# COMPARATIVE EVALUATION OF COLISTIN SUSCEPTIBILITY TESTING METHODS AND THE RESISTANT PATTERN OF COLISTIN AMONG CARBAPENEM-RESISTANT *Klebsiella pneumoniae* ISOLATES IN A TERTIARY CARE SETUP

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## Abstract

**Background:** Carbapenem-resistant *Klebsiella pneumoniae* (CR*Kp*) infections remain a significant challenge associated with morbidity and mortality among patients worldwide. Colistin remains the mainstay for complicated CR*Kp.* This study was done to relate the minimum inhibitory concentration (MIC) of colistin by gold standard technique broth microdilution (BMD) with automated system VITEK2C and its resistance pattern in CR*Kp* strains to evaluate discrepancies and further course of action.

**Materials & Methods:** This was a Cross sectional study, a total of 166 non repetitive isolates of CR*Kp* was collected for a period of 18 months (September 2020 to March 2022). Phenotypic confirmatory test for detection of carbapenemases was done by Modified Hodge test and automation by VITEK 2C. Colistin MIC was determined by BMD (Mikrolatest$^{R}$, Erba$^{R}$Mannheim) and VITEK 2C (Biomeriux) methods as per CLSI guidelines.

**Results: A total of 166 isolates of CR*Kp* were studied**. 89% of the isolates were susceptible to colistin by BMD whereas colistin resistance was 11% by BMD and 13% by VITEK2C and the range of MIC was between 4 and 16ug/ml.

**Conclusion:** Broth Micro dilution (BMD) technique is gold standard method for determining the MIC of Colistin. The results correlated with Vitek 2C except for 3% of isolates which showed minor errors which indicates that for MIC values of colistin

BMD is the preferred option. The high rate of resistance to carbapenems and colistin is worrisome. Nevertheless, the MIC of colistin helps the clinician to choose the drug in appropriate combinations.

**Keywords:** Colistin, *K. pneumoniae,* Broth microdilution, Vitek-2C**.**

## Introduction

According to the World Health Organization (WHO), Worldwide report of 2014, Surveillance of antimicrobial resistance *K. pneumoniae* was one of the most common species causing urinary tract infections, meningitis, pneumonia, surgical site infections and sepsis.[1] Among those, Carbapenem-resistant *Klebsiella pneumoniae* (CR*Kp*) infections remain a significant challenge associated with morbidity and mortality among patients globally.[2] Isolated from *Paenibacillus polymyxa subsp. colistinus*, a soil bacterium, in 1947, colistin is a circular polycationic antimicrobial agent regaining further attention as one of the last line therapeutic option against multidrug resistant (MDR) Gram-negative bacteria, specially the carbapenemase producers.[3] However, during the last few years, increase in colistin resistance has appeared worldwide, especially among *Klebsiella pneumoniae*, further limiting the treatment options.[4] In severe CRKp infections, colistin is often used in combination therapy with other antibiotics such as tigecycline, gentamicin, meropenem or fosfomycin. Hence with the rise in usage of Polymyxin-E, cases of colistin- resistant *Klebsiella pneumoniae* carbapenemase (KPC)– producing strains are reported worldwide.[5,6] Colistin is being progressively used as a last resort drug, so prompt and consistent colistin susceptibility testing (ST) is required in clinical laboratories to allow proper therapeutic management.[7]

Disk diffusion, which is frequently used in various clinical laboratories, created high error rates when related to MIC-based methods. It is not considered reliable for the detection of colistin susceptibility testing (ST).[8] Amid all commercial procedures, gradient diffusion strips are suitable for determining colistin MICs, but their presentation is not well established.[9] Some studies revealed very good correlations among the results of broth microdilution (BMD) and E-test and agar dilution (AD) techniques for colistin ST, while other studies questioned the reliability of Etest.[10]

To address this issue, CLSI (Clinical and Laboratory Standard Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) together suggested broth microdilution (BMD) as the gold standard technique for colistin susceptibility testing.[11] Our study was done to relate colistin susceptibility with broth micro dilution and VITEK 2C method in CR*Kp* to evaluate the inconsistencies and further course of action.

