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"Detection of Colistin Susceptibility in MDR Klebsiella Pneumoniae Isolates from Clinical Samples of CCU Patients in a Tertiary Care Hospital Kanpur"

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ABSTRACT

INTRODUCTION: -Colistin is generally used to treat infections with multidrug-resistant bacteria. Failure of drugs against Klebsiella pneumoniaebacteria has led to the unprecedented increase in the use of colistin (one of the last-resort drugs) and subsequent emergence and dissemination of colistin resistance.

AIM: -To estimate the prevalence of Colistin-Resistance Klebsiella pneumoniae (CRKp) in MDR isolates collected from different Critical care units (CCUs) and to determine the clinical outcomes of the patients.

MATERIALS AND METHODS: This study was conducted at Department of Microbiology and Medicine at RMCH&RC, Kanpur for 18 months between March 2022 to September 2023.Clinical samples from CCU were collected and processed by standard procedures to isolate and identify and to perform AST for Klebsiella pneumoniae spp.MDR Klebsiella pneumoniae species were examined for colistin susceptibility by the Broth Microdilution (BMD)method for the detection of Minimal Inhibitory Concentration (MIC). Data was analysed by comparing susceptibility categories (susceptible or resistant) and minimal inhibitory concentrations (MIC).

RESULT: Out of 240 *Klebsiella pneumoniae* isolates, 99 (41.3%) isolates were found to be Colistin Resistance by Disc Diffusion (Kirby Baur's) Method. The prevalence of Colistin Resistance Klebsiella pneumoniae (CRKp) was found by BMD for the detection of MIC-46/240 (19.1%) in the present study. CRKp drug-resistant isolates were commonly 11/46 (23.9%) isolated from samples of respiratory tract infections and the majority 38/46 (82.6%) were from Medicine ICU. The maximum number of CRKp isolates were found from urine cultures 25/46 (54.3%) followed by ET secretion 10/46 (21.7%) and Pus cultures 08/46 (17.3%).

CONCLUSION: Patients in the Medicine ICU were frequently infected and highest isolates were found from urine sample with CRKp strains. Prolonged hospital stay and direct antibiotic pressure in the hospital can lead to the development of CRKp variants.

KEYWORDS: Colistin resistance, Broth micro dilution, Critical care units.

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INTRODUCTION

Antimicrobial resistance (AMR) is rapidly increasing to a dangerous level worldwide, both in animals and humans.[1] although most antibiotics are consumed within the community, hospitals are important within the emergence of antibiotic resistance because antibiotics are often over-used.[2] The Critical care unit (CCU) often is called the epicenter of infections, due to its extremely vulnerable population (reduced host defenses deregulating the immune responses) and increased risk of becoming infected through multiple procedures and use of invasive devices.[3-4] During the past decades, a shift in the MDR dilemma has been noted from gram-positive to gram-negative bacteria, especially due to the scarceness of new antimicrobial agents active against resistant gram-negative microorganisms. In gram-negative bacteria, the resistance is mainly due to the rapid increase of extended-spectrum Beta-lactamases (ESBLs) in *Klebsiella pneumonia*.[5-6] *Klebsiella pneumonia* carbapenemase (KPC) by *Enterobacteriaceae* becomes problematic, because KPC beta-lactamases result in decreased susceptibility or resistance to virtually all beta-lactam antibiotics; and many strains of *Enterobacteriaceae* were already resistant to a wide range of non-beta-lactam antibiotics.[7] However, with increasing global incidence of Cabapenems resistance, Colistin is now widely used because the last resort antibiotic for the treatment of Cabapenems-resistant *Enterobacteriaceae*.[8] In 1959, the US Food and Drug Administration approved Colistin to treat various types of diarrhea and urinary tract infections. Failure of Cabapenems against Gram-negative bacteria has led to the unprecedented increase in the use of Colistin (one of the last-resort drugs) and subsequent emergence and dissemination of Colistin resistance.[9] Resistance to Colistin in gram-negative bacteria is attributed by two-component systems (TCSs) including PhoPQ and PmrAB, because the regulatory systems, reduce the charge of lipid A and subsequently reduce the binding affinity of Colistin to LPS.[10-11] Infections with drug resistant organisms remain an important problem in clinical practice that is difficult to solve.[12] The intensive care unit (ICU) often is called the epicenter of infections, due to its extremely vulnerable population (reduced host defenses deregulating the immune responses) and increased risk of becoming infected through multiple procedures and use of invasive devices distorting the anatomical integrity-protective barriers of patients (intubation, mechanical ventilation, vascular access, etc.). In addition, several drugs may be administered, which also predispose for infections, such as pneumonia, e.g., by reducing the cough and swallow reflexes (sedatives, muscle relaxants) or by distorting the normal nonpathogenic bacterial flora (e.g., stress ulcer prophylaxis) [13]. A nosocomial infection may be a common problem in critically ill patients, particularly in people who are hospitalized within the Critical care units (CCUs) and might cause death and morbidity during this group of patients.[14-15] The ongoing emergence of resistance in the community and hospital is considered a major threat for public health. Due to the specific risk profile of its residents, the ICU also is deemed the epicenter of resistance development. The ICU has even been described as a factory for creating, disseminating, and amplifying antimicrobial resistance.[16]

