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PREVALENCE OF COLISTIN RESISTANT KLEBSIELLA PNEUMONIAE IN VARIOUS CLINICAL SAMPLES IN RURAL MADHYA PRADESH

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1. INTRODUCTION:-

Klebsiella spp. is a gram negative, non-motile, encapsulated, lactose fermenting and facultative anaerobe belonging to the *Enterobacteriaceae* family. [1] Important virulence factors of the *Klebsiella pneumoniae* are capsular polysaccharides, lipopolysaccharide (LPS) and siderophores [2]. *Klebsiella pneumoniae* cause various infections like pneumonia, urinary tract infections, meningitis, wound infections, osteomyelitis, bacteremia, septicemia, gastroenteritis and many more infections in developing countries [3]. It is also one of the main sources of ventilator-related pneumonia (VAP) among patients in intensive care units (ICUs) and causes 83% of hospital-acquired (HA) pneumonia [4].

Recently, the emergence of multidrug-resistant (MDR) phenotype as the consequence of antibiotics overconsumption in the treatment of human diseases caused a global challenge to health systems. [5]

Carbapenems are the choice drugs to treat such infections. However, with increasing global incidence of carbapenems resistance, colistin is now widely used as the last resort antibiotic for the treatment of carbapenem-resistant *Enterobacteriaceae*. Among carbapenem-resistant *Klebsiella pneumoniae* isolates, the rate of resistance to colistin has been reported to be 15% which is very worrying. [6]

Colistin uses lipopolysaccharide (LPS) as the bacterial target and disrupts the negative charge of the outer membrane. Several studies proposed two main mechanisms for colistin resistance including LPS modifications and LPS removal. Other possible mechanisms are over expression of efflux pumps, overproduction of capsule polysaccharide and production of colistinase. [7]

Recently, a plasmid transmitted resistance has been reported as the mobilized colistin resistance (*mcr*), and has been designated as *mcr-1*, which is the most prevalent *mcr* type. [8]

The aim of this study was to investigate the prevalence of the colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* isolates which is collected from human clinical specimens in Central zone of India.

2. MATERIAL AND METHODS:- This study was performed at department of Microbiology, LN Medical College Bhopal M.P. from Jan 2022 to Dec 2022. A non-duplicate, non-repeat successively

and good quality clinical samples were collected from different ward/ outpatient departments (OPD)/ inpatient department (IPD) of J K hospital associated with L N medical college M.P.

2.1 Specimen: A total of 19225 samples were included in this study. The different samples urine, pus, blood, sputum and other type of samples were collected from J K hospital, Bhopal, Madhya Pradesh. All samples were collected aseptically and transported as soon as possible for bacteriological examination.

2.2 Inclusion criteria: All type of clinical samples received at the laboratory were checked for labeling such as patient name, client registration number, date, time and quality of samples. Patients of all age groups and gender who were admitted in or visiting the hospital for treatment were included in this study.

2.3 Exclusion criteria: Unlabeled and inappropriate/ leaked samples were rejected.

2.4 Specimen processing: All the samples were collected and processed by maintaining universal precautions. Samples were transported to the laboratory within two hours and processed immediately or refrigerated at 4°C to 8°C. Simultaneously all the samples were kept in peptone broth for 24-48 hours and tested for turbidity in broth and then processed on blood agar, chocolate agar and MacConkey agar plates for pus, Blood, sputum etc. and urine samples were inoculated on cysteine-lactose-electrolyte-deficient agar (CLED) and incubated in aerobic conditions at 37°C for 24-48 hours. Identification of isolated micro-organisms were done on the basis of colony characteristics, Gram staining and battery of different biochemical tests as per the standard protocol at species level. [9]

