"TO STUDY THE PREVALENCE OF KLEBSIELLA PNEUMONIAE AND ITS ANTIBIOGRAM WITH SPECIAL REFERENCE TO CARBAPENEM RESISTANCE IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL".

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ABSTRACT

Introduction: The emergence of multidrug-resistant (MDR) strains of *K. pneumoniae* that produce carbapenemase and extended-spectrum β-lactamase (ESBL) has made the pathogen a significant hazard to both public and clinical health.Carbapanem-resistant *K. pneumoniae* (Cr-KPN) is a pathogen that affects people worldwide, with prevalence in low, middle and upper income countries. Resistance to carbapenem is mediated by two primary mechanisms. First, Cr-KPN is able to produce β-lactamases with the ability to hydrolyze cephalosporins such *AmpC* cephalosporinase. The second mechanism is mediated by the production of a β-lactamases capable of hydrolyzing most β-lactams antibiotics including carbapenems.

Aim and Objective: To Study the Prevalence of *Klebsiella Pneumonia* and its Antibiogram with Special Reference to Carbapenem Resistance in Patients Attending a Tertiary Care Hospital.

Material and methods: This was a cross sectional study carried out in the Department of Microbiology for a period of 1 year i.e, during August 2021 to August 2022 at Index Medical College, Hospital and Research Centre (IMCHRC), Indore (M.P.) The study included all the patients of OPD and IPD. Samples like urine, sputum, ET tube, pleural fluid,pus, CSF, blood and Ascitic fluid were included in this study. Carbapenemase resistance was detected phenotypically by MHT, mCIM and eCIM methods according to the CLSI guidelines 2021.

Results: In the present study a total of 430 clinical samples were studies, out of which 160 (37.20 %) samples showed growth of *K. pneumoniae* while 270 (62.8 %) samples showed growth of bacteria other than *K. pneumoniae* or sterile samples. 69.37% (n=111) of total Klebsiella isolates were carbapenem resistant (suggestive of Carbapenemase producers) by Kirby-Bauer disc diffusion with the prevalence of 69.37%. It was also observed that the ratio of Males 68 (61.2%) were more as compared to the females 43 (38.7%) with the most common age group being ›60 years in both the sexes with Sputum being the highest clinical isolated followed by urine, pus and least for Pleural fluid, CSF, blood, ET Aspirate.

The results indicate the phenotypic detection mCIM combined with eCIM showed high sensitivity and specificity to detect carbapenemase producing K.pneumoniae compared with MHT.

Conclusion: There aren't many options for treating infections caused by *K. pneumoniae* that produces carbapenemase; tigecycline and colistin may be the best medications. In addition, to determine the best empirical antibiotic therapy and stop the spread of MBL-producing bacteria in a hospital setting, routine surveillance of these microorganisms is required.

Keywords: MDR,Prevalence, *K.pneumoniae*, ESBL, CRKP

INTRODUCTION

Klebsiella is an important opportunistic pathogen that is found to be the causative of hospital acquired pneumonia, urinary tract infections, skin and soft tissue infections and bacteremia [1]. *Klebsiella* is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the family *Enterobacteriaceae*. It has various virulence factors such as polysaccharide capsule, endotoxin, cell wall receptors and iron-scavenging systems [2]. *Klebsiella pneumoniae* is a superbug, which complicates treatment of infections worldwide & limits therapeutic options. Hospital acquired infections caused by multidrug resistant strains of *Klebsiella* species are associated with high rates of morbidity and mortality [3,4].

Multidrug-resistant *K. pneumoniae* isolates show a high resistance to a broad spectrum of drugs including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides [5]. The rapid spread of mobile carbapenemase-containing genes, the limited treatment options, and the high mortality rates for infections associated with carbapenemase-producing isolates make them one of the major public health threats.

Alarming global data demonstrate the prevalence of diseases brought on by common and diverse pathogenic microorganisms that have evolved resistance to antibiotics.. Carbapenems are effective against a wide range of bacteria. The unique structure of carbapenems, which provides protection against the majority of lactamases, including metallo- and extended spectrum -lactamases, is due to the interaction between the carbapenem and a -lactam ring . In light of the fact that carbapenems are among the most successful drugs for treating infections caused by bacteria, the emergence and spread of antibiotic resistance to these drugs is a serious public health issue.

Until now, carbapenems were considered as the most effective drug against the MDR *K. pneumoniae* and frequently used as the drugs of last resort [6]. However, the over dependence on carbapenems has led to an undesirable increase in the carbapenem-resistant isolates [7].

