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Research Article

# COLISTIN RESISTANCE IN CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* STRAINS

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**ABSTRACT**

**Objective:** There is an increasing use of colistin consequent to increase in the infections caused by carbapenem-resistant *Klebsiella pneumoniae.* The present study was conducted to determine the minimum inhibitory concentration (MIC) of colistin and the resistance pattern of colistin in carbapenem-resistant *K. pneumoniae* (CRKP) strains in our intensive care unit (ICU).

**Methods:** Antibiotic susceptibility testing for other antimicrobial agents was done by Kirby-Bauer disk diffusion method. MIC of colistin was determined by agar dilution method. The results of antibiotic susceptibility testing were interpreted as per Clinical Laboratory Standard Institute guidelines 2016 and MIC of colistin were interpreted as per European Committee on Antimicrobial susceptibility testing. The carbapenem resistance was phenotypically detected by modified hodge test and imipenem/imipenem ethylenediaminetetraacetic acid disk method.

**Results:** Out of 518 *K. pneumoniae*, 329 were resistant to carbapenems, and 91 isolates showed resistance to colistin. The MIC of colistin ranged between 4 and >512 ug/ml and $MIC_{90}$ was 16 ug/L and $MIC_{50}$ was 4 ug/ml. A majority of the colistin-resistant isolates were found in multidisciplinary ICU (85/91).

**Conclusion:** The emergence of colistin-resistant strains is a major problem due to limited treatment options for infections caused by CRKP carbapenemase producing *K. pneumoniae*. Colistin should not be used alone, combination therapy should be preferred.

**Keywords:** Colistin, Carbapenemase producing *Klebsiella pneumoniae*, Minimum inhibitory concentration of colistin.

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## INTRODUCTION

*Enterobacteriaceae* are one of the major agents of hospital and community-acquired infections; these cause increased morbidity and mortality, especially with increased resistance rates [1]. Extended spectrum betalactamases (ESBLs) lead to multidrug resistance by transfer between bacteria and are seen in *Enterobacteriaceae* [2]. Carbapenems are important group of antibiotics used as a last option, especially in ESBL producing multi-drug resistant *Enterobacteriaceae* [3]. As a result of increased use of carbapenems, carbapenemases are widespread in *Enterobacteriaceae* family and particularly in *Klebsiella pneumoniae;* so effective treatment options are decreasing [2,4]. Carbapenemase-producing *K. pneumoniae* emerged in the late 1990s and has become a serious health problem worldwide [5].

Carbapenem-resistant *K. pneumoniae* (CRKP) can cause nosocomial infections and outbreaks with high mortality rates. Such infections occur mainly in patients admitted to intensive care units (ICU) with several underlying diseases and histories of having received prolonged courses of antibiotics [6]. Several resistance mechanisms have been described. Carbapenemase production (*K. pneumoniae* carbapenemase [KPC]), metallo-β-lactamase (MBL) and porin loss, combined with the overproduction of ESBL, are described as the most common mechanisms of carbapenem resistance [7,8].

Carbapenemases producing *K. pneumoniae* strains are considered endemic in some areas. For example, studies by the European Antimicrobial Resistance Surveillance (EARS-net) showed that the prevalence of carbapenem-resistant *K. pneumoniae* has increased from 1-2% to 15% in Italy in 2006-2009 [9]. Due to this resistance spreading rapidly around the world, there has been a need for new therapeutic agents. Polymyxin and colistin were out of use since the early 1970s due to its side effects of neurotoxicity and nephrotoxicity, but colistin has become a preferred antimicrobial agent with increase of infections with resistant *Enterobacteriaceae* [10-12]. However, excessive use of colistin has led to resistance to this drug [13]. The resistance to colistin seen in *K. pneumoniae* strains is reported to be due to reduced affinity of colistin to lipopolysaccharide target [5]. In this study, colistin resistance in CRKP strains has been identified and evaluated.

Since there are not many studies with determination of colistin minimum inhibitory concentration (MIC), we undertook this study to estimate and evaluate colistin resistance in our hospital by MIC.

## MATERIALS AND METHODS

The present study was carried out in a Tertiary Care Hospital of Karnataka, South India, with bed strength of 650. A total of 518 non-repetitive clinical isolates of *K. pneumoniae* were collected over a period of 3 years (2014-2016) from our ICUs, i.e. multidisciplinary ICU (MICU), neurosurgery ICU, intensive thoracic unit, neonatal ICU, pediatric ICU, coronary care unit, and renal ICU. These isolates were obtained from endotracheal aspirate (111), blood (80), urine (59), pus (22), bronchoalveolar lavage and sputum (21), catheter tips (15), fluids (13), and tissue specimens (8).

### *K. pneumoniae* was identified using standard biochemical methods

Disk-diffusion tests were performed to determine susceptibility to antimicrobials, according to the Clinical and Laboratory Standards Institute (CLSI). *Escherichia coli* ATCC 25922 was used as a quality control. Isolates were screened for the ESBL phenotype by the standard double-disk synergy test, and KPC was screened both by evaluating breakpoints and using modified Hodge test, according to CLSI guidelines [14].