2.5 Antimicrobial susceptibility testing: - Antimicrobial susceptibility testing was done on Mueller-Hinton agar (Hi media, India) using standard disk diffusion (Kirby Bauer's) technique. This test and interpretation of result was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]
K. pneumoniae isolates were further subjected to *in-vitro* antibiotic susceptibility assay by using modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standard Institute. Nitrofurantoin (300 µg), cefotaxime (30 µg), cotrimoxazole (25 µg), cefixime (5 µg), amoxycillin (10 µg), ofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), moxifloxacin (5 µg), ceftazidime (30 µg), amoxycillin/clavulanate (20/10 µg), amikacin (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), imipenem (10 µg), ertapenem (10 µg), piperacillin/tazobactam (100/10 µg), polymyxin-B (100 µg), and tigecycline (15 µg) discs were tested for susceptibility assay. In this method, broth culture of test bacteria (comparable to McFarland tube no.0.5, inoculums density 1.5 × 108 bacteria/ml) was uniformly carpeted on the surface of Mueller Hinton agar (MHA). Then, antibiotics discs were placed onto the lawn culture of the test bacteria by sterile forceps. The inoculated and seeded MHA plates were incubated at 37 ºC for 24 h. After incubation, zone of inhibition was measured and results were interpreted as sensitive, intermediate and resistant. [10]

2.5.1 Quality control: *E. coli* ATCC25922 was used as control reference strains for identification and drug susceptibility testing.

2.6 Minimum inhibitory concentration (MIC):-
Colistin MICs were measured by E-test strips (Liofilchem, Italy) and interpreted based on European Committee on Antimicrobial Susceptibility Testing (EUCAST, Ver. 6, 2016) guidelines. After measuring the MIC with E-test method, *K. pneumoniae* isolates with a MIC higher than 2µg/ml were considered as “resistant”. [11, 12]

2.7 Ethical issue: Study was approved from institutional ethical committee as per institutional ethical guidelines after getting proper patient consent.

3. Result: During this study, patients suffering from various bacterial infections were included. We have analyzed all the clinical samples which were collected from J K hospital, Bhopal, Madhya Pradesh.

In microbiology department 19225 total no. of clinical sample were processed (Urine, Blood, Pus, sputum and other samples). 40% showed various microorganism, out of positive isolates, 759 (20%) were identified as *K.pneumoniae* in Urine, 31(6%) in Blood, 90(18%) in pus, 129(27%) in sputum and 976(43%) in other samples as shown in the Table no.1

Out of above *K.pneumoniae* isolates, 4% Colistin-Resistant *K.pneumoniae* were identified from urine, 6% from blood, 2% from sputum and 1% from pus as shown in Table 2 & Figure no. 2. Most of the samples were collected from female gender (61%) than male (39%) and *K.pneumoniae* isolates belonged to female gender 66.95% and to the male 33.04% as shown in the Table no. 3 & Figure no.3.

Antimicrobial susceptibility testing showed in below Table. Maximum resistant showed by *K. pneumoniae* was towards penicillin group and least was shown towards Polymyxin as shown in table 4.

Table no:- 1 Distribution of *Klebsiella pneumoniae* in various clinical samples

S.N.	Type of samples	No. of samples	No. of positive samples	No. of *Klebsiella pneumoniae* (Kp)
1.	Urine	9605	4130(42%)	759 (20%)
2.	Blood	769	395(51%)	31 (6%)
3.	Pus	958	622(64%)	90 (18%)
4.	Sputum	678	464(68%)	129 (27%)
5.	Other samples	7215	2240(31)	976 (43%)
Total		19225	7851(40%)	1985 (25%)

Table no:- 2 Distribution of Colistin-Resistant *K.pneumoniae* in various clinical samples

S.N.	Type of samples	No. of *Klebsiella pneumoniae* (Kp)	No. of Colistin-Resistant *Klebsiella pneumoniae* (CRKp)
1.	Urine	759	31(4%)
2.	Blood	31	2(6%)
3.	Pus	90	1(1%)
4.	Sputum	129	3(2%)
5.	Other samples	976	0
Total		1985	37(1.86%)

Figure no:- 2 Distribution of Colistin-Resistant *K.pneumoniae* in various clinical samples

