Section A-Research paper

Clinical, Antimicrobial and Phenotypic Profile of *Klebsiella pneumoniae* from a Tertiary Care Hospital in Western Maharashtra.

Prajakta S. Pawar,
PG (MSC Med. Microbiology)
Krishna Institute of Medical Sciences, Karad.
Krishna Vishwa Vidyapeeth "Deemed To Be University", Karad, Maharashtra, India
Satyajeet K. Pawar,*
Associate Professor, (Dept. of Microbiology) Krishna Institute of Medical Sciences, Karad.
Krishna Vishwa Vidyapeeth "Deemed To Be University", Karad, Maharashtra, India
Satish R. Patil,
Prof. & Head
Department of Microbiology, Krishna Institute of Medical Sciences, Karad.
Krishna Vishwa Vidyapeeth "Deemed To Be University", Karad, Maharashtra, India
Tanaya P. Pawar
PG (MSC Med. Microbiology), Krishna Institute of Medical Sciences, Karad.
Krishna Vishwa Vidyapeeth "Deemed To Be University", Karad, Maharashtra, India
***Corresponding Author's Mailing Address:**
Dr. Satyajeet K. Pawar,
Associate Professor
Dept. of Microbiology,
Krishna Institute of Medical Sciences, Malkapur,
Karad, Maharashtra. India.
Pin. 415539.
Email: drskpawar@gmail.com
Phone Number: 9423034094

ABSTRACT

Background: *Klebsiella pneumoniae* has been observed to develop resistance to antimicrobials more effectively than most bacteria through the synthesis of enzymes such as Extended Spectrum β-Lactamase (ESBLs) and Carbapenemase. They are also able to pass this resistance mechanism to other bacteria in a rapid time. Antimicrobial resistance (AMR) has become a global health problem. The study has tried to address the gaps in understanding the resistant mechanism of *K. pneumoniae* isolates.

Objective: To study the antimicrobial profile of *Klebsiella pneumoniae* along with the phenotypic detection from various clinical specimens from a tertiary care hospital in Western Maharashtra.

Methods: 100 isolates of *Klebsiella pneumoniae* obtained from patients admitted in various medical, surgical and intensive care unit and patient attending outpatient department were studied. Identification of *K .pneumoniae* was done by microscopic, biochemical testing. For the testing of antimicrobial susceptibility Kirby-Bauer disk diffusion method was used. Phenotypic tests for ESBL, MBL and AmpC detection was done for the isolates.

Results: A total 100 isolates of *Klebsiella pneumoniae* were tested from different clinical specimens. Most of them were from 31-40 age group (26%) containing 8 females and 18 males and 30.77% were from ICU while remaining were from other departments like Medicine ward, Emergency department, OBGY, Surgery ward, Oncology ward. Maximum isolates were from urine 25% followed by ETT (22%), pus (22%), blood (18%). Maximum sensitivity was seen to nitrofurantoin 61 (61%) whereas maximum resistance was seen to ceftazidime 88 (88%).Out of total isolates of *Klebsiella pneumoniae* 27% were ESBL, 53% MBL and 24% AmpC producers. Multidrug resistance was seen in 88% of the *Klebsiella pneumoniae.*

Conclusion: The study reflects higher prevalence of MBL compared to ESBL and AmpC among the *K. pneumoniae* isolates. Nitrofurantoin, fosfomycin are to some extent useful drugs against this bug, but has to be used judiciously

Keywords: *K. pneumoniae*, Extended Spectrum Beta-lactamase (ESBL), Metallo-Beta Lactamase (MBL), AmpC Beta-lactamase, Multidrug resistance