Carbapenem resistance is mediated by the production of carbapenemases; β-lactamases with versatile hydrolytic capacities or by the combination of outer membrane porin expression disruption [8].

Frequent isolation of the carbapenemase-producing isolates poses a significant complication in the treatment. Furthermore, these genes have the potential for dissemination via mobile genetic elements such as plasmids and transposons [9]which increases the risk of widespread dissemination in hospital settings as well as in the community.Different carbapenemase genes are responsible for the production of carbapenemases, the most clinically signifcant carbapenemases are class A (KPC type), class B metallo-β-lactamases (MBLs), i.e., VIM, IMP, and NDM types, and class D carbapenemhydrolyzing β-lactamases (OXA-48-like enzymes) [10, 11]. The New Delhi metallo-β-lactamases (NDM), frst reported in India [12] and OXA-48, frst reported in Turkey [13] and both have been reported in *K. pneumoniae*. Since their frst detection, both of the carbapenemase genes have spread across the globe [12–15].

The aim of this study was to determine the Prevalence of *Klebsiella Pneumonia* and its Antibiogram with Special Reference to Carbapenem Resistance in Patients Attending a Tertiary Care Hospital.

MATERIAL & METHODS

Study design
This cross-sectional study was conducted from August 2021 to August 2022 at at Index Medical College, Hospital and Research Centre (IMCHRC), Indore (M.P.)

Inclusion criteria: Ths study population included patients of all ages and genders visiting the both OPD and IPD of the hospital.

Exclusion criteria: The patients under any antibiotics treatments were excluded from this study.

Sample Collection and Identification
Samples like urine, sputum, ET tube, pleural fluid,pus, CSF, blood and Ascitic fluid were included in this study.Samples were collected by employing standard microbiological protocol [16].Specimens were cultured in suitable culture media as per their requirements. Identification of *K. pneumoniae* was made based on colonial morphology, staining reactions, and various biochemical properties [17].

Antimicrobial Susceptibility Testing (AST)
The isolated *K. pneumoniae* isolates were subjected to an antimicrobial susceptibility testing by the modified Kirby–Bauer disc diffusion method as per latest CLSI guidelines 2021 [18].
The antibiotic disks used were ampicillin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), amikacin (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), piperacillin-tazobactum,imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), tigecycline (15 μg and colistin (10 μg). *K. pneumoniae* which showed resistant to at least 3 diferent classes of antibiotics were considered as multidrug-resistant strains (MDR).

Testing for Carbapenemase Producers Strains
Carbapenem drugs include ertapenem, meropenem, and imipenem discs were incorporated onto the AST plate for the screening of the carbapenemase producers. Isolates showing resistance to at least one of the carbapenem discs mentioned were suspected as carbapenemase producers and were processed for a further confirmatory test.
Carbapenemase production was confirmed phenotypically by the Modified Hodge Test (MHT),mCIM and eCIM methods.

Ethical Clearance: Institutional ethical clearance was obtained from Index Medical College, Hospital and Research Centre (IMCHRC), Indore (M.P.)

Statistical analysis : Data obtained from microbiological and molecular analyses were analyzed using appropriate statistical methods to determine the prevalence of *Klebsiella pneumoniae* infections, assess antibiotic susceptibility patterns, and investigate the prevalence of carbapenem resistance.