## Materials and methods

This was a Cross sectional study, a total 166 isolates of CR*Kp* were collected for a period of 18 months (September 2020 to March 2022) in the Department of Microbiology. All samples received were processed as per standard protocols.[12] K. *pneumoniae* was identified using standard procedures. Antibiotic susceptibility was carried out manually by disk diffusion method; phenotypic confirmatory test for detection of carbapenemases was done by Modified Hodge test and automation was carried out by VITEK 2C (Biomeriux, Marcy-l'Étoile, France) as per CLSI guidelines.[13] Minimum inhibitory concentration (MIC) of colistin was determined by both broth microdilution (BMD) (Mikrolatest$^{R}$, Erba$^{R}$Mannheim) and VITEK 2C (Biomeriux, Marcy-l'Étoile, France) techniques.

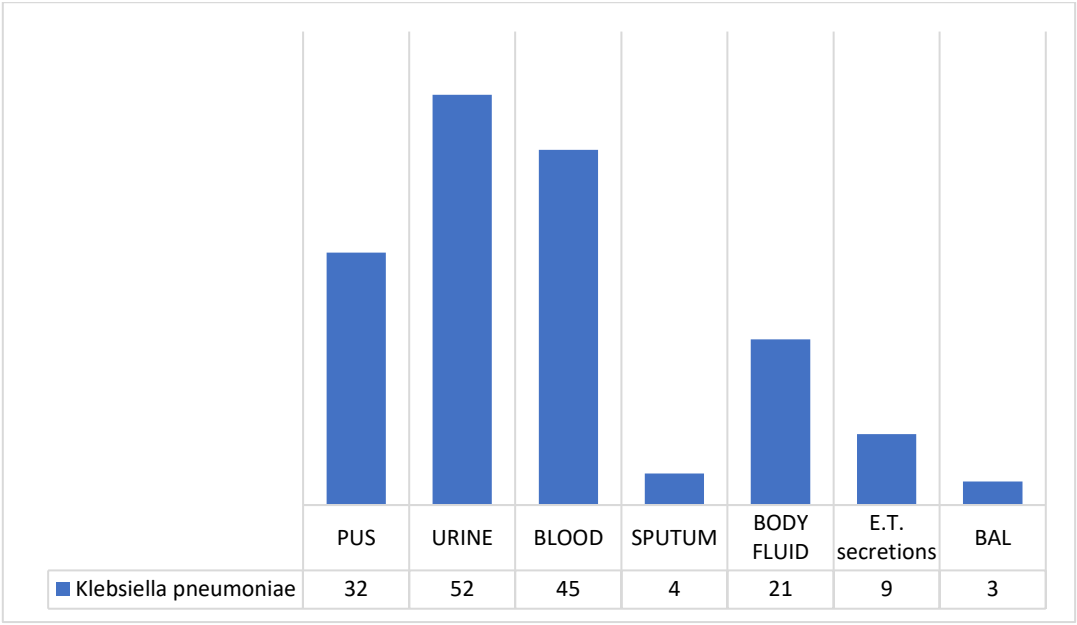
## Results

**A total of 166 isolates of Carbapenem Resistant *Klebsiella pneumoniae* were studied.**

Figure 1 shows the number of cases for each type of sample. Urine was found as the most common sample type in this study, constituting 31.32% of all isolates, while blood and pus with 27.10% and 19.27% respectively. Body fluid, with 12.65%, was another specimen type that comprised more than 10% of the cases. CRKp was most

commonly found in urine. However, some contributions were made by sputum, E.T. secretion and bal. Thus, urine, pus, body fluid and blood constituted 90% of the sample.

Figure 1. Source of infection for carbapenem resistant *Klebsiella pneumoniae* (CRkp) isolates.