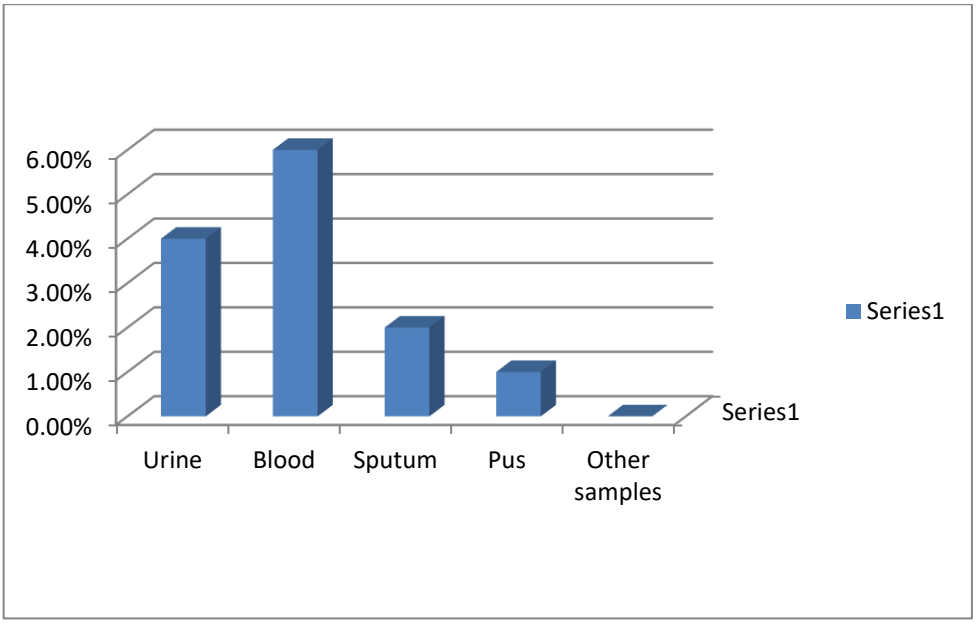

Table 3: Gender-wise distribution of clinical samples

Gender	**Samples (19225)**	***K. pneumoniae* (1985)**
Female	11789 (61%)	1329 (66.95%)
Male	7436 (39%)	656 (33.04%)

