Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 18s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

To Study the Emergence of Mcr-1 Gene In Carbapenemase Encoding Colistin Resistance Klebsiella Pneumoniae in Clinical Isolates at a Tertiary Care Centre

Dr. Deepak Kumar[1], Dr. Sarita Sinha[2], Dr. Pratima Rawat[3], Dr. Nashra Afaq[4], Dr. Mukesh Kumar Patwa[5], Ashish Pal[6], Faisal Abbas[7], Dr. Vikas D Kandpal*[8]

[1]Assistant Professor, Department of Microbiology, Uttar Pradesh, University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India.

[2]Assistant Professor, Department of Microbiology, Dr. Bhimrao Ramji Ambedkar Government Medical College, Kannauj, Uttar Pradesh, India.

[3]Senior Resident, Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Uttar Pradesh, India.

[4]Assistant Professor, Department of Microbiology and Central Research Laboratory, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India.

[5]Senior Resident, Department of Microbiology, King George Medical University, Lucknow,Uttar Pradesh, India.

[6]Research Scholar, Department of Microbiology, Malwanchal University, Indore, Madhya Pradesh, India.

[7]Research Scholar, Department of Microbiology, King George Medical University, Lucknow, Uttar Pradesh, India.

[*8]Senior Resident, Department of Microbiology, King George Medical University, Lucknow, Uttar Pradesh, India.

Corresponding Author: Dr. Vikas D Kandpal*

Email ID: drvikasdkandpal@gmail.com

Cite this paper as: Dr. Deepak Kumar, Dr. Sarita Sinha, Dr. Pratima Rawat, Dr. Nashra Afaq, Dr. Mukesh Kumar Patwa, Ashish Pal , Faisal Abbas, Dr. Vikas D Kandpal*, (2025) To Study the Emergence of Mcr-1 Gene In Carbapenemase Encoding Colistin Resistance Klebsiella Pneumoniae in Clinical Isolates at a Tertiary Care Centre. *Journal of Neonatal Surgery*, 14 (18s), 669-675.

ABSTRACT

Background: Colistin resistant K. pneumoniae has rapidly become one of the leading cause of nosocomial infection, community acquired infection and is major ongoing problem in the health care facilities globally, including India. Despite the critical importance of colistin as a last-resort antibiotic, limited studies have investigated colistin resistance in human infections. To bolster the number of so called “last resort” antimicrobial agents, polymyxins such as colistin are once again being administered clinically due to their potential effectiveness against MDR infections.
Aim and Objective: To study the prevalence of MCR-1 gene and carbapenemase encoding genes among colistin-resistance Klebsiella pneumoniae in clinical isolates.
Material and Methods: A total of 200 K. Pneumonia isolates were cultured and they were confirmed using the VITEK 2 system. The DNA was extracted using the Qiagen DNA extraction kit and the PCR was performed to detect the MCR-1 gene and Carbapenemase gene and the antibiotic susceptibilities, extended-spectrum beta-lactamase (ESβL), multidrug-re sistant (MDR), and CR-KP were determined by using the VITEK 2 system, disc diffusion, and the minimum inhibitory concentration (MIC) test according to the CLSI guidelines 2024.
Result: In the present study out of 200 K. pneumoniae isolates it was noted that males were 111(55.5%) and females were 89 (44.5%) stating males were more in number, out of which carbapenem resistant K.pneumoniae were 50 (25%) in which males were 30(60%) and females were 20 (40%) and the colistin resistance percentage was 12.5 % (25/200).Shows the total number of isolates collected from samples in which urine (69%), blood(14%), pus (6.5%), sputum(5%), wounds(3.5%), tissue (1%), CSF (1%).In the present study the rates of ESβL, MDR, and CR-KP were observed to be 25%, 25% and 100%, respectively. It was noted that only one MCR-1 gene was expressed out of the 50 colistin resistance isolates was detected.
Conclusion: The emergence of plasmid-mediated colistin resistance due to the mcr-1 gene poses a great threat to human health by causing the ineffectiveness of the last-resort antibiotic, polymyxins.

Keywords: K. Pneumonia, Colistin resistance, MCR-1 gene, CLSI, Carbapenem resistance.