INTRODUCTION

K. pneumoniae is a Gram-negative encapsulated bacteria which is normally observed in the mucosal surfaces of mammals and in soil, vegetation, water etc. In humans, *K. pnuemoniae* normally colonize the oropharynx and gastrointestinal tract from where it can easily enter into the circulation and different tissues causing infection which includes bacteremia, septicemia, surgical site infection, urinary tract infection, hospital acquired pneumonia, and ventilator-associated pneumonia. It is also responsible for increased frequency of opportunistic infections that occur among immunocompromised patient such as bladder neuropathy or diabetes mellitus.[1,2] *K. pneumoniae* has virulence factors which include capsule polysaccharide, lipopolysaccharide, outer membrane proteins, type 1 and type 3 fimbriae, and determining factors for iron procurement and nitrogen source utilization.[3] These virulence factors are known to be crucial for the pathogen to elude host immune system and for effective biofilm formation.[4]

The studies have shown that production of beta-lactamase enzymes among *K. pneumoniae* [5-7] is making it as one of the leading pathogen in community as well as hospital acquired infections. The main motive of this study was to analyze the resistant mechanism as well as ESBL, MBL, AmpC production of *K. pneumoniae.*

METHODOLOGY

Specimen collection:

Clinical samples including pus, sputum, wound swab, blood, urine and body fluids etc. from patients with active infection were collected from hospitalized patients i.e. surgical, medical, intensive care unit and outpatient department**.**

Ethical clearance:

The approval was taken from the Ethics Committee of Krishna Institute of Medical Sciences, "Deemed to be University" Karad. (Protocol number 051/2021-2022)

Sample processing:

Microscopy: Each specimen was smeared on clean, dry glass slide and was stained with Gram stain. The stained smear was microscopically examined under oil immersion lens for the presence of Gram negative bacilli.

Culture: All clinical specimens received in the laboratory were inoculated on culture media. Bacterial isolation was identified as per standard protocol described in Practical Microbiology of Mackie McCartney 14th volume.[8]

Biochemical characteristics of *Klebsiella pneumoniae*: Biochemical characterization done for the identification of *Klebsiella pneumoniae*. *Klebsiella pneumoniae* is non-motile and usually produce a prominent acidic polysaccharide based capsule.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test was performed on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method as recommended in Clinical and Laboratory Standard Institute (CLSI 2021).The zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standard 2021. [9]

Screening and confirmation of Extended Spectrum Beta-lactamase (ESBL) producers: Screening of ESBL was done as per the CLSI guidelines, if isolated Gram negative bacilli was resistant to ceftazidime (30 µg) then it was suspected to be an ESBL producer. Phenotypic confirmation of ESBL detection test was done by double disc diffusion test. After incubation, if the increase in inhibition zone with ceftazidime+ clavulanic acid disc was ≥ 5 mm than the ceftazidime alone, it was interpreted as ESBL producer.

Fig 1: Detection of ESBL production test

Screening and confirmation of Metallo Beta Lactamase (MBL): Screening and confirmation for the detection of MBL was done by EDTA- impregnated imipenem disc. For confirmatory test imipenem (10µg) disc was placed apart from imipenem-EDTA. Plates were incubated at 37˚C for 18-24 hrs. After incubation, if the zone of inhibition with imipenem-EDTA was ≥ 5 mm than imipenem discs alone, then the test was considered a positive result.

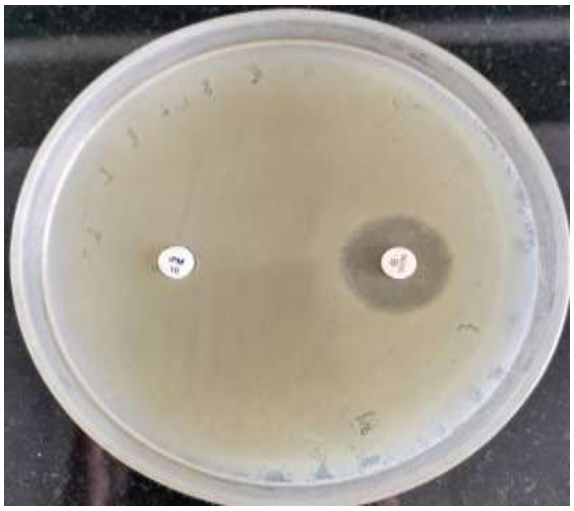

Fig 2: Detection of MBL production test

Screening and confirmation of AmpC Beta-lactamase production: This screening test was performed by cefoxitin (30 µg) disc test. If the isolated organisms showed less than 18 mm inhibition zone, it was further subjected to confirmatory test. After preparing the lawn a cefoxitin disc and a cefoxitin+ cloxacillin disc were placed 20mm apart and incubated at 37˚C for 18-24 hrs. After incubation, if the zone of inhibition with cefoxitin+ cloxacillin was ≥4 mm than the cefoxitin, then it was considered as AmpC producer.