RESULTS
A total of 430 clinical samples were studied, out of which 160 (37.20 %) samples showed growth of *K. pneumoniae* while 270 (62.8 %) samples showed growth of bacteria other than *K. pneumoniae* or sterile samples. There were 69.37% (111 of 160) of Klebseilla isolates showed carbapenem resistant, therefore the prevalence rate was found to be 69.37%. Among carbapenem imipenem(56%) was most effective. As shown in Graph 1.
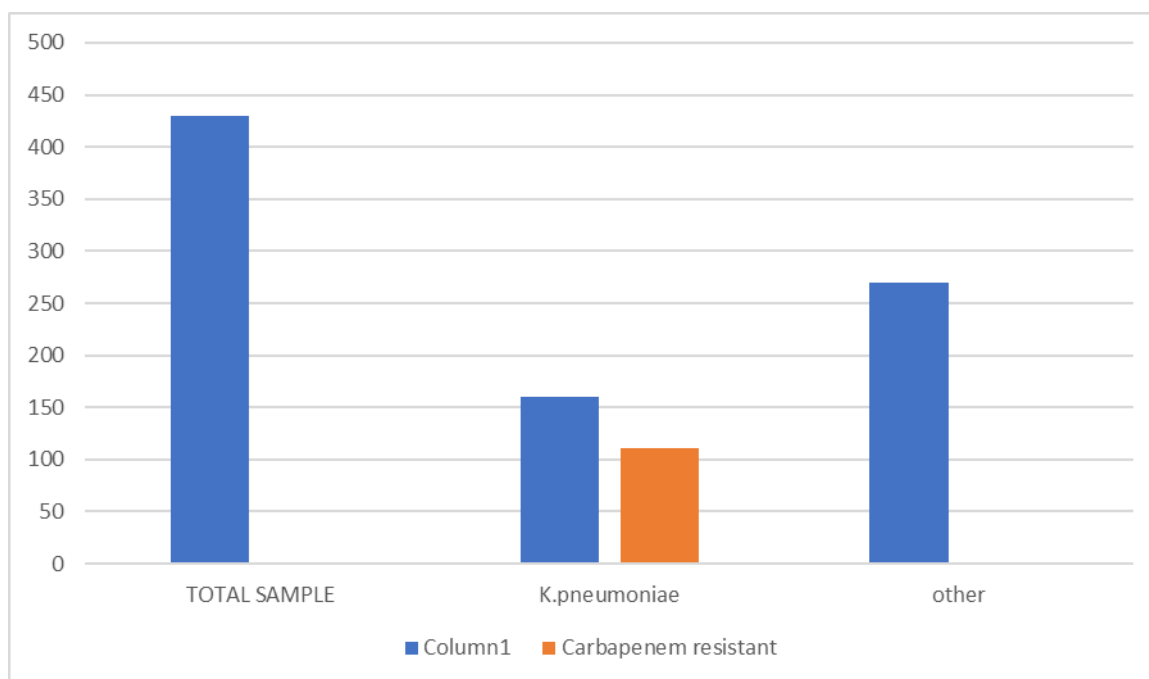
Graph 1: Prevalence and Carbapenem resistant *K.pneumoniae* isolates.

From the Graph 1 it was clear that out of 430 total sample, 160 were *Klebseilla* isolates & among them 111(69.37%) showed carbapenem resistant.

Among all the antibiotics used against *K.pneumoniae*, Colistin(100%) showed maximum sensitivity followed by tigycycline(98%) and piperacillin-tazobactum(92%). As shown in table 1 and Graph 2

| ANTIMICROBIAL AGENTS	NUMBER OF SENSITIVE ISOLATES	PERCENTAGE OF SENSITIVE ISOLATES
AMP	39	35.1%
GEN	31	27.9%
CIP	24	21.6%
COT	19	17.1%
AK	41	36.9%
CAZ	43	38.7%
CTX	53	47.7%
PTZ	89	80%
IMP	56	50.4%
MRP	41	36.9%
ERT	11	9.9%
TGC	99	89.1%
CL	111	100%

Table 1: Sensitivity pattern of *K.pneumoniae*. Among them colistin shows 100% sensitivity followed by tigycycline (89.1%)

The resistant was more in male 68(61.2%) than females 43(38.7%), (Table 2) and it was more in age group > 60 years(23.4%) followed by age group 51-60 (21.6%). (Table 3) The klebsiella isolates were frequently isolated from Sputum sample (43.2%) followed by urine(33.3%), pus (18.9%), and it was least isolated from pleural fluid (0.9%), CSF(0.9%), Blood(1.8%), and ET aspirate (0.9%) (table 4)

K.pneumoniae count	Frequency(n=111)	Percentage(%)
Male	68	61.2%
Female	43	38.7%
TOTAL	111	100%

Table 2:Gender wise distribution of *K.pneumoniae*. In this table it was observed that *K.pneumoniae* was most commonly isolated from males (61.2%).

