

To study the minimum inhibitory concentration of Colistin on MDR *klebsiella pneumoniae* by agar dilution and broth dilution method

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ABSTRACT

Background - The threat to human health posed by the global spread of multidrug-resistant (MDR) gram-negative bacteria is growing. In the healthcare environment, *K. pneumoniae* is also the most prevalent pathogen that is resistant to many drugs including carbapenems. In the past few decades, polymyxins have been the most widely utilised antibiotic choice for treating *K. pneumoniae* that is resistant to carbapenem. In fact, polymyxin E, also known as colistin, is frequently the only antibiotic to achieve sufficient serum levels and minimum inhibitory concentrations (MIC), making it a "last resort" treatment for MDR *K. pneumoniae* infections. Therefore, this study was conducted to check the MIC of colistin towards *K. pneumoniae*. **Materials** – In tertiary Care Hospital, samples that were received at Microbiology lab were processed using standard microbiological procedures and further studied. The MIC of agar dilution and Broth dilution was compared. **Result** – From the 250 MDR *K. pneumoniae* processed, Susceptibility of MDR *K. pneumoniae* to Colistin By Agar Dilution Method was 73.2 %. And Susceptibility of MDR *K. pneumoniae* to Colistin By Broth Dilution Method was 78.4 %. When Both of them were compared Broth Dilution was more susceptible to colistin than Agar dilution method.

Keywords: Colistin, MDR, MIC

INTRODUCTION

The rod-shaped gram-negative bacteria *K. pneumoniae* is one of the most frequent causes of infections linked to healthcare that affect people, including sepsis, pneumonia, meningitis, and urinary tract infections.^[1] Also one of the well-known human nosocomial pathogen is *Klebsiella pneumoniae*.^[2] One of the microbes linked to numerous serious infection cases in the intensive care unit (ICU) is also *Klebsiella pneumoniae*.^[3] Due to an increase in severe infections and a growing lack of effective therapies, *Klebsiella pneumoniae* has recently garnered popularity as an infectious agent.^[4] The emergence of *K. pneumoniae* strains resistant to carbapenems and other broad-spectrum antibiotics is a serious issue, particularly for patients who are very sick.^[3]

In the healthcare environment, *K. pneumoniae* is also the most prevalent pathogen that is resistant to both drugs and carbapenems. In the past few decades, polymyxins have been the most widely utilised antibiotic choice for treating *K. pneumoniae* that is resistant to carbapenem. In fact, polymyxin E, also known as colistin, is frequently the only antibiotic to achieve sufficient serum levels and minimum inhibitory concentrations (MIC), making it a "last resort" treatment for MDR *K. pneumoniae* infections.^[1] The minimum inhibitory concentration, also known as the MIC, of an antibacterial agent is measured in micrograms per millilitre, or µg/mL, and it is the concentration at which the test strain of an organism cannot grow at all under carefully regulated in vitro conditions.^[4] The two most widely used techniques for figuring out the minimal inhibitory concentration (MIC) of antimicrobial agents—which include antibiotics and other drugs with bactericidal or bacteriostatic properties—are agar and broth dilution.^[5] Superbugs or multidrug-resistant (MDR) bacteria, severely restrict available treatment options and are consequently linked to higher rates of morbidity, mortality, and economic burden. Conversely, limited treatment

options are driving physicians to use "last line" medications, mainly colistin.^[2]. Therefore this study has been conducted to check the MIC of colistin against *K. pneumoniae* by Agar and Broth Dilution method .

MATERIALS AND METHOD –

A Observational Descriptive study conducted in Department of Microbiology , MGM Medical college and Hospital Navi Mumbai During DEC 21- DEC22.