1. INTRODUCTION

ysis is most important for criminal investigations, as they provide crucial evidence for identifying guilty and giving justice Antimicrobial resistance (AMR) is a global threat that requires serious attention, particularly after the wide spread of the extended-spectrum beta-lactamase (ESβL) enzymes and the carbapenemase-producing strains. Globally, infectious diseases stand out as a prevalent factor leading to mortality, especially within developing nations. On a daily basis, an estimated 50,000 individuals, spanning various demographics, succumb to these diseases. Despite advancements in medical science, the persistence of infectious diseases remain a significant global apprehension [1].Thediagnostic proficiency of physicians is consistently tested by the complexity of infections. Klebsiella pneumoniae (KP) isa common cause of hospital-acquired infections including

pneumoniae, bloodstream infections, and urinary tract infections. Carbapenems belong to the group of beta-lactam antibiotics. They are considered to be an effective treatment of Gram-negative bacterial infections and confer exceptional stability against AmpC beta-lactamases and the extended spectrum beta-lactamases (ESBLs) [2]. However, due to a worldwide increase in the number of antibiotic resistant bacteria, carbapenem-resistant KP (CRKP) isolates have become a major problem. In clinical isolates, carbapenem resistance is most commonly caused by enzyme-mediated mechanisms . Carbapenemases encoded by horizontally transferable genes such as plasmids or transposons are able to inactivate carbapenems together with other beta-lactam antibiotics [2,3]. High morbidity and mortality, which are due to the widespread of CRE, have resulted in the re-emergence of the polymyxin E (colistin) drug, as the “last-line” treatment option for infections that are caused by the multidrug-resistant (MDR) bacteria. Subsequently, colistin consumption has increased remarkably in the human and animal fields. As a result, colistin resistance has emerged recently and it has now spread worldwide [4,5].

In addition to these mechanisms, the first plasmid-mediated colistin resistance gene, mcr-1, was identified on an IncI2 plasmid from Escherichia coli and K. pneumoniae in China[6]. Another plasmid-mediated colistin resistance gene mcr-2 was found in 2016 . It was suggested that dissemination of the mcr genes can lead to a serious escalation of the current antibiotic resistance crisis in the World [6,7]. Therefore, the characterization of carbepenem and colistin resistance mechanisms and understanding of the infection epidemiology are necessary for controlling dissemination of antibiotic resistant isolates. The aim of the present study was to evaluate the carbapenem resistance mechanisms and to investigate the frequency of the mcr-1 gene in CRKP isolates in a tertiary hospital.

2. MATERIAL AND METHOD

This study was a cross-sectional study carried out in the Department of Microbiology for a period of 12 months conducted between February 2024 to February 2025. A total of 200 CRKP strains isolated from hospitalized patients at a tertiary care centre were studied. The bacterial isolates were isolated from various clinical specimens, as showed in [Fig 1.] with most of the isolates coming from urine specimens (n = 138), followed by blood (n = 28), pus (n = 13), sputum (n = 10), wounds (n = 7), tissue, (n = 2), (CSF) (n = 2).

Shows the total number of isolates collected from samples in which urine (69%), blood(14%), pus (6.5%), sputum(5%), wounds(3.5%), tissue (1%), CSF (1%). They were identified by the VITEK 2 system . Antimicrobial susceptibility test was done by Kirby-Bauer disk diffusion mothod, ESβLs production were performed in doubl disk diffusion method .

Molecular Identification of MCR-1 gene

The DNA was isolated using the Qiamp DNA Blood Mini Kit (QIAGEN, Germany) as per the manufactures guidelines. The DNA was eluted in 60 µl elution buffer and preserve at -20 °C till PCR analysis. For amplification of the target gene, PCR was carried out in a 50 µL reaction mixture with 35 no. of cycles. The primers were purchased from “Saha gene’ and was reconstituted with sterile double distilled water based on the manufacturer’s instruction

Figure No.1: The Reagents used for the DNA Extraction

Primer

Gene	Sequence	Bp
***mcr*-1**	F:5'-CGGTCAGTCCGTTTGTTC-'3 R:5'-CTTGGTCGGTCTGTAGGG-'3	**309 bp [20]**

