6%), blaOXA48like (2.1%), blaNDM+OXA48like (1.4%) genes are the predominant genes in ESBL and carbapenemases respectively, Co-producing genes of ESBL and carbapenemases found in 38% (n=57) of isolates **Conclusion:** These findings emphasize the need to assess the genetic basis of antibiotic resistance in common organisms and track antibiotic resistance throughout time

KEYWORDS

MDR, ESBL, Carbapenemases, Multiplex PCR, CarbaNP test

INTRODUCTION

Klebsiella pneumoniae is most commonly isolated bacteria in hospital acquired infections and most of these strains are multidrug resistant (MDR). MDR K. pneumoniae was responsible for 8.7%- 10% global prevalence of nosocomial infections; the burden is increased Immuno compromised individuals (1).

K. pneumoniae has two main resistant mechanisms; one was expression of extended-spectrum β-lactamase (ESBL) which makes bacteria resistant to cephalosporins and monobactam. The other one was production of carbapenemases in which bacteria resistant tocarbapenems (2).

ESBLs are defined as enzymes that are able to hydrolyze extended spectrum cephalosporin like ceftazidime, ceftriaxone, cefotaxime and oxyimino monobactam and are inhibited by β- lactamase inhibitors such as clavulanic acid (3) and these enzymes are plasmid encoded. First ESBL genotype blaSHV-2 was detected in Germany, later blaTEM, blaCTX-M, blaOXA, blaGES, blaSFO, blaTLA, blaVEB and blaKLUC-5 found in various regions.

K. pneumoniae that produces ESBL is becoming more common across the world, with endemic rates of up to 50% in some areas (4). Carbapenems are drug of choice for treating ESBL infections. In recent years carbapenem resistance is increasing globally, according to World Health Organization (WHO) GLASS in 2021, Carbapenem resistant K. pneumoniae (CRKP) is one of the critically prioritized bacteria in clinical setups and reported higher mortality rates (5). Xu, L.et al reported 43.2% mortality rate compared to carbapenem susceptible isolates (6).The resistance is mainly due to carbapenemase enzymes and are plasmid-encoded. Globally distributed carbapenemases include blaKPC, blaVIM, blaIMP, blaNDM, and blaOXA$_{48like}$.

A comprehensive understanding of the genes associated with resistance mechanisms is essential for enhancing patient management. Polymyxins, Fosfomycin, tigecycline, rifampin, and carbapenems have been utilized with varying success in the treatment of multidrug-resistant K. pneumoniae infections, often as part of combination therapies.

New antibiotics, such as in ceftazidime/ avibactam, have shown effectiveness against Class A and class D carbapenemase-producing K. pneumoniae, particularly those harbouring blaKPC and blaOXA$_{48\text{-}like}$ enzymes. Nevertheless, effective treatment options for infections related to Class B(NDM) remain challenging to identify. The objective of this study is to pinpoint the genes implicated in extended-spectrum beta-lactamase (ESBL) and Carbapenem-resistant Klebsiella pneumoniae, with the aim of formulating antibiotic stewardship policies and implementing appropriate infection control measures.

MATERIALS & METHODS

Cross sectional study was conducted at Malla Reddy Narayana multispecialty hospital, Hyderabad from July-2022 to December-2022. A total of 150 non-duplicate multidrug resistant K. pneumoniae strains isolated from various clinical samples such as Blood, Urine, Pus, and Sputum was included. Multidrug resistance was identified by disk diffusion method as per the Clinical and Laboratory Standards Institute guidelines using disks of standard concentration (HiMedia, Mumbai, India) (7). The following antibiotics are tested Gentamicin (GEN), amikacin (AK) Amoxicillin–clavulanate (AMC), Aztreonam (ATM), Ceftazidime (CAZ), Cefixime (CFM), cefoxitin (CFO),Ciprofloxacin (CIP), Ceftriaxone (CTR), Cefuroxime (CXM), Cefotaxime (CTX), Ertapenem (ERT), Imipenem (IPM), Nalidixic acid (NAL), nitrofurantoin (NIT), Piperacillin–Tazobactam (PIT), Trimethoprim–sulfamethoxazole (COT).