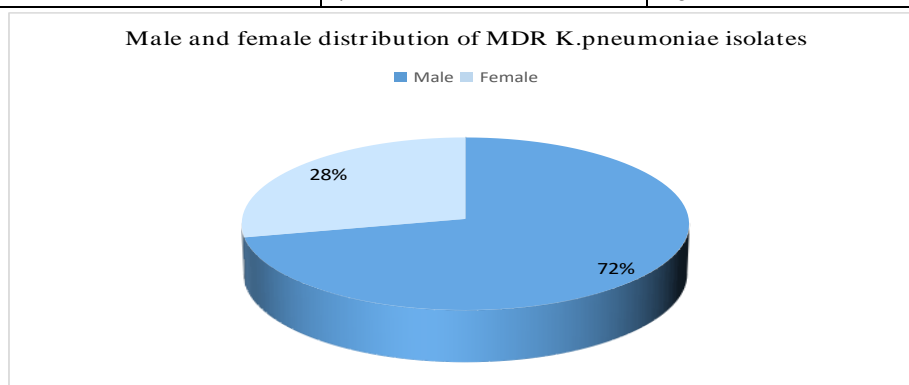
Study Procedure

1. **Sample collection** - Clinical samples such as urine, blood, pus, sputum, body fluids, wound swab, catheter and other tips, urethral and vaginal swabs, bone and bone marrow aspirate were collected and processed during the study period.^[6]
2. **Processing of samples** - All samples were processed and isolated according to Standard Microbiological Procedure .^[6,7]
3. **Antibiotic susceptibility testing (AST)** - *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 (2022) . Isolates showing resistance to at least one agent of three or more classes of antimicrobial agents were termed as multidrug-resistant (MDR).⁷
- **Agar dilution** ^[8] -
MIC was determined using AD in triplicate on Mueller-Hinton Agar (MHA) plates with colistin sulphate concentrations ranging from 0.25 to 16 µg/mL. Growth was controlled using drug-free MHA plates. Using a multipoint inoculator, 52 evenly spaced bacterial suspensions were added to each plate, resulting in a final concentration of 104 CFU/spot. Plates were incubated overnight at 37°C, and MIC values were reported as the lowest concentration that inhibited bacterial growth.
- **Broth dilution** ^[9] -
The broth dilution method involves diluting antibiotics (1, 2, 4, 8, 16, 32, and 64 µg/mL) in a liquid growth medium in test tubes.
A standardised bacterial suspension of 1.5 X 10⁵ CFU/mL was added to antibiotic-containing tubes. The tubes were incubated at 35-37 °C for 16-20 hours and turbidity was used to determine visible bacterial growth.
The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that inhibits observable growth of bacteria following overnight incubation
A standard strain with a known MIC value is used as a control to ensure proper reagents and conditions.

RESULT

TABLE 1 - Male and female distribution of MDR *K.pneumoniae* isolates [n =250]

Gender	Total Patient	Percentage
Male	179	71.6%
Female	71	28.4%

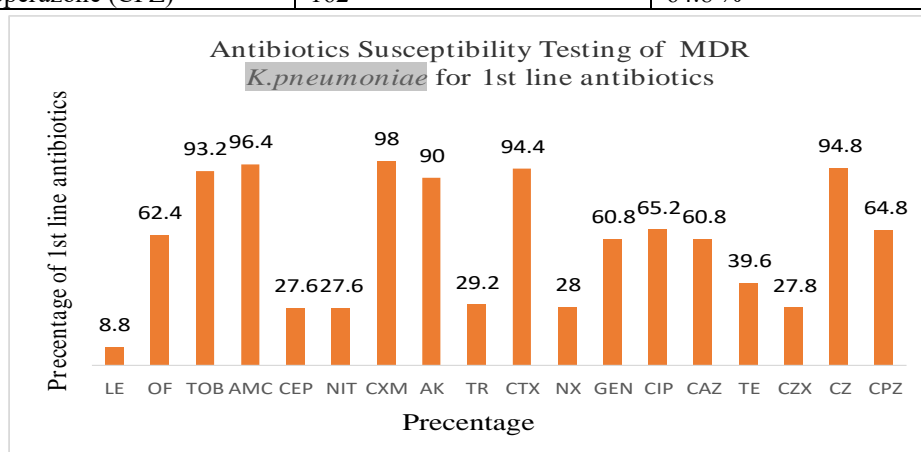


- Maximum isolates of *K.pneumoniae* were found in Males 71.6 % [179] than females

- **ANTIBIOTICS PATTERN**

Table 2 -Antimicrobial Susceptibility Testing of MDR *K. pneumoniae* for 1ST Line antibiotics –

Antibiotics	Total no of Isolates	Resistant
Levofloxacin (LE)	22	8.8 %
Ofloxacin (OF)	156	62.4 %
Tobramycin (TOB)	233	93.2 %
Amoxyclav (AMC)	241	96.4 %
Cephalothin (CEP)	69	27.6 %
Nitrofurantoin (NIT)	69	27.6 %
Cefuroxime (CXM)	245	98 %
Amikacin (AK)	205	90 %
Trimethoprim(TR)	73	29.2 %
Cefotaxime(CTX)	236	94.4 %
Norfloxacin (NX)	70	28 %
Gentamycin (GEN)	152	60.8 %
Ciprofloxacin (CIP)	163	65.2 %
Ceftazidime(CAZ)	152	60.8 %
Tetracycline (TE)	99	39.6 %
Ceftizoxime(CZX)	69	27.6 %
Cefazolin (CZ)	237	94.8 %
Cefoperazone (CPZ)	162	64.8 %

