International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 8, August 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.8.2024.118

**ORIGINAL RESEARCH**

# Study of efflux pump activity in klebsiella species

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Received Date: 17 July, 2024 Accepted Date: 23 August, 2024

## ABSTRACT
Klebsiella species isan emerging concern for the worldwide as it is one of the major causes of drug resistant. In infection with Klebsiella species, the fluoroquinolones are considered safe and adequate therapeutic option but several studies reported an increasing percentage resistant to these antimicrobials due to efflux pump activity which made these strains very difficult to treat.Objective of the study is to study the efflux pump activity in ciprofloxacin resistant isolates of Klebsiella species.A prospective and experimental study was carried out over a period of three years. A total of 200 isolates of Klebsiella species isolated from various clinical samples and confirmed by conventional biochemical tests. Antimicrobial susceptibility testing was done as per CLSI 2010 guidelines to determine ciprofloxacin resistance. The resistant strains were subjected to detection of efflux pump by inhibition of efflux pump activity with the use of Carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Minimum inhibitory concentrations (MICs) of all resistant strains to ciprofloxacin were measured with and without use of CCCP.71 out of 200 isolates of Klebsiella species were resistant to ciprofloxacin. Among these 71 isolates; 31 (43.66%) showed 2-32 fold reduction in the MICs value after use of CCCP as an efflux pump inhibitor. The other 40 remaining strains showed no reduction in their MICs value before or after use of CCCP.Efflux pump has a crucial role and it is one of the major causes of ciprofloxacin resistant in Klebsiella species. Routinely detecting the efflux pump and restricting the use of ciprofloxacin in such strains will certainly limits the development of further resistance as well as it also help clinicians to prescribe proper antibiotics to manage infections.

**Key words:** -Klebsiella species, Carbonyl cyanide 3-chlorophenylhydrazone, Ciprofloxacin, Efflux pump, Minimum inhibitory concentration.



## INTRODUCTION
Klebsiella is a gram-negative bacterium which is an opportunistic pathogen responsible for causing hospital & community acquired infections[(1)]. Klebsiella pneumoniae isan emerging concern for the worldwide as it is one of the major causes of drug resistant which are extremely difficult to eradicate using available antibiotics[(2-4)]. The extensive use of broad-spectrum antibiotics in patients has led to both increased carriage of Klebsiella and the development of multi-drug resistant (MDR) strains.

Bacteria can lead to antibiotic resistance through different mechanisms in which one of the major mechanisms is efflux pump. Antimicrobial compounds are pumped out of the cell through membrane spanning efflux pumps, resulting into lower internal concentration below the levels which are toxic to the bacteria. Due the activity of efflux pump activity, this helps and allows the bacterium to cope & survive even in the presence of low concentration of antibiotics. This results in to the delayed death of the bacterium due to increase exposure time to the antibiotics. Due to such phenomena, the bacterium may undergo mutations in order to achieve higher levels of resistance towards the used antibiotic [(5,6)].

Efflux pumps are increasing and one of the leading causes of antibiotic resistance within numerous clinically relevant bacterial species including K. pneumoniae[(7;8)]. The presence of efflux is often gauged in vitroby the use of efflux pump inhibitors (EPIs) such as reserpine, carbonyl cyanide 3-

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chlorophenyl hydrazone (CCCP) and phenylalanine-arginine beta-naphthylamide (PABN). When these inhibitors are used with the relevant antibiotics then it result in to increased susceptibilities, when compared to the antibiotic alone, in the presence of active efflux. However EPIs are only used to identify the presence of efflux pump activity in the laboratory only but it cannot be used clinically for treatment purpose due to their toxicity and potential to interfere with other cellular functions [(9)].

Fluoroquinolones are considered safe and adequate therapeutic option; however several studies reported an increasing percentage of Klebsiella strains are resistant to these antimicrobials due to efflux pump which made these strains very difficult to treat [(10-13)]. Finally we also decided to study the role of efflux pump in fluoroquinoles resistant strains of clinically isolated Klebsiellaspecies.