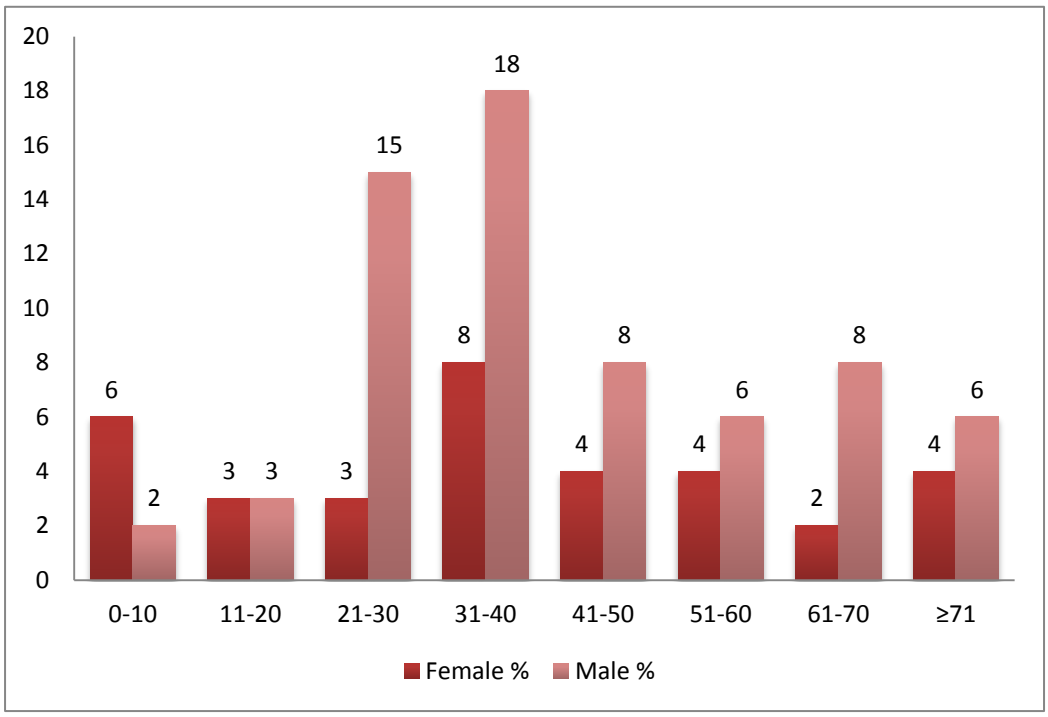

Fig 3: Detection of AmpC production test

OBSERAVATION:

Over a period of one year, 100 isolates of *Klebsiella pneumoniae* obtained from patients were studied. Maximum isolates were from 31-40 age group (26%) containing 8 females and 18 females.