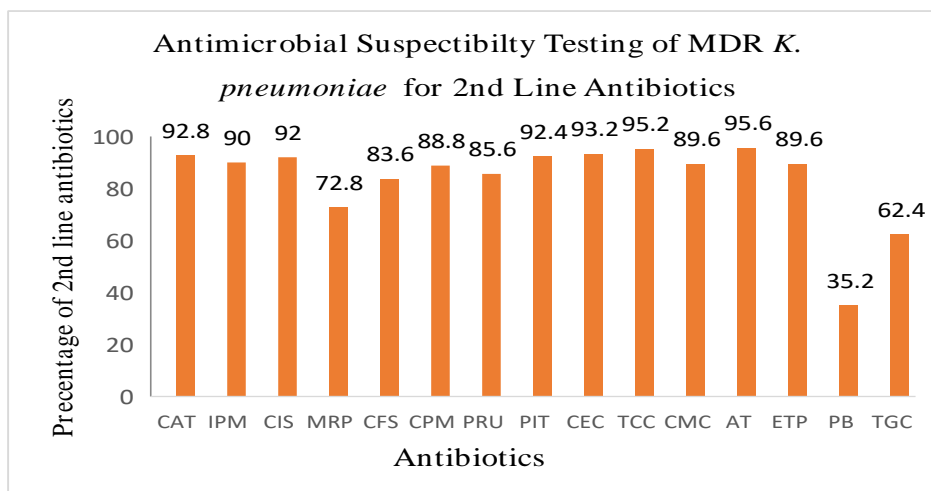


- Amongst 1st line antibiotics, Maximum resistance was observed for Cefuroxime (98%) and Minimum resistance was observed for Levofloxacin (8.8%).

TABLE 3 - Antimicrobial Susceptibility Testing of MDR *K. pneumoniae* for 2nd Line Antibiotics

Antibiotics	No of Isolates	Resistant
Ceftazidime/Tazobactam (CAT)	232	92.8 %
Imipenem (IPM)	225	90 %
Ceftriaxone/Sulbactam(CIS)	230	92 %
Meropenem (MRP)	185	72.8 %
Cefoperazone/Sulbactam (CFS)	209	83.6 %
Cefepime (CPM)	222	88.8 %
Prulifloxacin (PRU)	214	85.6 %
Piperacillin/Tazobactam (PIT)	231	92.4 %
Cefotaxime/Clavulanic acid (CEC)	233	93.2 %
Ticarillin/Clavulanic acid (TCC)	238	95.2 %
Cefixime/Clavulanic acid (CMC)	224	89.6 %
Azteonam (AT)	239	95.6 %
Etrapanem (ETP)	224	89.6 %

Polymyxin B (PB)	88	35.2 %
Tigecycline (TGC)	156	62.4 %

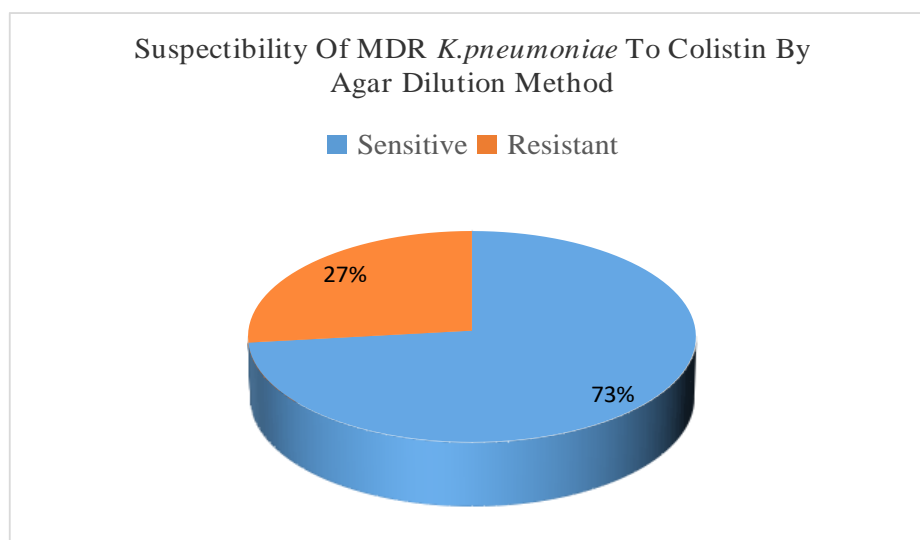


- Amongst 2nd Line antibiotics, Maximum resistance was observed for Aztreonam (95.6 %) while Minimum resistance was observed for Polymyxin B (35.2%).