## MATERIALS & METHODS

A prospective and experimental study was carried out over a period of three years (August 2013 to July 2016) in microbiology laboratory of MGM Medical College & Hospital Kamothe, Navi Mumbai, Maharashtra, India. The research topic was cleared by Ethical committee for research on Human subjects on 27$^{th}$October 2014 via letter no. MGM/HIS/RS/2014-15. Informed consents were taken from the patients before collection of the samples. Inclusion criteria: Sample showing pus cells and bacteria were included in the study. Exclusion criteria: Sample not showing pus cells and bacteria were excluded from the study. Out of 4440 samples, a total of 200 isolates of Klebsiella species were isolated from different clinical specimens which were characterized into different species by standard protocols[(14)]**.** The isolates were tested for their susceptibility to the ciprofloxacin using standard disc diffusion method. Out of 200 isolates, 71 were found ciprofloxacin resistant which were subjected for the detection of efflux pump activity. 0.5 McFarland standard of Ciprofloxacin resistant Klebsiella strain was lawn on the Muller Hinton Agar with & without containing carbonyl cyanide 3-chlorophenylhydrazone (CCCP) at a concentration of 25µg/ml. A ciprofloxacin E-strip (0.16 to 256µg/ml) were placed on both plate and incubated at 37$^0$C for 24 hrs. Reduction in the MIC value of MHA containing CCCP from MHA alone, i.e. ≥2 means positive for efflux pump activity **[(15)]**.

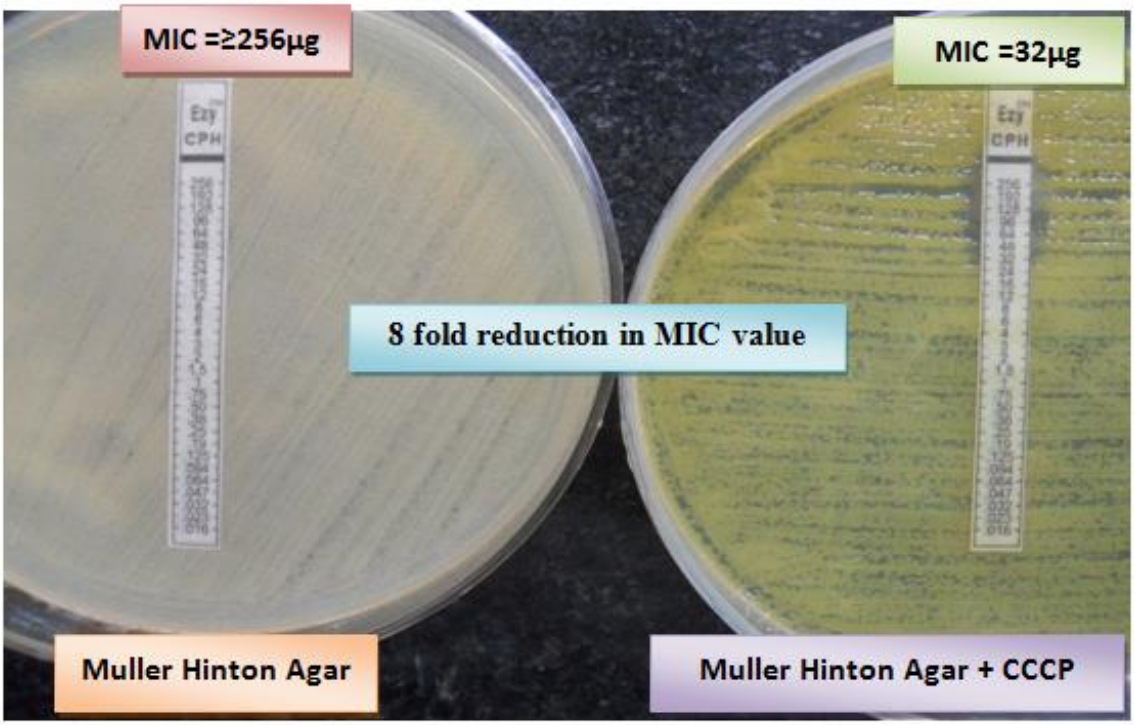


**Fig:-1 showing inhibition of efflux pump activity by CCCP**

## RESULT

Out of 1722 positive bacterial growth, 200 isolates of Klebsiella species were isolated from clinical samples with incidence rate of 11.6%. Among which 112 (56%) and 88 (44%) strains were from male and female respectively with the male female ratio 1.27:1. Out of 200, 24% (n=48) of the cases were from Out Patient Department (OPD) while remaining 76% (n=52) from the In Patient Department (IPD). Out of 200 isolates of Klebsiella, 135 isolates were resistant to ciprofloxacin.Among 71 isolates, 31 (43.66%) showed reduction in the MICs value after use of CCCP.