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MATERIALS AND METHODS

This study was conducted in the Department of Microbiology & Medicine at RMCH&RC, Kanpur for 18 months (March 2022 to September 2023). Total 240 Clinical samples from CCU were collected and processed by standard procedures and AST was performed according to the CLSI 2022. The MDR *Klebsiella pneumoniae* species were examined for Colistin susceptibility by the Broth Micro dilution (BMD) method for the detection of Minimal Inhibitory Concentration (MIC).

Identification of Klebsiella pneumoniae: -Culture was done by the streaking method on Blood agar, MacConkey's agar and urine sample for CLED agar and Nutrient agar then incubates the plates at 37°C for 18–24 hrs. Gram staining and Biochemical test was done for identification of *Klebsiella pneumoniae*.[17]

Antimicrobial Sensitivity Testing: Antibiotic sensitivity was performed for all the isolates by Kirby- Bauer disc diffusion method using Cation adjusted Mueller – Hinton agar plates. The panel of drugs used for antimicrobial sensitivity testing was:- Ampicillin,Ticarcillin, Piperacillin, Piperacillin/Tazobactam, Amoxyclav, Ampicillin/sulbactam, Cefoxitin, Ceftriaxone, Cefotaxime, Ceftazidime,Cefepime,Cefepime/tazobactam, Cefoperazone /sulbactam, Meropenem, Imipenem, Aztreonem, Polymyxin B, Polymyxin E (Colistin), Gentamicin, Amikacin, Tobramycin, Netilimicin, Tetracyclines, Tigecycline, Ciprofloxacin, Levofloxacin, Ofloxacin, Norfloxacin, Nitrofurantoin, Co- Trimoxazole.[17]

MIC determination of Colistin: The Standard Broth Micro Dilution method was used to determine the *in vitro* susceptibility to Colistin for aerobic gram negative bacterial isolates in Clinical Microbiology laboratory. Use Cation-Adjusted Mueller-Hinton broth (Ca-MHB) for routine broth dilution susceptibility testing of rapidly growing *Klebsiella pneumoniae*. Check the pH of each batch of MHB was checked and ensured that the final pH is between 7.2 to 7.4. Select the ATCC strains for QC that most closely resemble the patients' isolates being tested. Perform MIC with each batch of broth using a standard set of QC organisms, E.coli ATCC 25922 – MIC QC Ranges is 0.25 – 2 ug/ml.[18]