Figure no 3: Gender-wise distribution of clinical samples

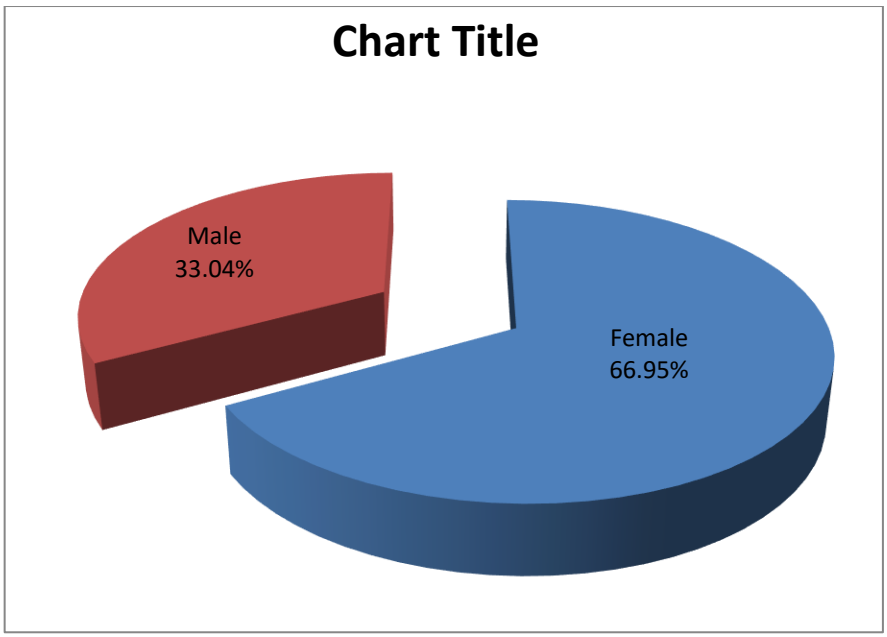

Table 4: AST pattern of *Klebsiella pneumoniae* in various clinical samples

S. No.	Group	Antibiotic	Sensitive (%)	Resistant (%)
1	**Penicillin**	Ampicillin	00	100
2		Amoxycillin-Clavulanic acid	30	70
3		Piperacillin/Tazobactum	42	58
4	**Cephalosporin**	Cefazolin	31	69
5		Cefuroxime	29	71
6		Ceftriaxone	24	76
7		Cefotaxime	28	72
8		Ceftazidime	29	71
9		Cefepime	49	51
10	**Carbapenems**	Imipenem	77	23
11		Meropenem	79	21
12		Ertapenem	72	28
13	**Combination**	Cotrimoxazole	26	74
14	**Chloramphenicol**	Chloramphenicol	62	38
15	**Tetracycline**	Tetracycline	41	59
16	**Aminoglycoside**	Amikacin	73	27
17		Gentamycin	75	25
18	**Fluoroquinolones**	Ciprofloxacin	35	65
19		Levofloxacin	33	67
20		Moxifloxacin	31	69
21	**Nitrofuran**	Nitrofurantoin	68	32
22	**Polymyxin**	Colistin	83	17

DISCUSSION:-

K.pneumoniae is associated with a wide range of severe infections including bloodstream infections, ventilator-associated pneumonia, community-acquired pneumonia, hospital-acquired pneumonia, complicated urinary tract infections (UTIs), and complicated intra-abdominal infections. Multi-drug-resistance (MDR), especially pan-drug resistance and MDR is emerging as a major challenge in the treatment of infections caused by *K.pneumoniae*. In this study, *K.pneumoniae* isolate was resistant to commonly prescribed broad-spectrum antibiotics.

Therefore, antibiotic resistance in *K.pneumoniae* has significant clinical and socioeconomic impacts. Emergence of ESBL producing *K.pneumoniae* has led to extensive use of carbapenem as a first line

empirical treatment. The increasing use of carbapenems for possible ESBL infections has led to a more serious problem of the emergence of carbapenemase-producing *K.pneumoniae*.[13] Carbapenemase-producing *K.pneumoniae* pose an exponentially increasing threat for public health worldwide. Carbapenemases are β-lactamases with the ability to hydrolyze penicillins, cephalosporins, monobactams, and carbapenems.[14] Carbapenems are considered last resort antibiotics for the treatment of infections caused by multidrug-resistant *K.pneumoniae*. In the last decade, the prevalence of colistin-resistant *K.pneumoniae* has increased rapidly. [15]

In our study the total number of *K.pneumoniae* isolates from various clinical samples is 1985 (25%) which is similar to study by Ravichitra *et al.* [16] This study shows colistin resistant *K.pneumoniae* (CRKp)is 37 (1.86%) in various clinical samples. out of 37, in urine 4%, blood 6%, pus and 1% in sputum sample. Bhaskar BH *et al*. [17] from manipal hospital demonstrated 28% resistance to colistin which was at higher side whereas Sodhi K *et al*. [18] reported 9% in urine. The prevalence rate of *K. pneumoniae* in blood sample is 6% as Mao Zhou *et al* [19] 7.8%, similar to the study conducted in Spain [20], but much lower than previous studies conducted in China (24.5% or 21.6%). [21]

In our study *K. pneumoniae* shows highest resistant to penicillin group drugs and maximum sensitivity to colistin.

CONCLUSION:-

K. pneumoniae is a medically important pathogen which causes various types of infections in humans like pneumonia, urinary tract infections, meningitis, wound infections, osteomyelitis, bacteremia, septicemia, and gastroenteritis. The prevalence of *K.pneumoniae* is important to track because of its increasing trend of resistance to multiple drugs. The alarming increase in the prevalence of multidrug-resistant *K. pneumoniae* infections that is very challenging and also important to determine genes to identify multidrug-resistant *K. pneumoniae* strains.

In summary, the recent emergence of a number of difficult to treat *K. pneumoniae* strains and infections is challenging the medical community to evaluate both host and bacterial factors critical during infection.

Colistin resistance is a critical issue to deal with nowadays. Many studies have proved this resistance in several bacterial species and in different countries around the world. The prevalence of colistin-resistant *K. pneumoniae* is increasing globally, representing a major concern, as colistin is one of the few remaining treatment alternatives for patients infected with MDR *K.pneumoniae*. Colistin resistance in *Klebsiella pneumoniae* isolates strongly suggests a necessity for the implementation of effective strategies to prevent and control the spread of such strains.

Our study encourages working on papers about different detection methods for the colistin-resistant gene in multidrug-resistant *K. pneumoniae*.

LIMITATIONS:- Molecular study should be done for genes responsible for Colistin-resistance is further required to know the accurate results.

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