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Antimicrobial susceptibility pattern of colistin resistance *Klebsiella pneumoniae* from clinical Isolates

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***Abstract*---**Background: There is very little information available in India about the prevalence of colistin-resistant *Klebsiella pneumonia* in patients and their susceptibility pattern. The increased use of colistin to treat infections are caused by multidrug-resistant Gram-negative bacteria has resulted in an increase of colistin resistance in *Klebsiella pneumoniae* in numerous countries. Materials and Methods: These isolates were collected from distinct clinical specimens and analyzed using the broth micro-dilution technique to establish their colistin minimal inhibitory concentration (mic). Result: Of 116 Klebsiella species, *Klebsiella pneumonia* was 96.55% while *Klebsiella oxytoca* was 3.45%. Among isolates, 09 (7.76%) were colistin resistant *Klebsiella pneumonia* by broth-micro dilution. In total, 09 case-patients were identified, 62.93% males and 37.07% females. The mean±SD of the age was 45.93±18.15. Carbapenem, Piperacillin-tazobactam and tigecycline were the most effective drug used for combine therapy to colistin resistance gram negative infections. Conclusion: This is the first study to look at the incidence of colistin-resistant *Klebsiella pneumoniae* in individuals in Jaipur. Infection caused by *Klebsiella pneumonia* highly resistant to many drugs. However, various colistin-based combined strategies have indeed been proven to be effective in curing these problems. To minimise colistin use and avoid misuse, a comprehensive antibiotic stewardship policy must be implemented.

Keywords*---Klebsiella pneumoniae*, Gram negative Bacteria, Multi-drug Resistance, Colistin Resistance, Broth-micro dilution method, Minimum Inhibitory Concentration.

Introduction

Antibiotic resistance is widely regarded as one of the most serious impacts on human health globally. Infectious diseases that are hard to treat, therapeutic problems, prolonged hospital admissions, and higher mortality are all consequences of rising antimicrobial resistance. Because of restricted medical interventions, Klebsiella spp., have been increasingly associated with high morbidity rates. Because of the advent and unprecedented spread of carbapenemases, the usage of last antimicrobial colistin for reclamation treatment has increased. [1–4].

Colistin, also known as polymyxin E, is a polypeptide antibiotic that was created in the 1950s. The antimicrobial was mainly sourced from the soil bacteria *Paenibacillus polymyxa* subsp. [5]. Though that was banned in clinical usage as in 1980s due to its high toxicity, it has recently been resumed for the management of multidrug resistant (MDR) bacteria, notably carbapenem-resistant Gram-negative bacterial infections [3, 4, 6, 7]. It works by disrupting bacterial cell membranes and/or inhibiting bacterium respiration [8, 9].
The very first plasmid-mediated colistin resistance gene, mcr-1, was discovered in *Escherichia coli* and *Klebsiella pneumoniae* in 2016 [10]. Colistin resistance is a catastrophic breach in our final line of protection, and unless we take immediate action, we will enter a post-antibiotic period in which ordinary diseases and mild injuries can kill.

Material & Methods

Study Design

To investigate the colistin resistance, among carbapenem resistant gram negative isolates, we performed a study on samples collected from 1 November 2021 to 31 May 2022. Non-duplicated Klebsiella strains that exhibited carbapenem resistance by disk diffusion test were collected from clinical specimens of the patients including blood, urine, sputum, pus, endotracheal aspirates, Ascitic fluid, Bile drain and various swab specimens. A total of 116 strains were included in this study which isolated from clinical samples.

Sample processing & antimicrobial susceptibility testing

Processing of the specimen was done on blood agar, and MacConkey's agar. Bacterial colonies were identified by colony morphology. All strains were subjected to species identification using routine biochemical tests. Antimicrobials used were tobramycin, meropenem, imipenem, gentamycin, amikacin, ciprofloxacin, aztreonam, piperacillin-tazobactam, ceftriaxone, cefotaxime, cefexime, cefuroxime, cefepime, ceftadizime, minocycline, tetracycline, co-trimexazole, amoxicillim, colistin, tigecycline, fosfomycin, nitrofurantoin and chloramphenicol were used to test antimicrobial activity as per CLSI guidelines.