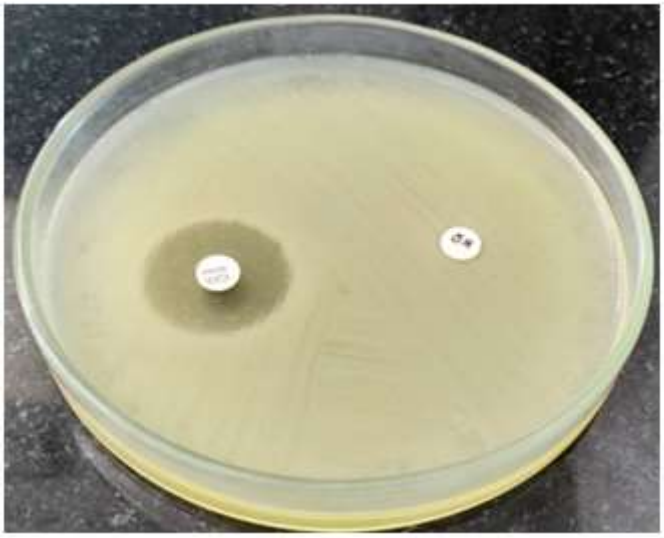

Fig 4: Age, Gender wise distribution

Maximum isolates were obtained from Surgery ICU 28 (30.77%) followed by Surgery ward 18 (19.78%), Medicine ward 14 (15.38%). 9 specimens were collected from outpatient department. (Fig 5)

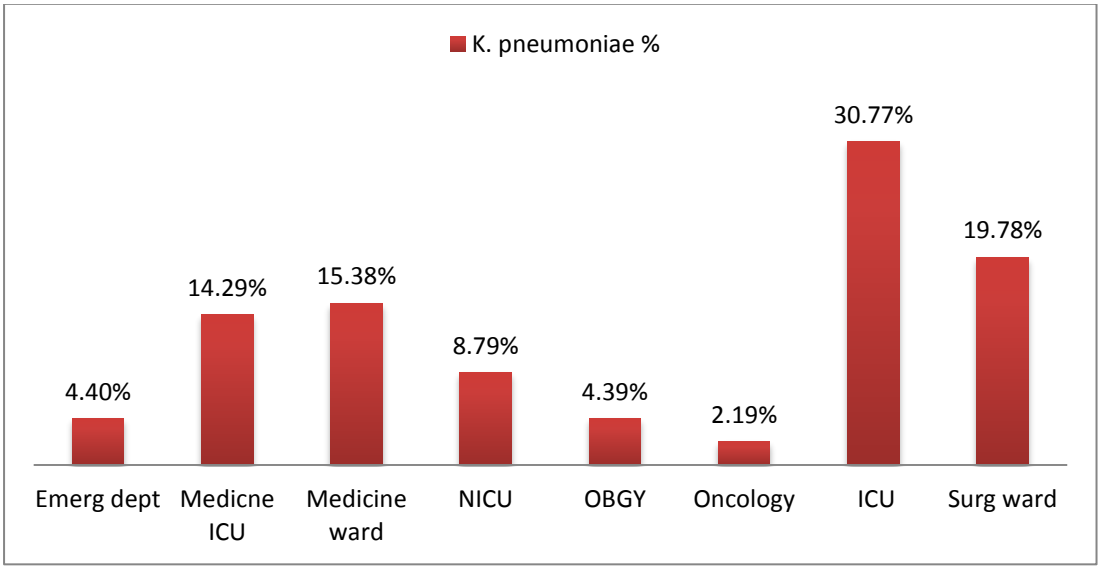

Fig 5: Ward wise distribution of *Klebsiella pneumoniae*

TABLE 1: Distribution of *Klebsiella pneumoniae* among various clinical specimens

Specimen	Number (n)	Percentage (%)
Urine	25	25
ETT	22	22
Pus	22	22
Blood	18	18
Sputum	8	8
CVP tip	2	2
Ascitic fluid	1	1
CSF	1	1
Pleural fluid	1	1
Total	**100**	**100**

Majority of isolates were from urine 25 (25%) followed by ETT 22 (22%), pus 22 (22%), blood 18 (18%). (Table 1)