Table no.1 Primer used for the mcr1 gene

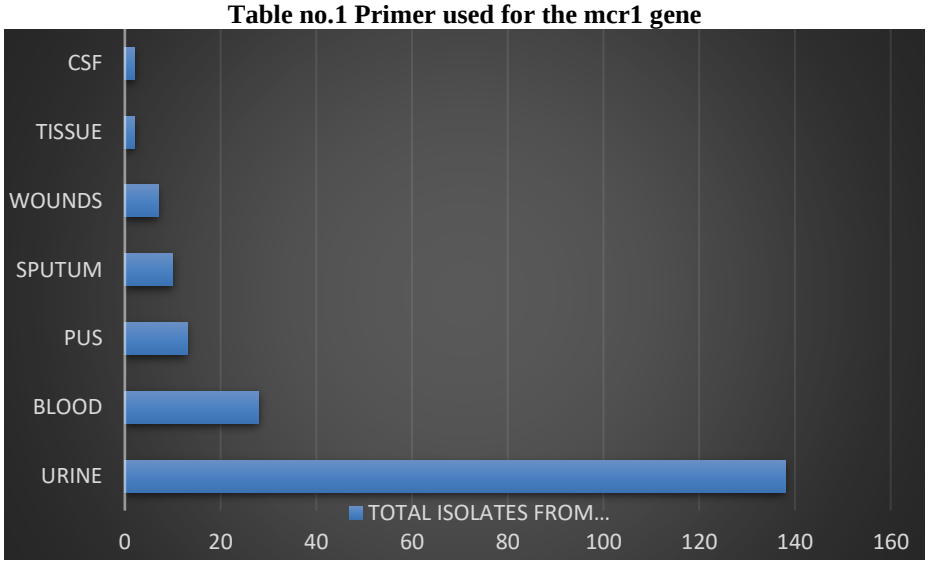

Fig 1: Shows the total number of isolates collected from samples in which urine (69%), blood(14%), pus (6.5%), sputum(5%), wounds(3.5%), tissue (1%), CSF (1%).

3. RESULTS

In the present study out of 200 K. pneumoniae isolates it was noted that males were 111(55.5%) and females were 89 (44.5%) [Fig2] stating males were more in number, out of which carbapenem resistant K.pneumoniae were 50 (25%) in which males were 30(60%) and females were 20 (40%) [Fig 3] and the colistin resistance percentage was 12.5 % (25/200).

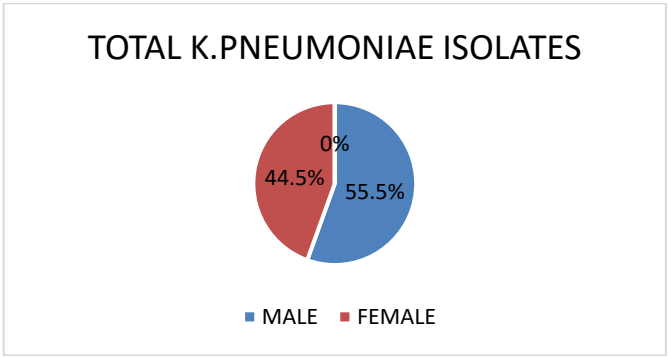

Fig 2. Showed the total number of K .pneumoniae isolates in which males (55.5%) and females were (44.5%).

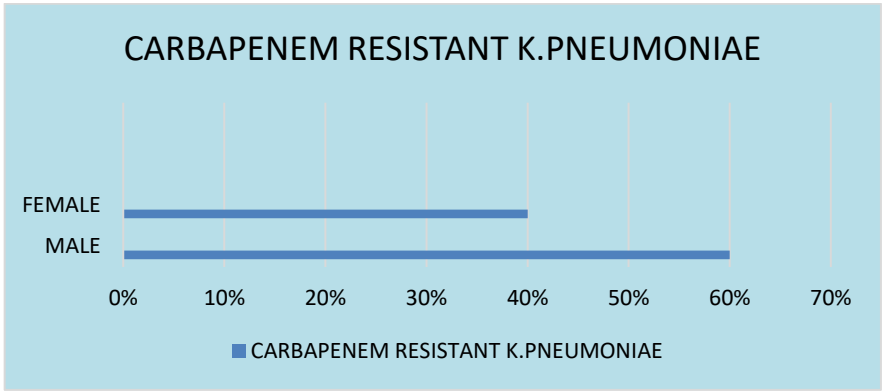

Fig 3: Showed the total number of isolates of CR-KP in which males were (60%) and females were (40%).