Testing for colistin activity

Screening of colistin resistance in *Klebsiella pneumoniae* was tested with 10µg colistin methanesulfonae (CMS) disk on muller hinton agar by kirby bauer Method. Suspected resistant strains were further confirmed by the reference method i.e broth micro dilution method. Colistin sulphate powder was used to determine minimum inhibitory concentration. Two folds dilution was prepared for colistin susceptibility testing in cation adjusted muller hinton broth. MIC ≤2 µg/ml was reported as sensitive while colistin resistance was defined as MIC of ≥4 µg/ml. Results of all colistin resistant strains, isolated during study period were included for data analysis in the study. For this, software MS Excel was used. Institutional ethical committee clearance was taken before conducting this study.

Results

In a total number of 215 culture positive samples 116 Klebsiella strains were collected. Among 116 Klebsiella strains, 62.93% were male and 37.07% were female population. The mean±SD of the age was 45.93±18.15. Among the Klebsiella strains, *Klebsiella pneumonia* was 96.55% while *Klebsiella oxytoca* was 3.45% (Table 1). Among the colistin resistant isolates 71.42% was from urine, 14.29% from pus and endotracheal aspirate (figure I). In present study the rate of colistin resistance was found to be 7.76% among *Klebsiella pneumonia* isolates while all *Klebsiella oxytoca* isolates were found to be sensitive for colistin. The MICs of resistant strains found to be >4 µg/ml (Figure II). Among source of samples majority of the isolates were from urine followed by pus, endotracheal aspirate, blood, sputum, swab and ascetic fluid (Table 2).

Table 1: Frequencies of ORGANISMS

Levels	Counts	% of Total	Cumulative %
KLEBSIELLA PNEUMONIAE	112	96.6 %	96.6 %
KLEBSEILLA OXYTOCA	4	3.4 %	100.0 %

Table 2: Frequencies of SAMPLES

Levels	Counts	% of Total	Cumulative %
PUS	24	20.7 %	20.7 %
URINE	43	37.1 %	57.8 %
SPUTUM	12	10.3 %	68.1 %
BLOOD	16	13.8 %	81.9 %
ASCITIC FLUID	1	0.9 %	82.8 %
ENDOTRACHEAL ASPIRATE	17	14.7 %	97.4 %

Levels	Counts	% of Total	Cumulative %
SWAB	3	2.6 %	100.0 %

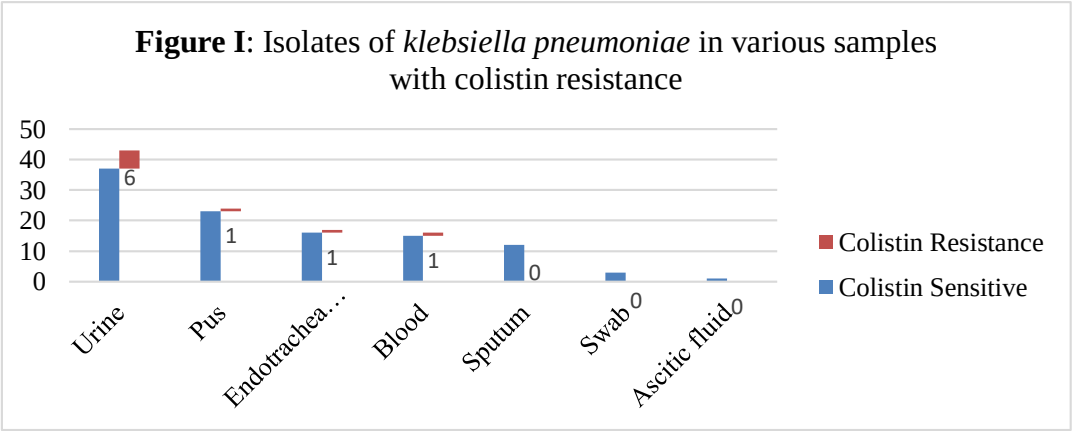

Figure I: Isolates of klebsiella pneumoniae in various samples with colistin resistance