Screening Methods for MDR- Klebsiella Pneumoniae:

Isolates resistant to Cefotaxime, ceftazidime, Imipenem, Meropenem considered as ESBL and carbapenem resistant strains respectively (8).

Table 1: Zone Sizes of ESBL and Carbapenem Resistance

Antibiotic	Resistance (zone size in mm)
Ceftazidime	≤17
Cefotaxime	≤22
Meropenem	≤19
Imipenem	≤19

I) Phenotypic Confirmatory Methods:

1) Detection of ESBL Production:

Combined Double-Disc Method

Ceftazidime (30µg) and Ceftazidime – clvulinic acid (30/10µg) and Cefotaxime (30µg) discs were applied on mulluer hinton agar distance of 20-24mm and incubated at 37^{0}C overnight. A zone diameter increases of ≥5mm for either antibiotic (such as ceftazidime and cefotaxime) tested with clavulanic acid as opposed to its zone when tested alone considered as ESBL producer (9).

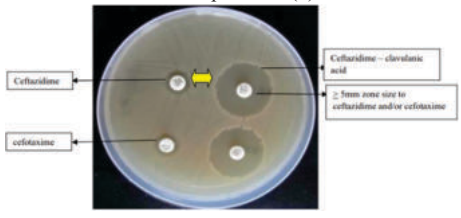


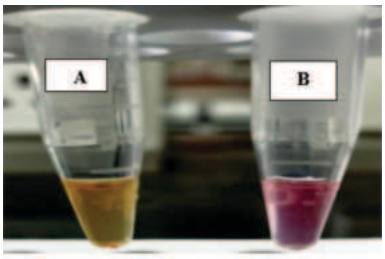
Fig 1: Combined Double Disc Test for ESBL Production

2) Detection of Carbapenem Resistance

CarbaNP test

100 µL of lyses buffer was added to tubes A and B, then colonies from MHA were emulsified in the buffer. Phenol red solution was added to tube A, and imipenem/cilastatin with phenol red to tube B. Tubes were incubated at 37°C, observing color changes every 15 minutes (10).

Tube B turning yellow color considered as positive



A: Positive B: Negative

Fig 2: CarbaNP Test for Carbapenemase Production

II) Detection of β-Lactamase Genes:

β-Lactamase genes were detected by multiplex real time PCR using a panel of primers for detection ESBL (blaSHV, blaTEM, blaCTX-M) and carbapenemase (blaKPC, blaVIM, blaNDM, blaIMP, and $blaOXA_{-48like}$) genes

I) Nucleic Acid Extraction:

DNA was isolated by spin column method by using Biopro nuclei acid extraction kit.4-5 colonies from overnight growth nutrient agar were suspended in 1.0 mL nutrient broth and incubated overnight at 37°C, and centrifuged at 8,000 rpm for 3 minutes. After removing the supernatant, the pellet was resuspended in nutrient broth, The bacterial cell was lysed with lysis solution and transfer to spin column and centrifuged at 8000rpm for 1minute. The DNA was washed with washing buffers, 60 µL DNA was eluted by adding elution buffer and centrifuged, the elution was used for detection of ESBL and carbapenemase genes (10).

ii) Multiplex PCR:

Multiplex Real time PCR was carried out to detect the ESBL (blaSHV, blaTEM, blaCTX-M) and carbapenemase (blaKPC, blaVIM, blaNDM, blaIMP, and $blaOXA_{-48like}$) genes separately (Hi media laboratories).The test was conducted using 25µL reaction mixture (Master Mix: 20µL and DNA template 5µL). The PCR thermal conditions used were 95◦C for 10 min for initial denaturation, 95◦C for 05 sec for denaturation, and 60 ◦C for 1 min for annealing and extension with 45 cycles. The cut off Ct values are <40 for ESBL and carbapenemase (Fig 3)

