



## RESEARCH ARTICLE

### COMPARATIVE EVALUATION OF COLISTIN SUSCEPTIBILITY TESTING BY DISK DIFFUSION AND ETEST AMONG CLINICAL ISOLATES OF CARBAPENAM RESISTANT *PSEUDOMONAS AERUGINOSA*

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#### ABSTRACT

Colistin is a polymyxin with bactericidal activity against most strains of *Pseudomonas aeruginosa*. The recent increase in multidrug-resistant strains of *P. aeruginosa* has prompted renewed interest in the use of colistin. Rapid and reliable colistin susceptibility testing (ST) is needed in routine clinical laboratories to allow appropriate therapeutic decision-making. The present study aimed to study the efficacy and reliability of disc diffusion method to test the susceptibility of colistin. **Materials and Methods:** A total of 50 clinical isolates of carbapenem non-susceptible *Pseudomonas aeruginosa* isolated from various clinical samples was identified using standard microbiological procedures. Antimicrobial susceptibility was tested by means of agar disc diffusion and E-test method.

**Results:** Around 82% isolates showed the sensitivity zone between 11-15mm (sensitive). The comparison between the Colistin Disks and E-strip showed 6 isolates to be sensitive by E-Strip method but found to be resistant by colistin disk method. Discussion: In this study most cases were found to be sensitive by both methods (86%). Nevertheless, 7 cases (14%) were resistant to the Colistin disk method. These were further tested for the Etest and 6/7 were found to be sensitive by the Etest.

**Conclusion:** The above study showed the disk diffusion method to be equally efficacious as the Etest with 86% of the isolates showing susceptibility by both the methods.

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## INTRODUCTION

Colistin is a polymyxin with bactericidal activity against most strains of *Pseudomonas aeruginosa*. Therapeutic use of parenteral colistin was minimal due to concerns about the high incidence of side effects, notably nephrotoxicity. The recent increase in multidrug-resistant strains of *P. aeruginosa* has prompted renewed interest in the use of colistin (WanutasanunTunyapanit, 2013). Rapid and reliable colistin susceptibility testing (ST) is needed in routine clinical laboratories to allow appropriate therapeutic decision-making. Disk diffusion, commonly used in many clinical laboratories, yielded high error rates compared to MIC-based methods and is considered unreliable for the detection of colistin resistance (Ali, 2010). Among commercial methods, gradient diffusion strips are convenient tests for determining colistin MICs, but their performance is not well established (Janet, 2013). Therefore, the study is undertaken to compare the resistance for colistin among carbapenam resistant of *Pseudomonas aeruginosa*.

## Objectives

- To study efficacy and reliability of disc diffusion method to test the susceptibility of colistin.
- To study minimal inhibitory concentration (MIC) of colistin using E-strips.

## MATERIALS AND METHODS

### Source of Data

The study will be conducted in the department of Microbiology, Victoria hospital. Various clinical samples sent to department of Microbiology, Victoria hospital will be included.

### Method of collection of data

### Design of study

It is a comparative study of *Pseudomonas aeruginosa* isolates from clinical samples.

**Study period:** From October 2016 to November 2016.

**Place of study:** Department of Microbiology, Victoria hospital, Bangalore, Karnataka, India.

**Sample size:** 50 isolates of carbapenam non-susceptible *Pseudomonas aeruginosa*.

**Methodology**

**Sample Collection and Processing**

A total of 50 clinical isolates of carbapenamnon-susceptible *Pseudomonas aeruginosa* isolated from various clinical samples are included in the study. The samples was processed by inoculating onto the conventional media and later isolates was identified using standard microbiological procedures (Mackie, 2015). Antimicrobial susceptibility was tested by means of agar disc diffusion method of Kirby Bauer according to the clinical and laboratory standards institute (2015) guidelines (Clinical And Laboratory Standards Institute, 2015). All clinical isolates of carbapenamnon-susceptible *Pseudomonas aeruginosa* were tested for colistin MIC (0.016-256µg/ml) by E-test method.

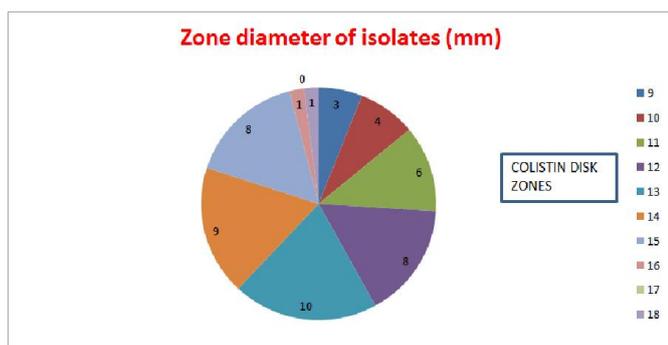
**Quality Control:** *Escherichia coli* ATCC 25922, *Klebsiellapneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853 was used as control strains throughout the study.