Out of 14 antibiotics that were used the highest resistance was found against Penicillin G(100%), followed by, Ceftazidime(50%), Cefepime(45%), Aztreonam(45%), Amoxicillin/Clavulanic acid(30%), Ciprofloxacin(20%), Tobramycin(15%), Gentamicin(20%), Piperacillin/Tazobactam(31%), and Cefoxitin(40%) showed medium susceptibility while Ertapenem(5%), Doripenem(8%), Imipenem(7%), and Meropenem(5%) showed the lowest resistance .

In general, the comparison of the colistin resistance results that were obtained through MIC with the 14 antibiotics suscept ibility results showed a statistically significant association between colistin resistance and the other classes of antibiotic resistance, the following antibiotics (Ceftazidime, Cef podoxime, Imipenem, Aztreonam, and Tobramycin) showed a statistically significant association with colistin resistance ($P \leq 0.05$).

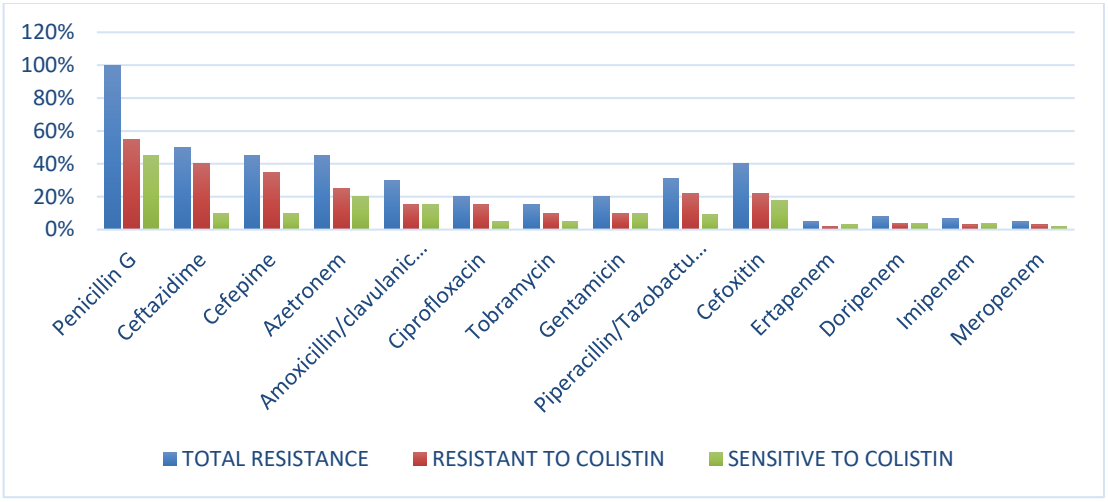

Fig 4: Showed the prevelance of colistin resistant and colistin sensitive antibiotics.

Carbapenem-resistance, ESβL, and the multidrug-resistant isolates detection Of the 200 K. pneumoniae that were isolated in the study, out of which 50(25%) were resistant to carbapenem drugs (CR-KP)[Fig5] . Based on the results of the VITEK 2 system, 50(25%) isolates were considered ESβL, and 100(50%) isolates were considered MDR. The percentages of ESβLs, MDR, and CR-KP in the co listin-resistant isolates were higher than in the colistin susceptible isolates .

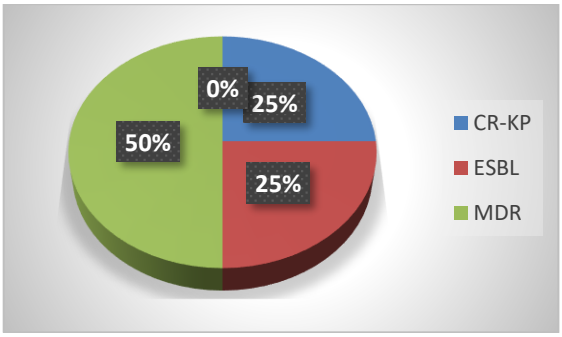

Fig 5: Showed the distribution of isolates as CR-KP , ESBL and MDR .