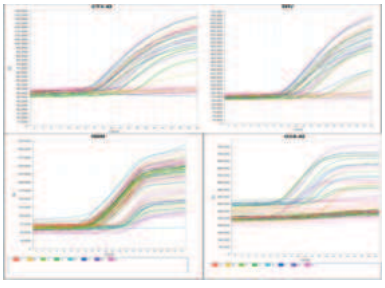


Fig 3: Multiplex PCR of β-Lactamase Genes

RESULTS:

Out of 150 K. pneumoniae isolates, 105 strains isolated from males, 45 strains isolated from females.35.3% (n=53) strains isolated from ICUs; 64.6% (n=97) strains isolated from non-ICU patients. Majority of strains isolated from pus samples 78.6% (n=118) followed by Sputum 10.6%(n=16), Urine 8.6%(n=13),and Blood 2%(n=3).Among 150 isolates 94.6%(n=142) isolates are ESBL producers, 51.3%(n=77) isolates harboring ESBL& Carbapenemase genes and 5.3% (n=8) isolates are colistin resistant.

Antibiotic pattern found 100% resistant to ceftazidime and ceftriaxone. Combination drugs also found high resistance. Amoxicillin/clavulanic acid 63.7% and Piperacillin/Tazobactam 58%. Other antibiotics such as ciprofloxacin (54.7%) and levofloxacin (50.5%), gentamycin (48.7%), Amikacin (42%), Meropenem and Imipenem (51.3%) resistant (Table 2) Tigecycline found highest susceptible (96.6%).

Double disc diffusion test and CarbaNP test for ESBL and carbapenemases are found 95% (n=143) and 97% (n=146) sensitivity and 100% specificity respectively.

Table 2: Antibiotic Susceptibility Pattern of K. pneumoniae

No. of Isolates n= 150			
Antibiotic	Sensitive	Resistant	% of Resistance
Gentamycin	77	73	48.7
Amikacin	87	63	42.0
Cefuroxime	35	115	76.7
Ceftriaxone	0	150	100.0
Ceftazidime	0	150	100.0
Cefotaxime	31	119	79.3
Cefepime	79	71	47.3
Ciprofloxacin	68	82	54.7
Amoxicillin/clavulanic acid	49	101	67.3
Piperacillin/Tazobactam	63	87	58.0
Co-trimoxazole	61	89	59.3
Imipenem	73	77	51.3
Meropenem	73	77	51.3
Tigecycline	145	5	3.3
Colistin	142	8	5.3

β-Lactamase Genes:

Total150 strains are tested for β-Lactamase genes production by RTPCR. Out of 150 isolates, 95% (n=142) isolates harboring either ESBL and carbapenemase genes alone or combination of ESBL and carbapenemase genes. blaCTX-M (15.5%) is the predominant gene in ESBL, followed by blaSHV (7.7%), blaTEM (1.4%), blaCTX+SHV (21.1%) and blaNDM (10.6%) is most prevalent gene in carbapenemase followed by $blaOXA_{48like}$ (2.1%), $blaNDM+OXA_{48like}$ (1.4%) genes. Co-producing genes of ESBL and carbapenemases found in 38% (n=57) of isolates. Distribution of other genes is depicted in table 3.

Table 3: ESBL & Carbapenem Resistant Genes

No. of K. pneumoniae strains n=142		
Type of Resistance	β-Lactamase gene	No. of stains and percentage
ESBL	blaCTX-M	22 (15.5%)
	blaSHV	11(7.7%)
	blaTEM	2(1.4%)
Carbapenemase	blaNDM	15(10.6%)
	blaOXA48like	3(2.1%)
	blaNDM+OXA48like	2(1.4%)
Multiple genes (ESBL & Carbapenemase)	blaCTX+SHV	30(21.1%)
	blaCTX+SHV+NDM	23(16.2%)
	blaCTX+SHV+NDM+ OXA48like	16(11.3%)
	blaCTX+NDM	11(7.7%)
	blaCTX+SHV+OXA48l ike	6(4.2%)
	blaCTX+OXA48like	1(0.7%)