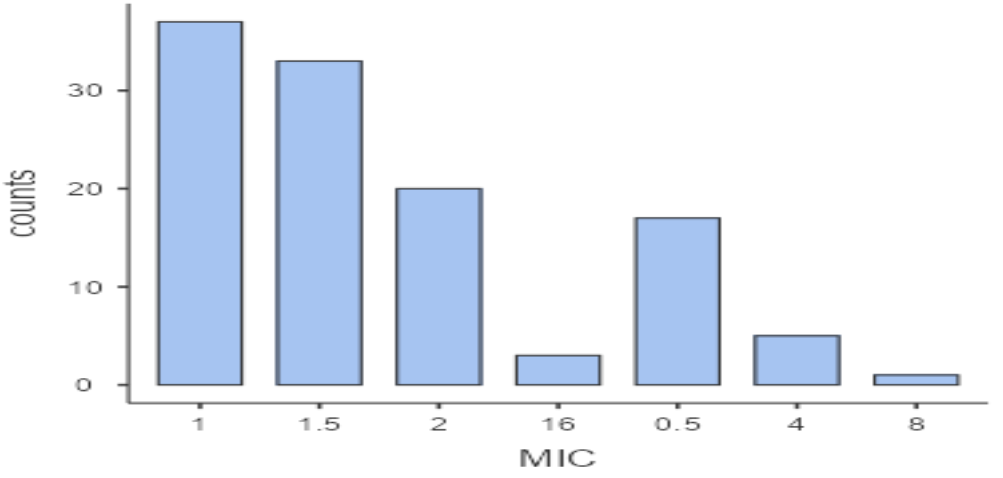

Figure II: Distribution of Colistin MICs of Klebsiella pneumoniae isolates

Table 3: Susceptibility pattern of Colistin Resistance *Klebsiella pneumoniae*

Antimicrobials	Sensitive (%)	Resistance (%)
TOBRAMYCIN	44.44	55.56
MEROPENEM	55.56	44.44

INIPENEM	55.56	44.44
AMIKACIN	33.33	66.67
GENTAMYCIN	44.44	55.56
CIPROFLOXACIN	22.23	77.78
AZTREONAM	33.33	66.67
PIPERACILLIN-TAZOBACTAM	55.56	44.44
CERTRIAXONE	0	100
CEFOTAXIME	0	100
CEFEXIME	11.12	88.89
CEFEPIME	0	100
CEFTADIZIME	11.12	89.89
MINOCYCLINE	33.33	66.67
TERTACYCLINE	33.33	66.67
CO-TRIMEXAZOLE	22.23	77.78
AMOXICILLIN	0	100
COLISTIN	0	100
TIGECYCLINE	55.56	44.44
FOSFOMYCIN	55.56	44.44
NITOFURANTOIN	33.33	66.67
CHLORAMPHENICOL	0	100

Colistin is used as last choice for treatment when most of the drugs fails to cure infection. In present study we analysed the susceptibility pattern of colistin resistant *Klebsiella pneumonia* species. Among colistin resistance Carbapenem was the most sensitive (55.56%) drug unfortunately in all samples. Fosfomycin was found to be 55.56% sensitive among urine samples while chloramphenicol was 100% resistance in blood samples (Table 3 & Figure III)