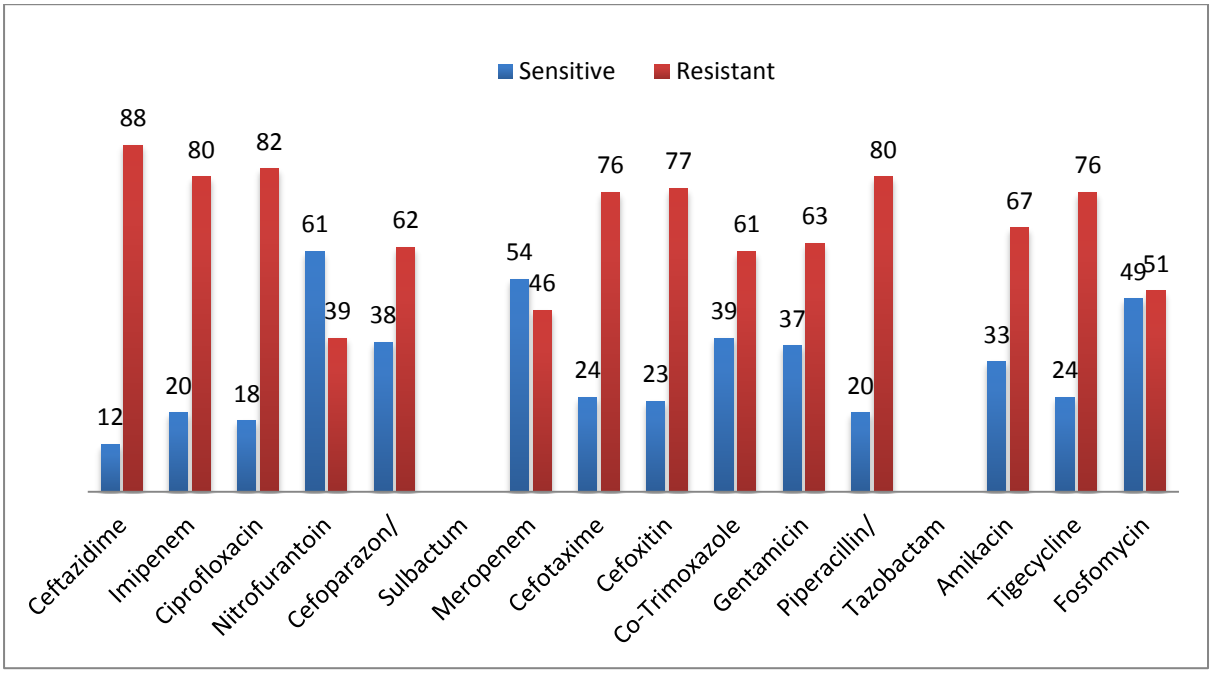

Fig 6: Antibiotic sensitivity profile *of Klebsiella pneumoniae*

Klebsiella pneumoniae showed maximum sensitivity to nitrofurantoin 61 (61%), meropenem 54 (54%), fosfomycin 49 (49%) whereas maximum resistance was seen to ceftazidine 88 (88%), ciprofloxacin 82(82%), imipenem 80 (80%). (Fig. 6)

TABLE 2: Distribution of ESBL producing and non-producing *Klebsiella pneumoniae* among various clinical specimens

Clinical samples	ESBL			
	Producer		Non-producer	
	(n)	%	(n)	%
Urine	6	6	19	19
ETT	5	5	17	17
Pus	5	5	17	17
Blood	10	10	8	8
Sputum	1	1	7	7
CVP tip	0	0	2	2
Ascitic fluid	0	0	1	1
CSF	0	0	1	1
Pleural fluid	0	0	1	1
Total	**27**	**27**	**73**	**73**

Among the 100 *Klebsiella pneumoniae* isolates 27% were ESBL producer and 73% were ESBL non-producers. Maximum ESBL producers were from blood 10 (10%) and ESBL non producers were from urine 19 (19%). (Table 2)

TABLE 3: Distribution of MBL producers and MBL non producers among various clinical specimens

Clinical Specimens	MBL +ve		MBL -ve	
	Number (n)	%	Number (n)	%
Urine	17	17	8	8
ETT	10	10	12	12
Pus	12	12	10	10
Blood	5	5	13	13
Sputum	4	4	4	4
CVP tip	2	2	0	0
Ascitic fluid	1	1	0	0
CSF	1	1	0	0
Pleural fluid	1	1	0	0
Total	**53**	**53**	**47**	**47**

Among the 100 *Klebsiella pneumoniae* isolates 53% were MBL producer and 47% were MBL non producers. Maximum MBL producing isolates were from urine 17 (17%) and maximum MBL Non producing isolates were from blood 13 (13%).