The MIC values obtained from Colistin testing were divided into two categories as per CLSI guidelines: (1) Intermediate (I) with MIC <= 2 µg/ml, and Resistant (R) with MIC >=4 µg/ml. The BMD technique identified 147 *Klebsiella pneumoniae* isolates as intermediate to Colistin and 145 were reported intermediate by VITEK2C as shown in table 1. According to this data, *Klebsiella pneumoniae* had a greater intermediate population which were identified using the BMD as compared to VITECK2C method. However, the VITEK2C technique detected more resistant cases in contrast to BMD.

Table 1: Colistin Intermediate and Resistant cases in CRE isolates by BMD and VITEK2C methods.

| | **BMD** | | | **VTK** | | |
|---|---|---|---|---|---|---|
| | **I (<=2)** | | **R (>=4)** | **I (<=2)** | | **R (>=4)** |
| ***Klebsiella pneumoniae*** | 147 | | 19 | 145 | | 21 |

* Intermediate (I) and Resistant (R).

All the isolates were subjected to serial MIC testing for Colistin by BMD and VITEK2C methods. The range of MIC with these two techniques are shown in Table 2. Here, *Klebsiella pneumoniae* shows discrepancy in MIC >=4 and in MIC>=16. 2 isolates showed a higher MIC range in VITEK 2C when compared to BMD.

Table 2: MIC value for colistin recorded for CRKp bacteria by BMD and VITEK techniques.

| **Isolate** | **#** | **MIC=0.5** | **MIC=1 µg/ml** | **MIC=2** | **MIC=4** | **MIC=8** | **MIC=16** |
|---|---|---|---|---|---|---|---|

| | Isolate | µg/ml | | | | µg /ml | | µg /ml | | µg /ml | | µg /ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BM | VT | BM | VT | BM | VT | BM | VT | BM | VT | BM | VT |
| *Klebsiella pneumoniae* | 166 | 102 | 102 | 38 | 38 | 7 | 7 | 5 | 6 | 5 | 5 | 9 | 10 |

**BM= BMD; VT=VITEK2C*

Statistical tests were performed to determine the significant difference between the two methods. The t-test was used to examine the MIC values calculated by both techniques. The MIC value for BMD and VITECK2C is shown in Figure 2 at two cutoffs: 0.5 and 1. It is observed that lower MIC values were captured more accurately using BMD method compared to VITEK2C.

Figure 2. MIC (µg/ml) values segmentation by BMD and VITEK2C techniques.

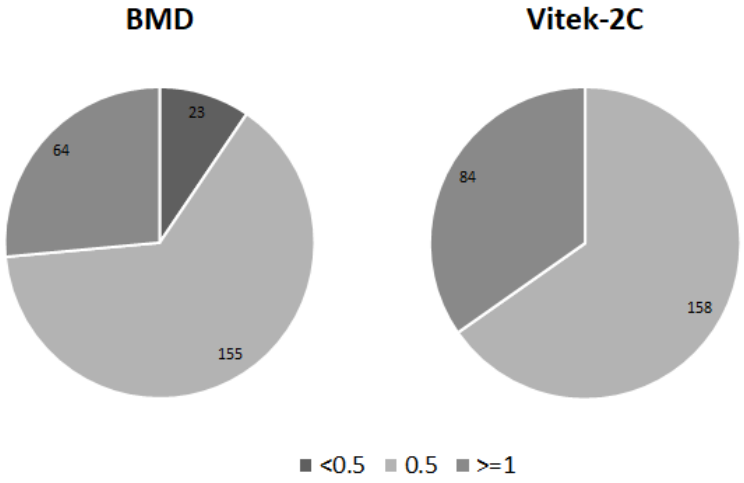



Table 3 provides the t-test outcome of two populations for mean testing. The null hypothesis (Ho) is defined as "zero mean difference" between the MIC values determined by BMD and VITEK2C populations, implying that both techniques produced similar results. The computed *p-value* for two tails is 0.00000494, which is less than the threshold critical value of 0.05 at 95% confidence interval. This implies that the null hypothesis (Ho) can be rejected, or alternative hypothesis ($H_a$) can be accepted that implies significant difference between the two populations of MIC values generated by two techniques.