Carbapenem resistant genes Overall, 50 out of the 200 clinical isolates were positive for the production of one or more of the five tested types of carba penemase genes. MCR-1 (n=1)

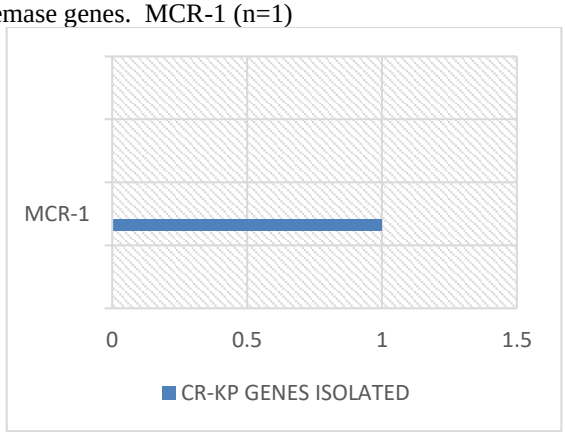

Fig 6: Showed the CR-KP genes isolated from positive samples.

The presence of the MCR- 1 gene was detected in one isolate only .

Polymerase Chain Reaction (PCR)

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water.
The cyclic conditions for MCR-1 gene, initial denaturation at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

Step	Program		Cycles
	MCR-1		
	Time	**Temperature**	
	15 min	95 ºC	
Initial denaturation	30 s	94 ºC	
Denaturation	1 min30 s	59 ºC	
Annealing	1 min 30 s	72º C	35
Extension			
Final extension	10 min	72º C	

Table No. 2 : The PCR cycling conditions to amplify MCR1gene fragment.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

- The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments.
- The resulting PCR product was subjected to 1% agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA).
- A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample.

4. STATISTIC ANALYSIS

Data along with statistic was recorded by the Microsoft Excel. The values were represented in Numbers percentage and bar diagram..

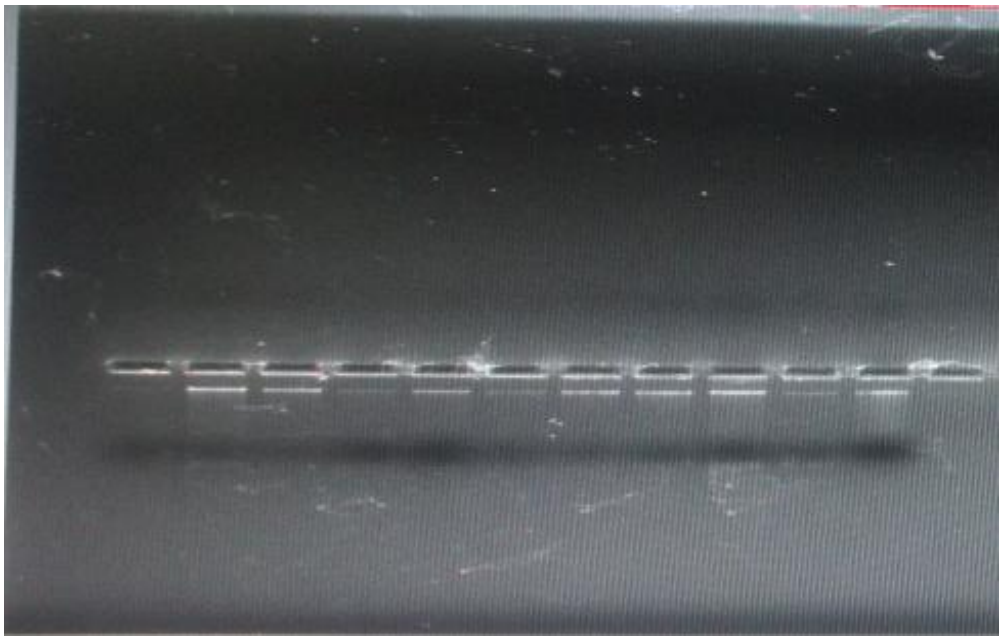

Fig 7: The DNA Extraction in Agarose gel

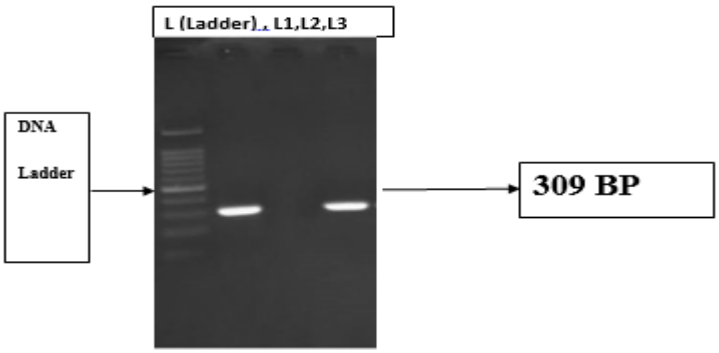

Fig 8: The Gene Exctraction mcr-1 309 bp

L is the Ladder, L1 corresponds to the Positive Control ATCC mcr-1 gene colistin resistant; L2 cosseponds to the Negative control for Mcr-1 gene; L3 is the sample positive for colistin resistant mcr- 1 gene