DISCUSSION

In recent years nearly all bacterial species are becoming more multidrug resistant strains; K. pneumoniae was one of the most prevalent MDR organisms in clinical settings (11). The World Health Organization (WHO) recognizes extended-spectrum β-lactam (ESBL)-producing and carbapenem-resistant K. pneumoniae (CRKP) as a critical public health threat (12). There is a great geographical variation in the blaCTX-M type of ESBLs in K. pneumoniae isolates. The most prevalent allele with a worldwide distribution is blaCTX-M (13). In the current study most, prevalent gene was blaCTX-M found in 15.5%followed by blaSHV found 7.7%and blaTEM found 1.4%. similar to our study Shahid et al. (14) reported more frequency of blaCTX-M (28.8%) over blaSHV (13.7%) and Varsha Gupta et al. reported 81% strains harboring blaCTX-M gene followed by blaSHV (54%) and blaTEM (52%) (15). Last few decades blaTEM was the most prevalent ESBL gene in the Indian bacterial population but was replaced by blaCTX-M. blaNDM was reported as most prevalence gene in India and this gene trait was more resistant to antibiotics than other genotypes. According to study conducted by Mohanty et al., the blaNDM gene was present in 65% of carbapenem-resistant K.

pneumoniae (17), in current study 10.6% of isolates harboring blaNDM and co-existence with ESBL genes were found in 33%. In recent years, the blaOXA-48like has been the second most prolific generator of carbapenemase (18), in current study 18.6% isolates carrying $blaOXA_{48like}$ (alone and co-production). The phenotypic method used in this study was CarbaNP test recommended by CLSI. The advantages of CarbaNP include speed in providing results, easy to perform, objectiveness in interpretation and increased sensitivity, specificity and detects almost all genotypes (10). CarbaNP test was reported 100% sensitivity in this study. The coexistence of different classes of β-lactamase genes in a single isolate was common in MDR strains; we found 38% isolates carrying ESBL and carbapenemase genes this may lead to diagnostic and treatment challenges for treating carbapenem resistant K.pneumoniae infections. This study suggested that the prevalence of the genes involving ESBL and carbapenemases and their prevalence varied in different studies.

CONCLUSION

These findings emphasize the need to assess the genetic basis of antibiotic resistance in common organisms and track antibiotic resistance throughout time. Determining the different classes of enzymes is necessary because different enzymes can have different treatment options. For example, MBL-producing K. pneumoniae can be treated with carbapenems in combination with colistin, tigecycline, and aminoglycosides, while carbapenems may not be a dependable treatment option for $blaOXA_{48like}$. Furthermore, as some studies imply, it is important to monitor the different carbapenemases that are common in the areas to determine if there is a shift in trend from one type to another, such as the blaNDM to $blaOXA_{48like}$ group shift in enzymes.