Table 3. T-test statistics for comparing the mean of MIC values population obtained from BMD and VITEK2C method.

| **t-Test: Paired Two Sample for Means** | | |
|---|---|---|
| | *MIC OF COLISTIN BY BMD* | *MIC OF COLISTIN BY VITEK2C* |
| Mean | 1.410124 | 1.859504 |
| Variance | 9.688725 | 13.35985 |
| Observations | 166 | 166 |

| | | |
|---|---|---|
| Pearson Correlation | 0.914584 | |
| Hypothesized Mean Difference | 0 | |
| df | 241 | |
| t Stat | -4.67313 | |
| P(T<=t) one-tail | 2.47E-06 | |
| t Critical one-tail | 1.651201 | |
| P(T<=t) two-tail | 4.94E-06 | |
| t Critical two-tail | 1.969856 | |

Table 4 categorizes the data based on the patient's ward location. The majority of patients in the given data were admitted in the ICU which comprised of 41% of total, while medical ward and surgical ward comprised of 31% and 28.31% respectively.

Table 4. Ward distribution of Intermediate and resistant patients based on MIC values to colistin using BMD and VITEK-2C method.

| Location | Total | BMD | | Vitek-2C | |
|---|---|---|---|---|---|
| | | Intermediate | Resistant | Intermediate | Resistant |
| ICU | 68 | 60 | 8 | 58 | 10 |
| Medical Ward | 51 | 44 | 7 | 44 | 7 |
| Surgical Ward | 47 | 41 | 6 | 41 | 6 |

Table 5 shows the data for other drugs used in this study, includes Amikacin, Tigecycline, and others; based on MIC values as: sensitive, intermediate, and resistant. As shown in the table 6, Tigecycline impacted as the most sensitive drug to the patients (77%), while all other drugs had minimum efficacy with resistance from the bacteria (>80%). The data for all remaining drugs were sufficiently considerable, and the observation was extremely noticeable. As, in Piperacillin/Tazobactam, the percentage of resistant samples is relatively high, implying that it has a lesser efficacy, while Tigecycline has a large percentage of sensitive samples, confirming its highest efficacy.

Table 5. MIC based classification across several drugs applied on the sample.

| Drug | Total | Sensitive | Intermediate | Resistance |
|---|---|---|---|---|
| AMIKACIN | 125 | 25(20%) | 39(29.6%) | 61(36.74%) |
| GENTAMICIN | 166 | 21(12.6%) | 3(1.80%) | 142(85.54%) |
| CIPROFLOXACIN | 166 | 2(1,20%) | 1(1.51%) | 163 (98%) |
| TRIMETHOPRIM/SULFAMETHOXAZOLE | 99 | 16(12.12%) | 0(0%) | 83 (87.8%) |

| | | | | |
|---|---|---|---|---|
| CEFUROXIME | 166 | 2(1.20%) | 7(4.21%) | 157 (94.57%) |
| CEFUROXIME AXETIL | 166 | 1(0.60%) | 3(1.80%) | 162 (97.59%) |
| CEFTRIAXONE | 166 | 0(0%) | 0(0%) | 166 (100%) |
| CEFOPERAZONE/SULBACTAM | 166 | 8(4.81%) | 3(1.80%) | 155 (93.37%) |
| CEFEPIME | 166 | 0(0%) | 0(0%) | 166 (100%) |
| AMOXYCLAV | 166 | 0(0%) | 1(0.60%) | 165 (99.4%) |
| PIPERACILLIN/TAZOBACTAM | 166 | 1(0.60%) | 0(0%) | 165 (99.4%) |
| IMIPENEM | 166 | 0(0%) | 0(0%) | 166(100%) |
| MEROPENEM | 166 | 0(0%) | 0(0%) | 166 (100%) |
| TIGECYCLINE | 166 | 128(77%) | 0(0%) | 38 (22%) |