5. DISCUSSION

Colistin is a cationic polypeptide antibiotic belonging to the class of polymyxins that was first used clinically in the late 1950s. Polymyxins have often been used to treat infections caused by Gram-negative bacteria resistant to other antibiotics. However, the use of colistin has declined due to serious systemic side effects, such as nephrotoxicity and neurotoxicity, and the availability of less toxic antibiotics that can be used for treatment.

In our study, most of the isolates were from urine specimens, followed by blood, pus, sputum, wounds, tissue, and CSF. In the study by Azam M et.al in 2021 also shown similar results, where most of the clinical K. pneumoniae were isolated from urine, blood, pus, and wounds, respectively [8].

In our study, the highest resistance was found against Penicillin G(100%) which was similar to the study by Vasaikar S et.al in 2017 in which the high resistance to penicillin is compatible with many other studies among the Klebsiella species isolates[9].

In our study, Ceftazidime, Cefepime, Aztreonam, Amoxicillin/Clavulanic acid, Ci profloxacin, Tobramycin, Gentamicin, Piperacillin/Tazobactam, and Cefoxitin showed medium susceptibility. In the study Vasaikar S et.al in 2017 showed that Cefpodoxime was (62.9 %), Cefotaxime (61.4 %), and Ceftazidime (61.4 %) which was similar to our study. Also in the study by Effah CY et.al in 2020 showed Cefepime (48 %) resistance, followed by Aztreonam (45.80 %), Ci profloxacin (34.10 %), and Gentamicin (25.10 %). These resistant rates are lower than previous results which reported that the prevalence of Cefepime resistance ranged from 57.7 % to 83.8 %, Aztreonam (59.9–83.4 %), Ciprofloxacin (48.6–70.1 %), and Gentamicin (49.2–66.3 %) . This might indicate an over misuse of antibiotics in these countries, or it might be the result of geographical variations[10].

In our study Carbapenem-resistance, ESβL, and the multidrug-resistant isolates detection Of the 200 K. pneumoniae that were isolated in the study, out of which 50 were resistant to carbapenem drugs (CR-KP). Based on the results of the VITEK 2 system, 50 isolates were considered ESβL, and 100 isolates were considered MDR. The percentages of ESβLs, MDR, and CR-KP in the co listin-resistant isolates were higher than in the colistin susceptible isolates . In addition, the colistin-re sistant isolates showed a higher prevalence of ESβL, MDR, and CR-KP than did the colistin susceptible isolates, and this was similar to the study by Elmonir W et.al in 2021 which showed a genetic relatedness of ESβL and CR-KP to the colistin resistant K. pneumoniae isolates. Furthermore, colistin re sistance in CR-KP is considered a significant global antimicrobial issue, as the therapeutic options are limited[11].

Patients having bacterial infections were successfully treating with antibiotics in the past [12,13]. However, currently, the fast emergence of resistant bacteria and the absence of new drugs have presented a significant threat to human health . Antimicrobial resistance (AMR) is the ability of bacteria attained over time to show resistance to antibiotics causing untreatable infections resulting in prolonged illness, increased mortality rate, and high expenditure . Urinary tract infection (UTI) is among the most prevalent bacterial infections, affecting about 150 million people annually worldwide. The overuse/misuse of antibiotics for this type of frequently occurring infections has contributed to the persistence of resistant pathogens [14]

Bacteria have developed different mechanisms, one of which is extended-spectrum β-lactamase (ESBL) that hydrolyzes the beta-lactam ring of a known class of beta-lactam antibiotics. Extended-spectrum β-lactamase is found in almost all species of Enterobacteriaceae, but its ratio is slightly higher in Klebsiella pneumoniae . In such a complex situation of Multidrug Resistance (MDR), colistin is considered the last resort antibiotic to date. On the other hand, colistin is being widely used in veterinary medicine that has already enhanced resistance to this antibiotic in bacteria [15,16].