❖ SUSCEPTIBILITY PATTERN –

TABLE 4 - Susceptibility of MDR *K.pneumoniae* to Colistin By Agar Dilution Method

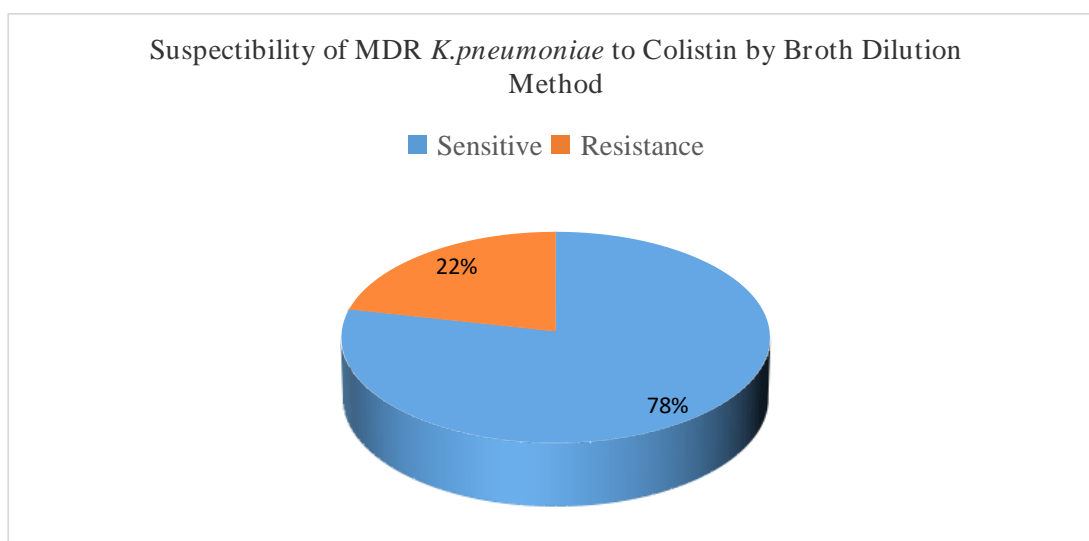
MIC Interpretation	No Of Isolates	Percentage
Sensitive	183	73.2 %
Resistant	67	26.8 %



- Susceptibility of MDR *Klebsiella pneumoniae* for Colistin was observed to be (73 %) by Agar Dilution Method .

TABLE 5 - Susceptibility of MDR *K.pneumoniae* to Colistin by Broth Dilution Method

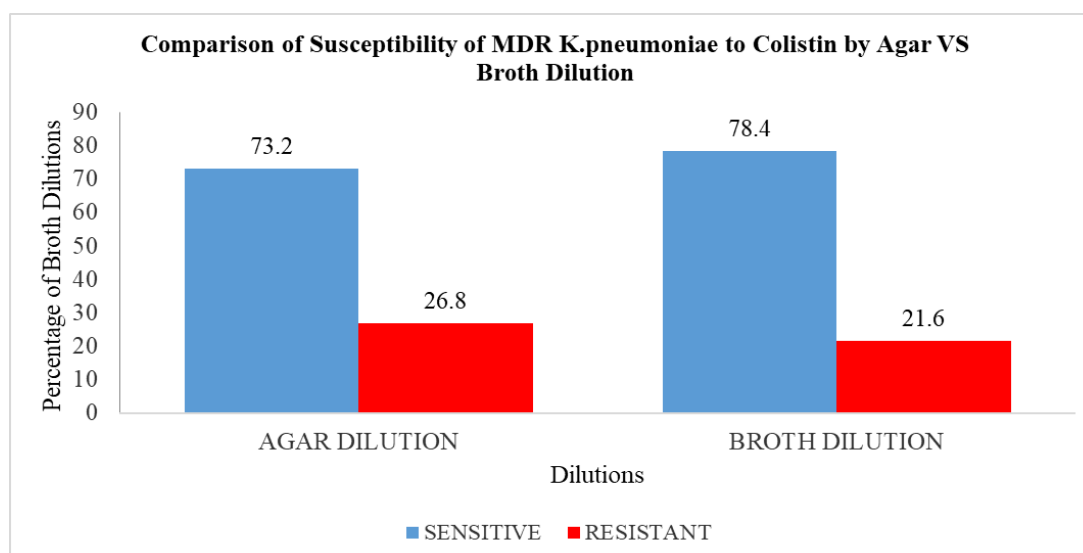
MIC Interpretation	No of Isolates	Percentage
Sensitivity	196	78.4 %
Resistance	54	21.6 %