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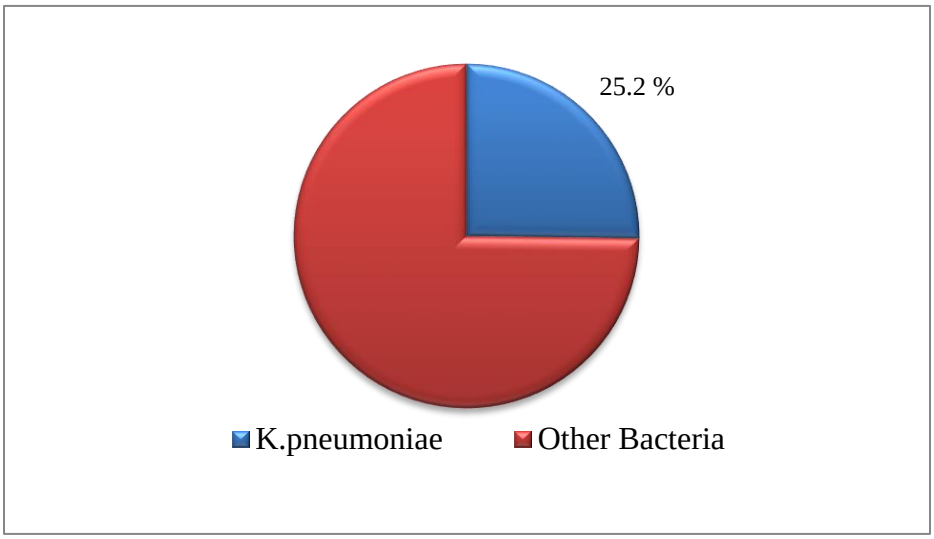
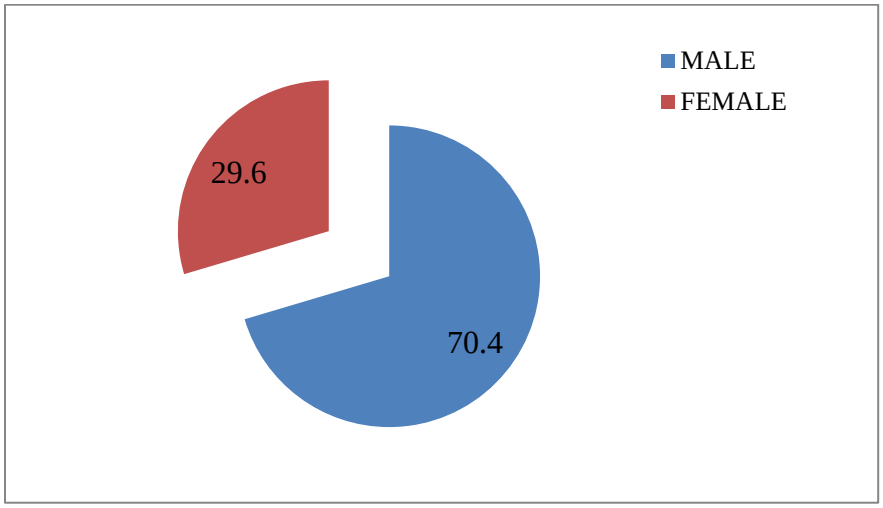
RESULTS

Graph No.1: Prevalence of Klebsiella pneumoniae among various clinical samples obtained for culture and sensitivity:



Out of 1281 samples obtained for culture & sensitivity within 18 month's duration in Clinical Microbiology laboratory, 959 were culture positive. Among these 959 culture positive cases, 240 (25.1%) were identified as Klebsiella pneumoniae.

Graph No.2: Gender wise, Distribution of Klebsiella pneumoniae



Gender wise, more number of Klebsiella pneumoniae infections were seen among males (70.4%) compared to that among females (29.6%) in our study