Imipenem plus ethylenediaminetetraacetic acid (EDTA) discs were used to detect the presence of MBL (HiMedia Laboratories, Mumbai). MICs of meropenem and colistin (Sigma-Aldrich Corporation, St. Louis, US) were determined by the agar dilution method according to the guidelines from the CLSI [14]. The colistin breakpoint was evaluated using breakpoints for *Enterobacteriaceae* recommended by the European Committee on Antibiotic Susceptibility Testing. (resistant: >2 ug/ml; sensitive: ≤2 ug/ml). *K. pneumoniae* ATCC 700603 was used as a quality control.

## RESULTS

Totally 518 isolates of *K. pneumoniae* were isolated during the study, of which 329 (63.5%) isolates were CRKP isolates. The clinical source and distribution of *K. pneumoniae* in different ICUs are shown in Tables 1 and 2.

The antibiotic susceptibility of CRKP to other drugs is shown in Table 3. Antibiotic susceptibility testing revealed that very few antibiotics were susceptible.

With regard to colistin 91/329 (27.65%), isolates were resistant and the MIC ranged between 4 and >512 ug/ml. $MIC_{90}$ and $MIC_{50}$ of colistin were 16 and 4 ug/ml (Fig. 1).

In the present study, we found that 85/91 (93.4%) isolates that showed resistance to colistin were from MICU and resistance to carbapenems also seen in MICU (83.3%).

**Table 1: Clinical sources of carbapenem-resistant *Klebsiella pneumoniae* (329)**

| Clinical specimen | N (%) |
|---|---|
| Tracheal aspirate | 111 (33.73) |
| Blood | 80 (24.3) |
| Urine | 59 (17.9) |
| Pus | 22 (6.7) |
| BAL and sputum | 21 (6.4) |
| Catheter tips | 15 (4.5) |
| Fluids | 13 (3.95) |
| Tissue | 8 (2.4) |

**Table 2: Distribution of CRKP in ICUs**

| ICU | N (%) |
|---|---|
| MICU | 274 (83.3) |
| NSICU | 25 (7.9) |
| ITU | 11 (3.34) |
| NICU | 8 (2.43) |
| PICU | 5 (1.5) |
| CCU | 3 (0.91) |
| RICU | 2 (0.60) |

ICU: Intensive care unit, MICU: Multidisciplinary intensive care unit, NSICU: Neurosurgery intensive care unit, ITU: Intensive thoracic unit, NICU: Neonatal intensive care unit, PICU: Pediatric intensive care unit, CCU: Critical care unit, RICU: Renal intensive care unit, CRKP: Carbapenem-resistant *K. pneumoniae*

**Table 3: Antibiotic susceptibility pattern of CRKP* (n=329)**

| Antibiotic | CRKP (%) susceptible |
|---|---|
| Cotrimoxazole | 22 (6.6) |
| Gentamicin | 58 (17.6) |
| Amikacin | 59 (17.9) |
| Netilimicin | 42 (12.7) |
| Tobramicin | 19 (5.7) |
| Ciprofloxacin | 9 (2.7) |
| Ofloxacin | 10 (3.0) |
| Levofloxacin | 22 (6.6) |

*All cephalosporins, betalacatamase, and betalactamase inhibitors were resistant. CRKP: Carbapenem-resistant *Klebsiella pneumoniae*

Out of 329 carbapenem-resistant isolates, 216 were positive for modified-Hodge test and 59 *K. pneumoniae* were screened positive for imipenem/imipennem EDTA test. The rest of the 54 carbapenem-resistant isolates were negative for both tests.

## DISCUSSION

*K. pneumoniae* is highly prevalent in hospitals and causes many nosocomial infections. The emergence of drug resistance in *K. pneumoniae* continues to be of critical concern for the choice of treatment options against infections caused by this bacterium.

Carbapenem resistance in *Klebsiella* spp. is an emerging problem and is a cause of concern as many nosocomial *Klebsiella* spp. are detected to be resistant to most other antibiotics.

Colistin is used as last resort of antimicrobials, especially in the present worrisome therapeutic scenario of multidrug resistant and pan drug-resistant Gram-negative infections. The drug acts on outer cell membrane of Gram-negative bacteria (GNB) and releases lipopolysaccharides [15]. This in turn results into disruption of cell membrane leading to leakage of cell content, causing cell lysis and finally cell death [16,17]. Within years after the reuse of colistin, there have been reports of colistin-resistant strains [18]. Indiscriminate antibiotic use in India is leading to cases of bacteria resistant to colistin.

ICUs are the epicenter for spawning multidrug resistance within hospitals. Many patients are transferred to the ICU from other healthcare facilities, where they have acquired resistant pathogens. Patients within the ICU undergo invasive procedures, treatment with antibiotic.