TABLE 4: Distribution of AmpC producer and non producer *Klebsiella pneumoniae* among various clinical specimens

Clinical specimens	Amp C +ve		Amp C -ve	
	Number (n)	%	Number (n)	%
Urine	6	6	19	19
ETT	3	3	19	19
Pus	6	6	16	16
Blood	4	4	14	14
Sputum	4	4	4	4
CVP tip	0	0	2	2
Ascitic fluid	0	0	1	1
CSF	0	0	1	1
Pleural fluid	1	0	0	0
Total	**24**	**24**	**76**	**76**

24 % AmpC producing and 76% Ampc non-producing *Klebsiella pneumoniae* were found in 100 isolates. Maximum AmpC producing isolates were from urine and pus 6 (6%), blood and sputum 4 (4%).

DISCUSSION

Klebsiella pneumoniae is a major nosocomial pathogen that causes difficult-to-treat infections worldwide and leads to several hospital-acquired and community acquired infections, including urinary tract infection, skin-soft tissue infection, wound, blood stream infection, meningitis, and ventilator-associated pneumonia in healthcare settings. [10] Also now a days various microorganism have tremendous properties to overcome the immune system and cause a disease. Some of the virulence factors of *Klebsiella pneumoniae* are showing the properties like capsular polysaccharide, lipopolysaccharide, fimbrial adhesions and their ability to form biofilm. Because of these abilities, finding an effective drug and treatment option has turned into a challenge globally.

β-lactamase enzymes, such as extended-spectrum β-lactamase (ESBL), AmpC β- lactamases and carbapenemase, are responsible for resistance to β-lactam antibiotics such as penicillins, cephamycin and carbapenem respectively. Carbapenem resistance due to metallo-beta-lactamase (MBL) production has been increasingly reported among clinical isolates from all around the world. Also rapidly increasing rate of MBL production among the members of *Enterobacteriaceae.*[11] This study was carried out to detect the antimicrobial susceptibility test

along with Extended-spectrum beta-lactamase (ESBL), Metallo-beta-lactamase (MBL), AmpC production among 100 isolates of *Klebsiella pneumoniae* from various clinical specimens obtained at Krishna Institute of Medical Sciences, Karad, Maharashtra.

For the antimicrobial susceptibility test, fourteen antibiotics of different classes were used for *Klebsiella pneumoniae.* Among the antibiotics used for the antimicrobial profile of *Klebsiella pneumoniae*, ceftazidime was found to be the most resistant 88 (88%) followed by ciprofloxacin 82 %, imipenem and piperacillin/tazobactam 80 % each, cefoxitin 77 %, cefotaxime and tigecycline 76 %. Fosfomycin 51 %, meropenem 46 % and nitrofurantoin 39 % are being least effective. This report is supported by the study conducted by Houri Alizadehetal. [11] that found *K. pneumoniae* isolates were resistant to ceftazidime and ciprofloxacin with 80.9% and 80.7% of resistance respectively**.**

β-lactamase enzymes like extended-spectrum beta-lactamase (ESBL) , AmpC beta-lactamase, cephamycin and carbapenem are liable for resistance to β-lactam antibiotics like carbapenem, penicillins, cephamycin. [12,13] Resistance to carbapenems due to the production of β-lactamase enzymes has been reported worldwide.[11] It is important to manage the control, infection and treatment options for drug resistant strains. For the phenotypic detection isolates of *Klebsiella pneumoniae* were screened for ESBL, MBL and AmpC β lactamase and most of the isolates were found positive.

The prevalence of extended-spectrum beta-lactamase (ESBL) producing microorganism has expanded consistently around the world and the members of *Enterobacterales* are main suspects of these enzymes. [14] Because of the rapid increase in these enzymeproduction has become a major issue in human infection. So it is important to understand the prevalence of antimicrobial resistance to deal with microorganisms.