In the current study it was onbserved that in the IPD wards, Maximum number of isolates isolated from ICU wards (64.8%) while only 35.1% were isolated from OPD wards. As shown in Table 5. The results indicate the phenotypic detection mCIM combined with eCIM showed high sensitivity and specificity to detect cabapenemase producing *Klebseilla pneumoniae* compared with Modify hodge test(MHT).
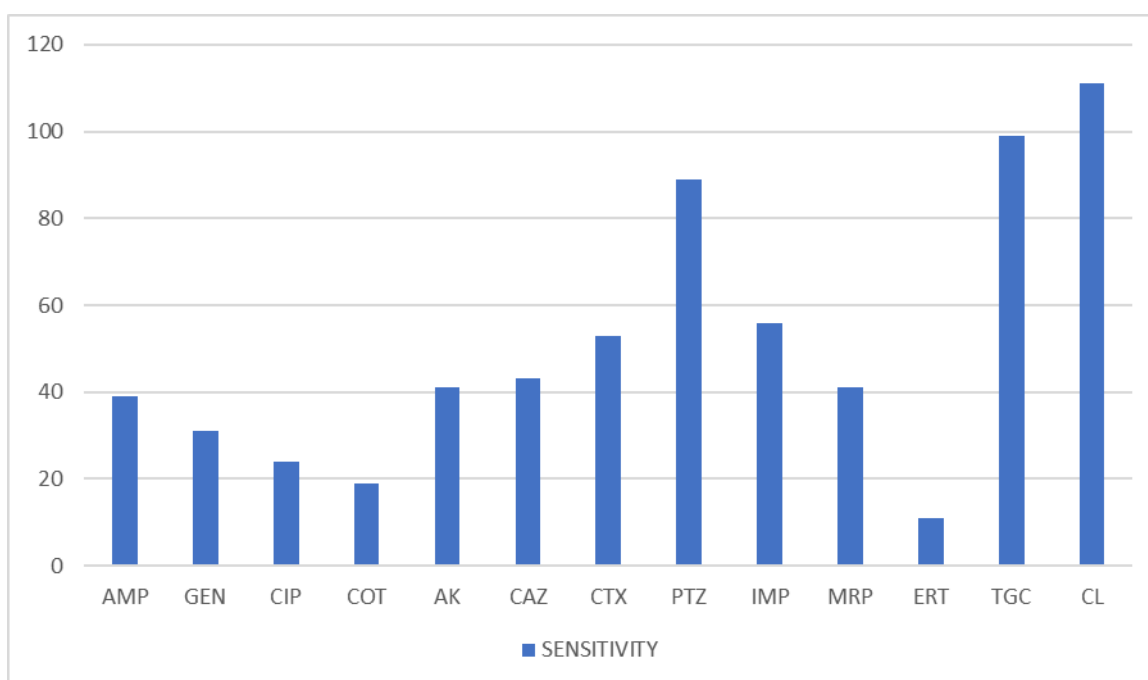
Graph 2: Sensitivity pattern of *K.pneumoniae*. As shown in this graph, *K.pneumonia* shows maximum sensitivity towards Colistin(100%), tigycycline (89.1%%) followed by piperacillin-tazobactum(80%).

AGE GROUP	FREQUENCY	PERCENTAGE
0-10	8	7.2%
11-20	7	6.3%
21-30	18	16.2%
31-40	12	10.8%
41-50	16	14.4%
51-60	24	21.6%
>60	26	23.4%
TOTAL	**111**	**100%**

Table 3 : Age wise distribution. In present study,it was observed that maximum number of klebseilla isolates were isolated in age group > 60 years of age(23.4%) followed by age group 51-60 years(21.6%) and least from the age group of 11-20 (6.3%).

SAMPLE	FREQUENCY	PERCENTAGE
SPUTUM	48	43.2%
PUS	21	18.9%
URINE	37	33.3%
PLEURAL FLUID	1	0.9%
CSF	1	0.9%
BLOOD	2	1.8%
ET SECRETIONS	1	0.9%
TOTAL	**111**	**100%**

Table 4 : Sample wise distribution. In this table it was observed that maximum number of isolates were isolated from sputum sample (43.2%) followed by urine (33.3%) and least from pleural fluid(0.9%),CSF(0.9%), blood(1.8%) & ET secretions(0.9%).

WARD	FREQUENCY	PERCENTAGE
IPD ICU	72	64.8%
OPD	39	35.1%

Table 5: Ward wise distribution. In this table,it was observed that maximum number of isolates were isolated from ICU (IPD) ward (64.8%) than from OPD ward (35.1%).

DISCUSSION

The rapid spread of multidrug-resistant Gram-negative bacteria continues to be one of the most significant challenges to global health [19]. While these bacteria have developed a different mechanism to avert the bactericidal effects of commonly prescribed antibiotics, the increasing

The prevalence of carbapenemase-producing Gram-negative bacteria is of particular concern [20]. The rapid spread of mobile carbapenemase-containing genes, the limited treatment options, and the high mortality rates for infections associated with carbapenemase-producing isolates make them one of the major public health threats [21].