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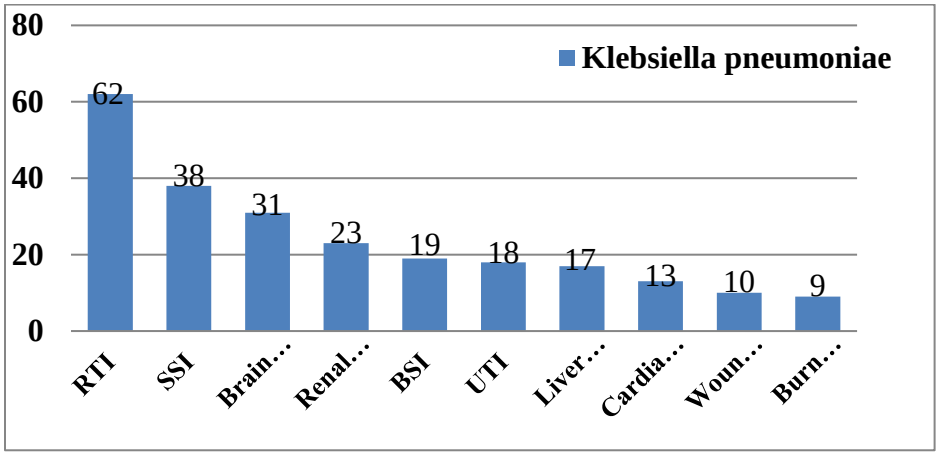
Table No. 1: Source wise distribution of Klebsiella pneumoniae (n=240)

SOURCE	K.PNEUMONIAE	PERCENTAGE (%)
Medicine ICU	**191**	**79.5%**
Neuro ICU	27	11.2%
Pedia ICU	13	5.5%
Burn ICU	09	3.8%
TOTAL	**240**	**100%**

Most of the Klebsiella pneumoniae were isolated from medicine ICU 191 (79.5%), followed by Neuro ICU 27 (11.2%), Pedia ICU 13 (5.5%) and 09 (3.8%) were isolated from Burn ICU

Graph No. -2: Distribution of Klebsiella pneumoniae in various Clinical infections (n=240)



Klebsiella pneumonia was most commonly isolated in by Respiratory tract infections (RTI) percentage was 62 (25.8%) followed by Surgical site (SSI) infections percentage was 38 (15.8%), followed by Brain infections percentage was 31(12.9%), Renal infections percentage was 23 (9.58%), Blood stream infections (BSI) percentage was 19 (7.91%), Urinary tract infections (UTI) percentage was 18 (7.50%), Liver infections percentage was 17 (7.08%), Cardiac infections percentage was 13 (5.41%), Wound infections percentage was 10 (4.16%) and Burn infections percentage was 09 (3.75%).

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Table-2: AST pattern of Colistin drug in Klebsiella pneumoniae species by Disc Diffusion (Kirby Baur's Method). (n=240)

COLISTIN	SENSITIVE	INTERMEDIATE SENSITVE	RESISTANT	TOTAL (%)
Disk Diffusion (Kirby Baur's Method)	72 (30.0%)	69 (28.7%)	**99 (41.3%)**	240 (100%)

Out of 240 isolates of *Klebsiella pneumoniae* species were Colistin sensitive 72 (30.0%), Colistin intermediate sensitive 69 (28.7%) and Colistin resistance was 99 (41.3%) by Disk Diffusion (Kirby Baur's Method).

TABLE-3: Broth Micro Dilution (BMD) method for the detection of Minimum Inhibition Concentration (MIC) of Colistin Resistance Klebsiella Pneumoniae isolates (CRKp) (n=99).

MIC FOR COLISTIN (µg/ml)		16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	Total
Klebsiella Pneumoniae (99)		**13**	**18**	**15**	11	17	9	4	12	-	-	**99**

Out of 240 isolates, 99 (41.3%) Klebsiella pneumoniae isolates were found to be resistant to Colistin by Disc Diffusion Method which was confirmed by MIC from Broth Micro Dilution method (BMD). Among the 99 isolates, 13 isolates had 16 (µg/ml) as MIC, 18 isolates had 08 (µg/ml) as MIC, 15 isolates had 4 (µg/ml) as MIC, 11 isolates had 02 (µg/ml) as MIC, 17 isolates had 01 (µg/ml) as MIC, 09 isolates had 0.5 (µg/ml) as MIC, 04 isolates had 0.25 (µg/ml) as MIC and remaining 12 isolates had 0.125 (µg/ml) as MIC. Out of 99 isolates, 46 (19.1%) Klebsiella pneumoniae isolates were found to be Colistin resistance confirmed by Broth Microdilution method (BMD) for the detection of Minimum Inhibition Concentration (MIC) value.