In our study, The presence of the MCR- 1 gene was detected in one isolate only . However, none of the MCR-2 and MCR-3, genes were detected in any of the isolates. This might be due to the chromosomally mediated colistin resistance mechanism rather than the plasmid-mediated genes. Further study is needed to sequence the genetic alterations mechanism of colistin resistance in the K. pneumoniae of clinical origin.

6. CONCLUSION

The presence of the mcr-1 gene in bacteria isolated from human samples highlights the urgent need for surveillance studies on a larger scale to overcome the inappropriate use of colistin-containing formulations and prevent further spread of resistance to this antibiotic.

7. DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

REFERENCES

[1] Denton M. Enterobacteriaceae. Int J Antimicrob Agents. 2007 May;29 Suppl 3:S9- S22. doi: 10.1016/S0924-8579(07)72174-X. Erratum in: Int J Antimicrob Agents. 2007 Dec;30(6):568.

[2] Bush K, Jacoby GA. MINIREVIEW Updated Functional Classification of β- Lactamases, Vol. 54; 2010, p. 969–76.

[3] Queenan AM, Bush K, Queenan AM, Bush K. Carbapenemases: the Versatile β Carbapenemases: the Versatile β-Lactamases; 2007, 20.

[4] Diene1 SM, Rolain1 JM. Carbapenemase genes and genetic platforms in Gram- negative bacilli. Enterobacteriaceae 2014.

[5] Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. Clin Microbiol Rev 2008;21:449–65

[6] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16: 161–168.

[7] Richter SE, Miller L, Uslan DZ, Bell D, Watson K, Humphries R, McKinnell JA (2018). Risk factors for colistin resistance among Gram-negative rods and Klebsiella pneumoniae isolates. J Clin Microbiol 56: 9.

[8] Azam M, Gaind R, Yadav G, Sharma A, Upmanyu K, Jain M, et al. Colistin re sistance among multiple sequence types of Klebsiella pneumoniae is associated with diverse resistance mechanisms: a report from India. Front Microbiol 2021:12.

[9] from Iran; 2018, p. 1–8. ⟨https://doi.org/10.1089/mdr.2017.0404⟩. [28] Vasaikar S, Obi L, Morobe I, Bisi-Johnson M. Molecular characteristics and an tibiotic resistance profiles of klebsiella isolates in Mthatha, Eastern Cape Province, South Africa. Int J Microbiol 2017:2017.

[10] Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob 2020;19:1–9.

[11] Elmonir W, Abd El-aziz NK, Tartor YH, Moustafa SM. Emergence of colistin and carbapenem resistance in extended-spectrum β-lactamase producing Klebsiella pneumoniae isolated from chickens and humans in Egypt. Biology 2021;10(5):373.

[12] Sanganyado E, Gwenzi W. Antibiotic resistance in drinking water systems: Occurrence, removal, and human health risks. Sci Total Environ. 2019;669:785-97.

[13] Hala S, Antony CP, Alshehri M, Alsaedi A, Al Thaqafi OA,Al-Ahmadi G, Kaaki M, Alazmi M, Alhaj-Hussein TB, Yasen M, Zowawi MH, Al-Amri A, Pain A (2020) An emerging clone (ST2096) of Klebsiella pneumoniae clonal complex 14 with enhanced virulence causes an outbreak in Saudi Arabia. J Infect Public Health 13: 363–364.

[14] Urooj M, Shoukat M, Imran M, Ansar M, Faryal R (2022) Emergence of hypermucoviscous colistin-resistant high-risk convergent Klebsiella pneumoniae ST-2096 clone from Pakistan. Future Microbiol 17: 989-1000. doi: 10.2217/fmb-2021-0292.

[15] Kumar S, Anwer R, Azzi A (2023) Molecular typing methods& resistance mechanisms of MDR Klebsiella pneumoniae. AIMS Microbiol 9: 112-130.

[16] Erkut Afyoncu et al. Investigation of colistin heteroresistance and the colistin resistance genesmcr-1 to mcr-5 in Escherichia coli and Klebsiella pneumoniae isolates in a tertiary hospital in Turkey. J Infect Dev Ctries 2024; 18(11):1687-1694.