In this study almost 27 (27%) were ESBL producer among all the isolates and this is similar to previous studies reported by Rabina Dumaru *et al.* [15] and with Achiraya siriphap et al. [14] with 30.61% and 30.2 % respectively. The findings of Krishus Nepal et al. [16] (38.5%) is slightly more than present study. Present ESBL prevalence was greater than the findings of Susmits Kuinkel et al. [11]which was 17.5%.

Infection caused due to carbapenem resistant and Gram negative microorganisms have been reported in many countries around the world with different morbidity and mortality rates.[17] In this study the prevalence of MBL producing isolates were 53% among all the isolates. This finding is in harmony with Nahla A. Melake et al.[18] with 48.8% MBL producing isolates. Similarly Krishna Dhunganaet et al. [19] reported 39.3% of MBL producing isolates. There are also some quite low rates of MBL producing *Klebsiella pneumoniae* in comparison to our study which were reported with Susmits Kuinkel et al. [11] and Houri Alizadeh et al. [10] having values 32.5% and 30.8% respectively. In the present study, almost half (49.05%) of the MBL positive isolates were from ICU setup. These patients are more likely to be receive carbapenem group of antimicrobials as a therapeutic agent. Studies have shown that overuse of these groups may result in higher prevalence of MBL production. [10]

The enzymes AmpC makes organisms resistant to penicillins, cephalosporins (second and third generations) and cephamycins. In our study almost 24% isolates were AmpC producer, which are comparable with Susmits Kuinkel et al. [11] with 20% AmpC producers. The lowest value of AmpC producer comparing with our study was seen in Madhavi S. Hullur et al.[20] with 14.67%. AmpC prevalence. In many bacteria, the enzyme AmpC is inducible and by mutation it can be expressed at high level.

CONCLUSION

It is important to focus on antimicrobial therapy, which will be beneficial to patient best treatment options. The study reflects higher prevalence of MBL compared to ESBL and AmpC among the *K. pneumoniae* isolates. This suggest very limited therapeutic options even with the last resort of antimicrobials like carbapenem groups in near future. Nitrofurantoin, fosfomycin are to some extent useful drugs against this bug, but has to be used judiciously. In view of these findings, study recommends for screening of ESBL, MBL and AmpC phenotype detection along with antimicrobial sensitivity testing for *Klebsiella pneumoniae* infection.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

DATA AVAILABILITY

The article contains the appropriate and proper data obtained during the experiment which supports the result, discussion and conclusion of the research article.

FUNDING

None.

ACKNOWLEDGEMENT

The authors are thankful to Department of Microbiology, Krishna Institute of Medical Sciences, "Deemed To Be University", Karad, Malkapur, Karad for providing necessary research facilities to carry out the work.

REFERENCE

1.Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, HatiVP, et al. Biofilm formation and antibiotic resistance of Klebsiellapneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia.BioMed Central.InBMC proceedings 2019;13(11):1-8.

2.Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saee Y, Shirvani F. et al. Evaluation of biofilm formation among *Klebsiellapneumoniae* isolates and molecular characterizationby ERIC-PCR. JundishapurJournal of Microbiology. 2016;9(1)e30682 doi: 10.5812/jjm.30682.

3.Ragupathi NK, Sethuvel DP, Dwarakanathan HT, Murugan D, Umashankar Y, Monk PNetal. The influence of biofilm formation on carbapenemresistance in clinical Klebsiella pneumoniae infections: phenotype vs genome-wide analysis. doi: https://doi.org/10.1101/2020.07.03.186130

4.Murphy CN, Clegg S. *Klebsiella pneumoniae* and type 3 fimbriae: nosocomial infection, regulation and biofilm formation. Future microbiology. 2012;7(8):991-1002.

5.Marchaim, D.; Navon-Venezia, S.; Schwaber, M.J.; Carmeli, Y. Isolation of imipenem-resistant Enterobacter species: Emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. Antimicrob. Agents Chemother. 2008, 52, 1413–1418.