## Discussion

Carbapenem resistant pathogens are a major problem in ICU patients and *Klebsiella pneumoniae* remains the most frequently encountered etiological agent.[5]

Colistin, remains the last option of antimicrobial agent, specifically in the present troublesome therapeutic situation for treatment of multi-drug and pan drug-resistant gram-negative infections. Colistin molecule acts on outer cell membrane of Gram-negative cell wall and displaces the divalent cations of calcium and magnesium. This as a result causes disruption of cell membrane leading to outflow of cellular constituents, causing cellular lysis and finally a bactericidal effect. Within years after the repetitive use of this drug, colistin-resistant strains were reported.[14] Trends towards raised colistin MICs have been observed globally emphasizing the importance of accuracy of colistin susceptibility outcomes.[4]

In this study, we assessed the efficacy of colistin susceptibility techniques against carbapenem-resistant *K. pneumoniae* using automated VITEK2C method with gold standard BMD. In a study done by Shaheen shaikh *et al*, found that Carbapenem resistant *Klebsiella pneumoniae* (28.88%) was most common isolate followed by *E. coli* (18.88%).[15] Here, this study shows that urine forms the bulk of the sample with CR*Kp* causing UTI comprising about 31.32%. Zilberberg *et al*. did a study on carbapenem resistant *Enterobacteriaceae* which showed similar results of UTI where most common isolate was *Klebsiella pneumoniae*.[16] Present study shows, carbapenem resistance was more common from ICU's (41%) followed by medical (31%) and surgical ward (28.31%). Similar findings were seen in a study by Dizbay *et al*, the authors studied healthcare-associated infections formed by CR*Kp* in ICU and their risk factors where they found carbapenem resistance is considerably high in ICU set-ups and more common (78.57%), and resistance was more in respiratory specimens.[17]

In the current study, Colistin showed 147 and 145 intermediate cases by BMD and VITEK 2C out of 166 isolates that implied 89% and 87% sensitivity to colistin. However, colistin resistance was 11% by BMD and 13% by VITEK2C and the MIC was between 4 and 16ug/ml which was more common in ICUs. We found that both the methods were statistically different from one another regarding MIC calculation and BMD as more sensitive to calculate lower MIC values compared to VITEK2C.

We also studied the range of other drugs in addition to colistin and found tigecycline produced only 23% resistance. A study by Taneja *et al*.[18] from North India found that 16% of the CR*kp* isolates were resistant to both tigecycline and colistin. Giani *et al*.[19] conducted a cross-sectional survey on CRE in Italy 2011, where they found 22.4% of the KPC producing *Klebsiella pneumoniae* isolates were resistant to colistin. Another study done by

Capone *et al*. in Italy observed 36.1% of the isolates were resistant to colistin which was different from our study.[20] Bhaskar *et al* found 27% of the CR*Kp* were resistant to colistin.[21]

With high emergence and spread of CR*Kp*, management options are declining. Due to rise of multi-drug resistance organisms, colistin has been irrationally used over the years leading to slow development of colistin resistance. The high rate of resistance to carbapenems and colistin is worrying, but accurate and reliable MIC of colistin and other carbapenems will help the physician to choose the drug in appropriate combinations.[5,6,21]

## Conclusion

Broth Microdilution (BMD) technique remains the gold standard technique for defining the colistin minimum inhibitory concentration. The results of Vitek2C when compared with BMD except for 2 % of isolates which revealed minor errors, this specifies that resistance to colistin by any commercial methods or automation like Vitek, BMD must be used for correlation and to recheck the result. Therefore, all clinical laboratories must be aware of those inconsistencies regarding MIC testing and consider applying a reference method for colistin susceptibility. The current study also showed slow emergence of colistin resistance among CR*kp* isolates which are upsetting in ICUs. For those resistant cases, it's better to use colistin in combination therapy with other antimicrobials. Apart that, necessary infection control safety measures and increased awareness should be done to prevent further rise in the drug resistance against this last resort of antimicrobial is important. A limited and rational use of colistin is the need of hour.

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