REFERENCES

1) Itani, R., Hani, Rahaf Kibrit, Hamza Raychouni, Shuhaiber, P., Dib, C., Hassan, M., Mukattash, T. L., & Abdalla El-Lakany. (2024). Risk factors associated with multidrug-resistant Klebsiella pneumoniae infections: a multicenter observational study in Lebanese hospitals. BMC Public Health, 24(1). https://doi.org/10.1186/s12889-024-20474-0
2) Ghafourian, S., Sadeghifard, N., Soheili, S., & Sekawi, Z. (2015). Extended Spectrum Beta-lactamases: Definition, Classification and Epidemiology. Current issues in molecular biology, 17, 11–21.
3) Hou, X. H., Song, X. Y., Ma, X. B., Zhang, S. Y., & Zhang, J. Q. (2015). Molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology], 46(3), 759–768. https://doi.org/10.1590/S1517-838246320140138
4) Huy, T.X.N. Overcoming Klebsiella pneumoniae antibiotic resistance: new insights into mechanisms and drug discovery. Beni-Suef Univ J Basic Appl Sci 13, 13 (2024). https://doi.org/10.1186/s43088-024-00470-4
5) WHO. World Health Organization. Global Antimicrobial Resistance and Use Surveillance System(GLASS) Report 2021, Vol (2022).
6) Xu, L., Sun, X., & Ma, X. (2017). Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. Annals of clinical microbiology and antimicrobials, 16(1), 18. https://doi.org/10.1186/s12941-017-0191-3
7) Hou XH, Song XY, Ma XB, Zhang SY, Zhang JQ. Molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates. Braz J Microbial. 2015 Jul 1;46(3):759-68. doi: 10.1590/S1517-838246320140138. PMID: 26413058; PMCID: PMC4568872.
8) CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022
9) Thomson, K. S., & Sanders, C. C. (1992). Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. Antimicrobial agents and chemotherapy, 36(9), 1877-1882.
10) Sarva, K., Marimuthu Ragavan, R., Tadi, L. J., Raja, S., & Narasingam, A. (2023). Performance of Carbapenemase Nordmann-Poirel, Modified Carbapenem Inactivation, and EDTA Carbapenem Inactivation Methods for Detecting Carbapenem-Resistant Klebsiella pneumoniae Isolates. Microbial drug resistance (Larchmont, N.Y.), 29(11), 504–509. https://doi.org/10.1089/mdr.2023.0040
11) Li Y, Kumar S, Zhang L, Wu H, Wu H. Characteristics of antibiotic resistance mechanisms and genes of Klebsiella pneumoniae. Open Med (Wars). 2023 May 12;18(1):20230707. doi: 10.1515/med-2023-0707. PMID: 37197355; PMCID: PMC10183727.
12) World Health Organization. (2024). WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance, to guide research, development and strategies to prevent and control antimicrobial resistance. World Health Organization. https://iris.who.int/handle/10665/376776. License: CC BY-NC-SA 3.0 IGO
13) Bush K, Bradford PA. Epidemiology of β-lactamase-producing pathogens. Clin Microbiol Rev 2020;33:e00047-19x
14) Shahid M, Singh A, Sobia F, Rashid M, Malik A, Shukla I, et al. bla (CTX-M), bla (TEM), and bla (SHV) in Enterobacteriaceae from North-Indian tertiary hospital: High occurrence of combination genes. Asian Pac J Trop Med 2011;4:101-5.
15) Varsha Gupta, Meenakshi Singh, Priya Datta, Anku Goel, Sanjay Singh, Kashinath Prasad, Jagdish Chander, Detection of Various Beta-Lactamases in Escherichia coli and Klebsiella sp.: A Study from Tertiary Care Centre of North India, Indian Journal of Medical Microbiology, Volume 38, Issues 3–4, 2020, Pages 390-396, ISSN 0255-0857, https://doi.org/10.4103/ijmm.IJMM_20_253.
16) Verma S, Kalyan RK, Gupta P, Khan MD, Venkatesh V. Molecular Characterization of Extended Spectrum β-Lactamase Producing Escherichia coli and Klebsiella pneumoniae Isolates and Their Antibiotic Resistance Profile in Health Care-Associated Urinary Tract Infections in North India. J Lab Physicians. 2022 Oct 20;15(2):194-201. doi: 10.1055/s-0042-1757416. PMID: 37323593; PMCID: PMC10264127.
17) Mohanty, S., Gajanand, M., & Gaind, R. (2017). Identification of carbapenemase-mediated resistance among Enterobacteriaceae bloodstream isolates: A molecular study from India. Indian journal of medical microbiology, 35(3), 421–425. https://doi.org/10.4103/ijmm.IJMM_16_386
18) Jaggi, N., Chatterjee, N., Singh, V., Giri, S. K., Dwivedi, P., Panwar, R., & Sharma, A. P. (2019). Carbapenem resistance in Escherichia coli and Klebsiella pneumoniae among Indian and international patients in North India. Acta microbiologica et immunologica Hungarica, 66(3), 367–376. https://doi.org/10.1556/030.66.2019.020