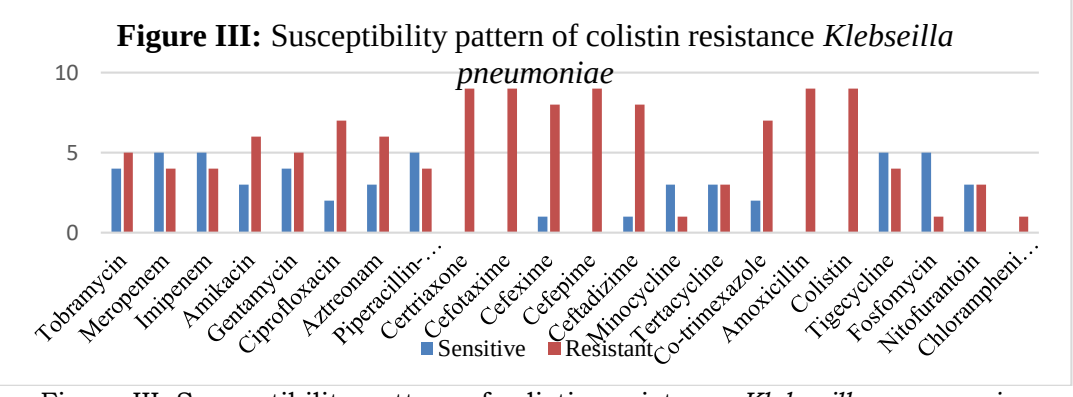

Figure III: Susceptibility pattern of colistin resistance *Klebseilla pneumoniae*

Discussion

Due to the obvious global growth of multi drug resistance Gram-negative infections, colistin is being utilised more frequently to treat these infections. As a result, the establishment of colistin resistance has indeed been documented all over the globe. The incidence of colistin resistance within strains isolated of enterobacteriaceae in Jaipur was studied, although only *Klebsiella pneumoniae*

were included. *Klebsiella pneumoniae* is an opportunistic bacterium that infects both respiratory system, urogenital tract, and circulation. The digestive tract acts as a reservoir for hospital-acquired infections such as *Klebsiella pneumoniae*. Infections caused by antimicrobial-resistant *Klebsiella pneumoniae* have grown dramatically in the previous several decades, leading to an increase in the use of colistin as a last-resort medicine. Simultaneous resistance to last-resort treatments is a potentially serious issue across the world, and our investigation discovered considerable levels of ongoing colistin resistance, with a frequency of 7.76 percent. Because of the lack of antibiotic monitoring, this is a major problem. This also shows the emergence and spread of potentially pan-drug resistant bacteria, restricting the ability to treat common diseases.

A study from north India [11] also reported prevalence of 4% colistin resistance among *Klebsiella pneumoniae*. This prevalence was less than prevalence in present study. Another study from India [12] also reported 5.6% of *Klebsiella pneumonia* among gram negative isolates. This reported prevalence is very much similar to present study. Ramesh et al. have reported colistin resistance in almost all gram negative organisms in a study across two centres in South India [13].In the study from Western India, prevalence of colistin resistant gram-negative bacilli was 9.98% [14].

In present study we found the reduce susceptibility for many potent antimicrobial drug. These findings supported by other studies [14, 15].Our overview of the microbial resistance patterns especially colistin resistant *Klebsiella pneumoniae* in a tertiary care hospital suggest that specific infection control strategies, periodic targeted surveillance and stringent antibiotic usage policies including restricted antimicrobial policy and antibiotic cycling, may be the appropriate measures for dealing with infections caused by CRE in ICU settings in the future.

Conclusion

Carbapenem-Resistant Klebsiella pneumonia has been created in recent years by the misuse and overuse of carbapenems among *Klebsiella pneumoniae*. Colistin is among the few remaining alternatives, and that there are growing worries regarding its dose and resistance. Given the rising indications of colistin resistance from clinics throughout the globe, including ours, giving colistin to all patients suspected of having sepsis and other infections is unfeasible and will exacerbate the grave scenario of colistin resistance. A reasonable solution would be to identify the greatest risk group and limit empirically colistin use to these individuals. Antibiotic medication monitoring, vigorous microbiology department engagement in antibiotic monitoring and management programme, and tight infection prevention and control policies are required to avoid future growth in resistance to these last-resort antimicrobials. This will be the first report on the prevalence of colistin resistance in *Klebsiella pneumonia* and its susceptibility pattern in human isolates from Jaipur. These findings shed light on the current situation of potent drug like colistin.