Combinations and exposure to other patients with resistant pathogens [19]. Multiple mechanisms exist for ICU pathogens to acquire antibiotic resistance. These mechanisms include enzymatic inhibition of drugs, alteration of proteins targeted by antibiotics, changes in metabolic pathways, antibiotic efflux, alterations in porin channels, and changes of membrane permeability [20].

In the present study, we found 63.5% (329/518) *K. pneumoniae* was multidrug resistant. The majority of the carbapenem resistant isolates were isolated form tracheal aspirate (33.73%), followed by blood (24.3%) and urine (17.9%). Gupta *et al*. found resistance to meropenem (22.16%) was more compared to imipenem (17.32%) in his study from different bacteria.

He also found that a significantly high resistance was seen in ICU patients, 37.3% and 31.9% for meropenem and imipenem, respectively [21]. El-Mahdy *et al.* in his work on cancer patients found only 4% of them were resistant to carbapenems [22]. Bashir *et al*. in his research study found 27.9% isolates were carbapenem-resistant [23].

Dizbay *et al*. [24] analyzed nosocomial infections produced by carbapenem-resistant *Klebsiella* spp. in ICU and their risk factors. They found that carbapenem resistance is significantly high in ICUs and more

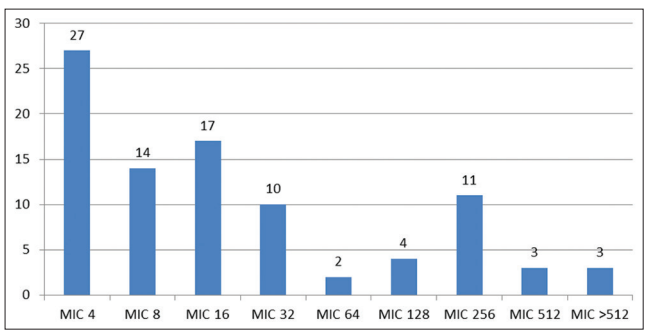


**Fig. 1: Minimum inhibitory concentration (MIC) of colistin. MIC90 – 16 ug/ml and MIC50 – 4 ug/ml**

frequent (78.57%), and resistance was more often seen in respiratory tract specimens.

In the current study, all the isolates were screened for MIC of colistin. The prevalence of colistin resistance in *K. pneumoniae* from ICUs was (91/329) 27.65%, and the MIC ranged between 4 and >512 ug/ml.

Taneja *et al*. in a study from North India found 16% of the carbapenem-resistant strains were resistant to both tigecycline and colistin [25]. Giani *et al*. in Italy conducted a nationwide cross-sectional survey on CRE, carried out in mid-2011, the overall percentage of colistin resistance among KPC-KP was found to be 22.4% [26]. In another study in Italy Capone *et al*. observed 36.1% resistance to colistin in CRKP [18]. In a recent study by Kontopidou *et al*., 51 (34%) patients were colonized by pathogens with an intrinsic resistance to colistin [13].

Ghafur *et al*. reported a series of 13 patients with colistin resistance from South India [27].

With the increased emergence and spread of CRKP, treatment options are dcecreasing. As a reserve agent, colistin has been the drug of choice in the treatment of CRKP, but with the rise of carbapenem resistance, the colistin usage has been increased over the years leading to slow emergence of colistin resistance. Especially, if these antibiotics are not used in combinations, they are not enough as therapeutic agent's leads to treatment failure and high mortality rates. The high rate of resistance to colistin and carbapenems is worrisome, but the MIC of colistin and carbapenems helps the clinician to choose the drug in appropriate combinations.

Shah *et al*. conducted a study in a tertiary care hospital in Mumbai, the purpose of this study was to evaluate the efficacy of colistin-carbapenem combination against Carbapenem-resistant GNB (CRGNB) infection in a clinical study and an *in vitro* synergy study using Etest. Overall, 60.6% clinical success was observed in patients receiving colistin-carbapenem combination against CRGNB infection [28].

Tumbarello *et al*. [29] in his study emphasized the importance of combination therapy in carbapenemase-producing *Klebsiella pneumonia*. Combination therapy with tigecycline, colistin, and meropenem (MIC upto 8 ug/ml) was associated with lower mortality.

In another study by Daikos *et al*. [30] stated that combination therapy with carbapenem MIC of 8 was more beneficiary in severely ill patients by lowering the mortality rate.

## CONCLUSION

The present study showed the emergence of colistin resistance among clinical isolates of *K. pneumoniae* which are alarming in ICUs. It is important to determine the MIC of colistin to formulate an effective treatment protocol, even for the resistant isolates. For the resistant cases, colistin should be used in combination with other antimicrobials for therapy. Necessary infection control precautions and increased awareness to prevent further rise in the drug resistance against this last resort of antimicrobials is important. A restricted and rational use of the colistin is the need of hour.

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