- Susceptibility of MDR *Klebsiella pneumoniae* for Colistin was observed to be (78.4%) by Broth Dilution Method .

TABLE 6 - Comparison of Susceptibility of MDR *K.pneumoniae* to Colistin by Agar VS Broth Dilution

Method of Dilution	Susceptible	Resistant
Agar Dilution	73.2 %	21.6 %
Broth Dilution	78.4 %	26.8 %



When Agar and Broth Dilution methods were compared, the susceptibility of MDR *K. pneumoniae* to colistin was higher in Broth dilution method (78.4%) than Agar dilution method (73.2%). Additionally, resistance to colistin was observed more for broth dilution method (26.8%) as compared to agar dilution method (21.6%).

DISCUSSION

- The emergence of multidrug-resistant gram-negative bacteria poses a growing hazard to human health. Among gram-negative bacteria, the rod-shaped bacterium *K. pneumoniae* is a leading source of healthcare-associated illnesses in humans, including urinary tract infections, pneumonia, meningitis, and sepsis. *K. pneumoniae* is the most prevalent multidrug-resistant and carbapenem-resistant infection in healthcare settings.^[1]
- This study was conducted in MGM Medical College, total of 250 samples with growth of MDR *K. pneumoniae* were received in Microbiology laboratory from DEC 22 - APRIL 23 .
- Out of 250 samples, Maximum isolates of *K.pneumoniae* were found in Males 71.6 % [179] as compared to females (28.4%). A study by Mutasim E. Ibrahim et.al also reported that the maximum number of isolates in men as compared to women ^[10]. Our study also correlates with the study conducted by Sureka Indrajith et.al^[11]
- In our study, Among 1st line antibiotics, Maximum resistance was observed for Cefuroxime (98%) and Minimum resistance was observed for Levofloxacin(8.8%). Another study conducted by H. schumacher et.al also reported resistance to cefuroxime ^[12].
- Our study showed maximum resistance to aztreonam (95.6%) among 2nd line antibiotics, and the least resistance was observed for polymyxin B (35.2%). Another study conducted by Alice Elena Ghenea et.al also reported resistance to aztreonam ^[13] . One more study conducted by Alexandre Prehn et.al Zavascki et.al showed similar resistance to polymyxin B. ^[14]
- According to CLSI guidelines, the MIC breakpoint for colistin is $\leq 2\mu\text{g/ml}$ ^[38]. Out of 250 MDR *K. pneumoniae* isolates, Our study revealed 183 susceptible (73.2%) and 67 resistant (26.8 %) isolates to colistin by agar dilution method, and 196 susceptible (78.4%) and 54 resistant (21.6 %) isolates by Broth Dilution method. Another study conducted by Rita Elias et.al reported 131 susceptible and 10 resistant isolates by agar dilution method and 99 susceptible and 16 resistant isolates were found by broth dilution method.^[15]

CONCLUSION

- For the 1st line antibiotics, maximum resistance to MDR *K. pneumoniae* was observed for cefuroxime (98%) and minimum resistance was for levofloxacin (8.8%)
- For 2nd line antibiotics, maximum resistance to MDR *K. pneumoniae* was observed for aztreonam, and minimum was for Polymyxin B (35.2 %)
- Susceptibility of MDR *K. pneumoniae* to colistin was 78.4% with the broth dilution method, and 73% with agar dilution method.
- When agar dilution and broth dilution methods were compared, resistance to colistin for MDR *K. pneumoniae* was more for Agar dilution method (26.8%) than Broth dilution method (21.6%) .
- As infections with MDR *K. pneumoniae* is increasing day by day, antibiotic susceptibility testing with colistin should be followed as routine practice in clinical Microbiology Laboratory.
- To reduce the rate of infection with MDR *K.pneumoniae* , strict infection control practices should be followed .

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