In this study a total of 430 clinical samples were studied, out of which 160 (37.20 %) samples showed growth of *K. pneumoniae* while 270 (62.8 %) samples showed growth of bacteria other than *K. pneumoniae* or sterile samples. There were 69.37% (111 of 160) of Klebseilla isolates showed carbapenem resistant,

The prevalence of *Klebsiella* in this study was 69.37%. This was in agreement with previous studies from Nepal [22] but in contract with the study by Vibas et al. [23 which] reported a much higher prevalence rate while Another study by priyadarshini et al showed 7.1% prevalence among Klebsiella species.[24] Prevalence of ESBL producing *Klebsiella* was found to be 36.0% in a study by Shireen et al.[25] In India, prevalence of ESBL producing *Klebsiella* spp. is reported varying from 6% to 87.% [24].

Among the various *Klebsiella* isolates, maximum numbers were isolated from the age group > 60 years of age . The results of the present study correlates with the study conducted in Italy which showed 58% of subjects to be of more than 60 years of age [26]. Another study conducted by Shireen rana et al showed majority of the subjects to be between 31-40 years of age. A recent study in South India shows maximum samples to be between 40-60 years of age [25] . A study by *Susethira et al* showed maximum age group to be between 41-50 years [27] which was in support to the present study.

Our study showed the percentage of males (61.2%) was comparatively higher than the females (38.7%). This study was in accordance to the another study performwd by other research investigator where males (n=68) were comparatively more than females (n=53) but in contrast with the another study where showed females (n=298) to be more than males(n=280). [25]

In this study, the highest percentage of *K. pneumoniae* was found in sputum sample(43.2%), followed by urine(33.3%) & pus(18.9%) This was in contrast to that reported by Ferreiria et al. [28].

K. *pneumoniae* was isolated at the highest rate from the ICU ward, followed by the OPD. This was in similar tothe study performed by Khan et al. [29].

Among different antibiotics used, colistin was 100% effective against *K. pneumoniae* while 98% and 92% isolates were sensitive to tigecycline& piperacillin-tazobactum respectively. Thus, colistin and tigycycline could be the drug of choice for carbapenemresistant *K. pneumoniae* isolates. Many researchers have also reported high sensitivity against colistin [30, 22, 31].

A study by Shireen rana et al showed similar high resistance pattern to Ampicillin among the ESBL producers. Another study conducted by Manjula et al which showed resistance of (75.6%). [24]

This study also showed 45.6% resistance pattern among ESBL producing and 39.1% among non ESBL producing *Klebsiella pneumoniae* [25]. A similar study showed 88.8% (ESBL producer) & 68.8% (Non ESBL producer) of resistance pattern to quinolones.[24]

Second and third generation cephalosporins are the common drugs of choice for *Klebsiella pneumoniae* infections.[4]

Ciprofoxacin showing maximum ineffectiveness was in agreement with previous finding in Nepal [32].

MDR is a global issue and a relatively high burden seen in developing countries [20]. Various prevalence of MDR *K. pneumoniae* have been frequently reported in Nepal [22, 33, 34]. High prevalence of MDR could be due to the easy availability and blind irrational use of antibiotics without proper culture report and prescription [35, 36].

Out of 88 *K. pneumoniae* isolates, half were MHT positive. Shanmugan et al. [37] reported a higher prevalence (82.6%) of MHT-positive *K. pneumoniae* in India; however, lower prevalence of carbapenemase-producing *K. pneumoniae* is also reported in India [15], and also in Nepal [38].

The variation among the distribution *of K. pneumoniae* is considerable as the studies used for comparison are from different geographical locations with differing numbers of specimens used. Furthermore, the use of antibiotic therapy among patients, topographical situations, sanitation and the location of the study can also explain the variation in the distribution of *K. pneumoniae*.

Since carbapenems have long been known to be effective against gram-negative bacteria that are resistant to many drugs, the growth in resistance is particularly concerning [21]. Further jeopardising the progress gained in treating critically ill hospitalised patients—where the risk of contracting multidrug-resistant infections is particularly high—is the lack of effective substitute antibiotics, which makes it harder to contain multidrug-resistant germs. Thus, in order to develop efficient control measures and stop the development of AMRs, it is crucial to obtain a thorough grasp of the prevalence of carbapenem resistance among gram-negative bacteria. The current study outlined the carbapenem susceptibility patterns among Gram-negative bacterial isolates in a tertiary care hospital in response to this pressing need, offering vital insights into the state of carbapenem resistance today and opening the door for preventative measures.

CONCLUSION

This study provides valuable insights into the prevalence of *Klebsiella pneumoniae* infections and the emergence of carbapenem resistance among patients attending a tertiary care hospital. The findings underscore the urgent need for comprehensive strategies to combat antimicrobial resistance, including enhanced surveillance.

Declarations:
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work.

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