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| Table 1: EFFLUX PUMP ACTIVITY | | | | | |
|---|---|---|---|---|---|
| | | **Fold Reduction in MICs** | **No. of isolates** | **MIC of Ciprofloxacin (µg/ml)** | **MIC of Ciprofloxacin after addition of 25 µg/ml CCCP** |
| **Positive** | 31 (43.66%) | 2 Fold | 9 | 8 | 4 |
| | | | 6 | 16 | 8 |
| | | | 2 | 32 | 16 |
| | | | 2 | 48 | 24 |
| | | 4 Fold | 1 | 96 | 24 |
| | | | 2 | 32 | 8 |
| | | | 5 | 16 | 4 |
| | | 8 Fold | 1 | 256 | 32 |
| | | | 1 | 128 | 16 |
| | | 16 Fold | 1 | 256 | 16 |
| | | 32 Fold | 1 | 256 | 8 |
| **Negative** | 40 (56.34%) | 0 Fold | 17 | 256 | 256 |
| | | | 4 | 128 | 128 |
| | | | 1 | 96 | 96 |
| | | | 2 | 64 | 64 |
| | | | 2 | 48 | 48 |
| | | | 3 | 24 | 24 |
| | | | 4 | 16 | 16 |
| | | | 2 | 12 | 12 |
| | | | 5 | 8 | 8 |
| Total | 71 (100%) | | | | |

**Table 2: Sample wise distribution of Efflux pump activity**

| S.No. | Sample | Total | Ciprofloxacin (CIP) Resistant | Positive |
|---|---|---|---|---|
| 1 | Vaginal swabs | 2 | 1 | 1 (100%) |
| 2 | Blood | 17 | 3 | 2 (66.7%) |
| 3 | Pus | 37 | 23 | 12 (52.2%) |
| 4 | Accessory devices | 6 | 2 | 1 (50%) |
| 5 | Sputum | 51 | 9 | 4 (44.4%) |
| 6 | Urine | 53 | 17 | 6 (35.3%) |
| 7 | Endotracheal aspirate | 25 | 15 | 5 (33.3%) |
| 8 | Stool | 4 | 1 | 0 |
| 9 | Throat swabs | 5 | 0 | 0 |
| Total | | 200 | 71 (35.5%) | 31 (43.66%) |

**Table 3: Species wise distribution Efflux pump activity**

| S.No. | Species of Klebsiella | No. of isolates | Ciprofloxacin Resistant | Positive |
|---|---|---|---|---|
| 1 | Klebsiellapneumoniaepneumoniae | 169 | 61 | 29 (47.54%) |
| 2 | KlebsiellapneumoniaeOzaenae | 7 | 1 | 0 |
| 3 | Klebsiellaoxytoca | 24 | 9 | 2 (22.22%) |
| Total | | 200 | 71 | 31 (43.66%) |

## DISCUSSION

Increasing resistance to fluoroquinolones has become a serious concern among pathogens causing nosocomial infections. Due to resistance of these antibiotics causes treatment failure which leads to increase hospital stay and treatment costs. One of the major causes of fluoroquinolones resistance is due to efflux pump. For this, different types of inhibitors can be used like Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) or phenylalanine arginine β-naphthylamide (PABN). To understand the role of efflux pump which may impart resistance or decreased susceptibility to ciprofloxacin, we carried out the present study which was isolated from different clinical specimens.

Our findings are similar to some researcher's, other reported higher values than ours. This variation could be attributed to differences in the biological behaviour or genetic expression of the bacterium, antibiotic prescription pattern and protocols in various places. All these factors are responsible for development of resistance in the bacteria. The results of our study in reduction of MICs after using CCCP are consistent with other studies. Our study is similar to Iraj Pakzad

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et.al. from Iran [10] (47.5%). Higher values were shown by Hai-quin Zhong et.al. from China [16] (100%), Annarita et.al. from Italy [17] (100%) followed by S. Aathithan et.al. from UK [18] (92.31%) and El-Naggar et.al. from Egypt [19] (71.43%).

## CONCLUSIONS

Efflux pump has a crucial role and it is one of the major causes of ciprofloxacin (fluoroquinolones) resistant. It was detected phenotypically in strains of Klebsiella species isolated from various clinical samples. Routinely detecting the efflux pump in isolated strains of Klebsiella species and restricting the use of fluoroquinolones in such strains will certainly limits the development of further resistance toward these antibiotics. So, it is important to continuously monitor resistance pattern and enhancing the infection control for these strains in health care units. It is also important to report efflux pump producers on routine basis which will help clinicians to prescribe proper antibiotics to manage infections caused by them.

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