6. Fisher, M.A.; Stamper, P.D.; Hujer, K.M.; Love, Z.; Croft, A.; Cohen, S.; Bonomo, R.A.; Carroll, K.C.; Petti, C.A. Performance of the Phoenix bacterial identification system compared with disc diffusion methods for identifying extended-spectrum beta-lactamase, AmpC and KPC producers. J. Med. Microbiol. 2009, 58, 774–778.

7. Sanchez, G.V.; Master, R.N.; Clark, R.B.; Fyyaz, M.; Duvvuri, P.; Ekta, G.; Bordon, J. Klebsiella pneumoniae antimicrobial drug resistance, United States, 1998–2010. Emerg. Infect. Dis. 2013, 19, 133–136.

8.Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, Mackie, McCartney's, editors. Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. 131–150.

9.Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; CLSI document M100-S31.2021

10. Alizadeh H, Khodavandi A, Alizadeh F, Bahador N. Molecular Characteristics of Carbapenem-Resistant Klebsiellapneumoniae Isolates Producing blaVIM, blaNDM, and blaIMP in Clinical Centers in Isfahan, Iran. Jundishapur Journal of Microbiology. 2021;14(2).doi: 10.5812/jjm.114473

11. Kuinkel S, Acharya J, Dhungel B, Adhikari S, Adhikari N, Shrestha UT et al. Biofilm formation and phenotypic detection of ESBL, MBL, KPC and AmpC enzymes and their coexistence in Klebsiella spp. isolated at the National Reference Laboratory, Kathmandu, Nepal. Microbiology Research. 2021;12(3):683–697

12. Caneiras C, Lito L, Melo-Cristino J, DuarteA. Community- and hospital-acquired Klebsiella pneumoniae urinary tract infection in Portugal: virulence and antibiotic resistance. Microorganism. 2019;7(5):138. https://doi.org/10.3390/microorganisms7050138

13. Raut S, Gokhale S, Adhikari B. Prevalence of extended spectrum beta-lactamases among E. coli and Klebsiella spp. isolates in Manipal Teaching Hospital, Pokhara, Nepal. J. Microbiol. Infect. Dis. 2015;5(2):69–75.

14. Siriphap A, Kitti T, Khuekankaew A, Boonlao C, Thephinlap C, Thepmalee C et al.High prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and *Klebsiella pneumoniae* isolates: A 5-year retrospective study at a Tertiary Hospital in Northern Thailand. Front. Cell. Infect. Microbial 12:955774. Doi: 10.3389/fcimb. 2022.955774

15. Dumaru R, Baral R, Shrestha LB. Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. BMC Research Notes. 2019;12(1):1-6.

16. Nepal K, Pant ND, Neupane B, Belbase A, Baidhya R, Shrestha RK et al. Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiella pneumoniae isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. Annals of clinical microbiology and antimicrobials. 2017;16(1):1-7.

17. Fatima S, Prasanthi K, Nagamani K. Comparative evaluation of biofilm production in multidrug resistant and sensitive Gram negative clinical isolates. Int.J.Curr.Microbiol.App.Sci (2015) 4(6): 918-926.

18. Melake NA, Mahmoud AB, Elraghy NA, Labib AZ, Hassan DM, Elbrolosy AM. Detection of Klebsiella pneumoniae carbapenemases and metallo-β-lactamases among Klebsiella pneumoniae isolates from hospitalized patients at Menoufia University Hospitals, Egypt. Menoufia Medical Journal. 2016;29(4):801.

19. Dhungana K, Awal BK, Dhungel B, Sharma S, Banjara MR, Rijal KR.et al. Detection of *Klebsiella pneumoniae* carbapenemase (KPC) and metallobetalactamae (MBL) producing Gram negative bacteria isolated from different clinical samples in a Transplant Center, Kathmandu, Nepal. Nepal. ASMI. 2019;2(12):60-69.

20. Hullur MS, Natarajan A, Sreeramulu P**.** Phenotypic Characterization of Virulence Factors and Antibiogram of Klebsiella pneumoniae Isolates from Various Clinical Samples – A Cross Sectional Study *J Pure Appl Microbiol.* 2022;16(3):1783-1791.