DISCUSSION

Prevalence of Klebsiella pneumoniae was found to be 25.2% in our study. In a study conducted by **Vijayashree V.et al**[19] 32.5%, it was found to be in India and 32.3% **Zhezhe Lin et al**[20] in China. This similarity or difference in findings might be due to geographical location, isolation technique for bacteria in various hospital set-up and disease pattern in particular places. Gender wise, more number of Klebsiella pneumoniae infections were seen among males (70.4%) compared to that among females (29.6%) in our study. This might be due to more number of Pus and Urine samples obtained for culture and sensitivity. Similar report has been found in

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other study also **Hera Nirwati et al.**[21] were 64.0% male & female 36.0%. Source wise distribution of Klebsiella pneumoniae shows, Klebsiella pneumoniae were mostly isolated from Medicine ICU (79.5%), followed by Neuro ICU (11.2%) and Pedia ICU (5.5%). Isolation of more number of Klebsiella pneumoniae from medicine department could be due to admission of critical patients in this department. As Klebsiella pneumoniae infections are commonly found in ICU patients due to their poor immune status similar to other organism related infections. Similar report has been shown by **Beena Hosdurg Bhaskar et al**[22] Medicine ICU (83.5%) ,Neuro ICU (2.5%),Pedia ICU (1.5%) & **Yulia Rosa Saharman et al**.[23] Medicine ICU unit(59.5%)Surgical ICU unit(40.5%). Klebsiella pneumoniae show resistance to various antibiotics. Out of 240 isolates, Resistance to Colistin was found to be 99 (41.3%) by disc diffusion method. **V. Praveen Kumar et al**[24] had shown that resistance to this drug 32.9% by disc diffusion method too in India. Similarity or variation might depend upon random use of these antibiotics in the past. High resistance to these antibiotics in India, especially, might be due to easy access of antibiotics in medical shops over the counter without prescription of clinicians. Unregulated use of antibiotics in animals and poultry might be the other reason for high resistance to these antibiotics **(Laxminarayan R et al)**[1]**.** For overcoming this problem (Colistin resistance), Tigecycline could be useful drug as Klebsiella pneumoniae had shown 74.1% sensitivity to it in our study. Other studies also have recommended use of Tigecycline 93.6% sensitive in case of Colistin resistance **Mutasim E. Ibrahim et al.**[25] In the present study out of 240 isolates, 46 (19.1%) were found to be Colistin resistance Klebsiella pneumoniae (CRKp) by Broth Micro Dilution method. Supportive results were seen in a study conducted by **Hadir A. El-Mahallawy et al**, [26] where the prevalence of CRKp was 19.9%. Another study which showed similar observation was conducted by **Abeer K. Abu-El-Azayem et al.**[27] and **Basak Baykara et al.**[28] which reported the CRKp prevalence to be 14.1% & 42.0% respectively. Various studies from different parts of India, **Poonam AR et al**[29] (35.2%), **Bidyutprava Rout, et al**[30] (18.9**%), Punyatoya Kar et al.** [31] (13.5%)

CONCLUSIONS

The present study represents the CRKp among the MDR isolates from a tertiary care hospital in North India. We found that the prevalence of Colistin resistance was high (19.6%) among Klebsiella pneumoniae isolates from samples of CCU patients. CRKp was common isolated from patients with urinary tract infections of medicine ICU patients. It can be concluded that prolonged hospital stay and direct antibiotic pressure in hospitals can lead to the development of CRKp variants and are considered to be nosocomial in origin. Active involvement of the microbiology department in antibiotic surveillance, as well as stewardship programs and strict infection control practices to prevent further increase in resistance to these last resorts of antimicrobials.

CONFLICT OF INTEREST:

All the authors of this article declared no conflict of interest.

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