References

1. Trecarichi EM, Tumbarello M: Therapeutic options for carbapenem-resistant Enterobacteriaceae infections. *Virulence.* 2017; 8(4): 470–84.
2. Paul M, Daikos GL, Durante-Mangoni E, *et al.*: Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem resistant Gram-negative bacteria: an open-label, randomised controlled trial. *Lancet Infect Dis.* 2018; 18(4): 391–400.
3. Zhang X, Gu B, Mei Y, *et al.*: Increasing resistance rate to carbapenem among blood culture isolates of Klebsiella pneumoniae, Acinetobacter baumannii and *Pseudomonas aeruginosa* in a university-affiliated hospital in China, 2004–2011. *J Antibiot (Tokyo).* 2015; 68(2): 115–20.
4. Theuretzbacher U, Van Bambeke F, Cantón R, *et al.*: Reviving old antibiotics. *J Antimicrob Chemother.* 2015; 70(8): 2177–81.
5. Poirel L, Jayol A, Nordmann P: Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin Microbiol Rev.* 2017; 30(2): 557–96.
6. Biswas S, Brunel JM, Dubus JC, *et al.*: Colistin: an update on the antibiotic of the 21st century. *Expert Rev Anti Infect Ther.* 2012; 10(8): 917–34.
7. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. International Journal of Health Sciences, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938
8. Giamarellou H: Multidrug-resistant Gram-negative bacteria: how to treat and for how long. *Int J Antimicrob Agents.* 2010; 36 Suppl 2: S50–4.
9. Deris ZZ, Akter J, Sivanesan S, *et al.*: A secondary mode of action of polymyxins against Gram-negative bacteria involves the inhibition of NADH-quinone oxidoreductase activity. *J Antibiot (Tokyo).* 2014; 67(2): 147–51.
10. Hancock REW, Chapple DS: Peptide Antibiotics. *Antimicrob Agents Chemother.* 1999; 43(6): 1317–23.
11. Widyaningrum , I. ., Wibisono, N. ., & Kusumawati, A. H. . (2020). Effect of extraction method on antimicrobial activity against staphylococcus aureus of tapak liman (elephantopus scaber l.) leaves. International Journal of Health & Medical Sciences, 3(1), 105-110. https://doi.org/10.31295/ijhms.v3n1.181
12. Liu YY, Wang Y, Walsh TR, *et al.*: 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.* 2016; 16(2): 161-8
13. Mathur P, et al. Multiple importations and transmission of colistin-resistant Klebsiella pneumoniae in a hospital in northern India. Infection Control & Hospital Epidemiology. (2019), 1–7.
14. Arip, M., Cembun, .-., & Emilyani, D. (2018). Strategy to improve knowledge, attitude, and skill toward clean and healthy life behaviour. International Journal of Social Sciences and Humanities, 2(3), 125–135. https://doi.org/10.29332/ijssh.v2n3.222
15. Sodhi K, et al. Pattern of colistin resistance in Klebsiella isolates in an Intensive Care Unit of a tertiary care hospital in India. J Infect Public Health (2019), https://doi.org/10.1016/j.jiph.2019.10.013

16. Ramesh N, Gothandam KM, Prasanth M, Ramkumar S, Karthikeyan S, BozdoganB, et al. Colistin susceptibility of gram-negative clinical isolates from TamilNadu, India. Asian Biomed 2016;10:35–9.
17. Pawar SK, Karande GS, Shinde RV, Pawar VS. Emergence of colistin resistantgram negative Bacilli in a tertiary care rural hospital from Western India. IndianJ Microbiol Res 2016;3:308–13.
18. P.G. Soundari, A. Ghafur, P. Krishnan, et al., Intestinal carriage of colistin resistant Enterobacteriaceae in hospitalized patients from an Indian Centre, *Diagnostic Microbiology & Infectious Disease*(2020), https://doi.org/10.1016/j.diagmicrobio.2020.114998