**RESULTS**

The most isolates (20%) showed the disk zones of 13mm (sensitive), whereas the least commonly seen was 16 &18 mm zone sizes, seen in just 2% of the isolate each respectively. Around 82% isolates showed the sensitivity zone between 11-15mm.

**Colistin Disk Zone Sizes**

| Colistin disk zone sizes (mm) | Number of isolates | Percentage of isolates (%) |
|-------------------------------|--------------------|----------------------------|
| 9                             | 3                  | 6                          |
| 10                            | 4                  | 8                          |
| 11                            | 6                  | 12                         |
| 12                            | 8                  | 16                         |
| 13                            | 10                 | 20                         |
| 14                            | 9                  | 18                         |
| 15                            | 8                  | 16                         |
| 16                            | 1                  | 2                          |
| 18                            | 1                  | 2                          |

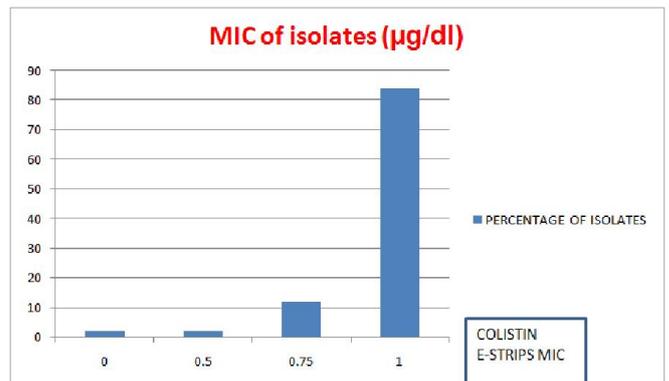


The colistinEtest showed most isolates to have an MIC value of 1.0. Only 1 isolate was found to be completely resistant and showed Zero MIC value. The comparison between the Colistin

Disks and E- strip showed 6 isolates to be sensitive by E-Strip method but found to be resistant by colistin disk method. However, 1 case was found to be resistant on both the study types- colisitn disk and E-strip method.

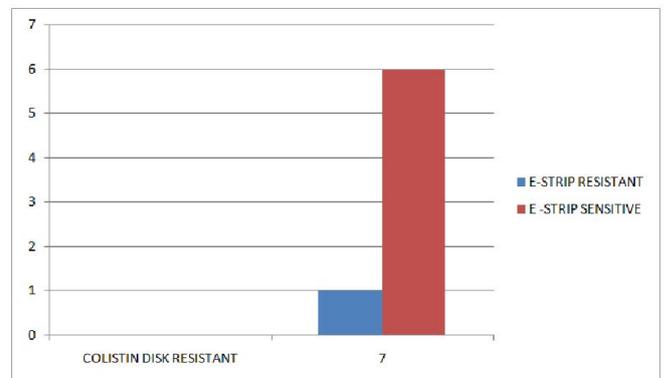
**Colistin e-strips mic**

| Colistin E-Strip mic (µG/DL) | Number of isolates | Percentage of isolates |
|------------------------------|--------------------|------------------------|
| 0                            | 1                  | 2                      |
| 0.50                         | 1                  | 2                      |
| 0.75                         | 6                  | 12                     |
| 1.0                          | 42                 | 84                     |



**Comparison between resistant colistin disk zones vs e-strip mic**

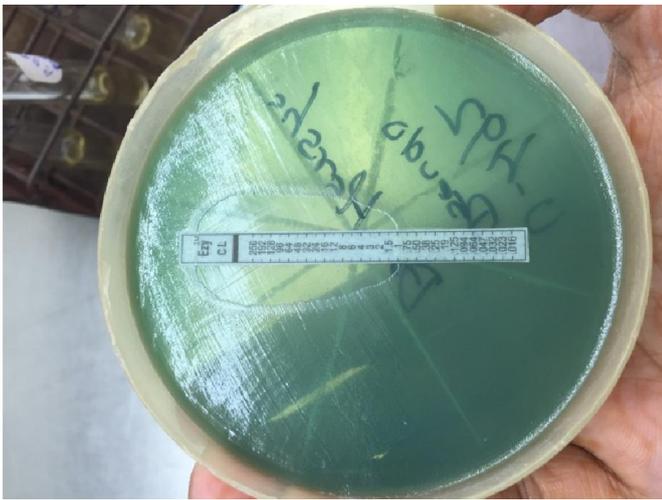
| Isolates with colistin disk zones resistant | E-strip mic resistant | E-strip mic sensitive |
|---|-----------------------|-----------------------|
| 7   | 1                     | 6                     |



**Etest with MIC**



The colistinEtest showed most isolates to have an MIC value of 1.0. Only 1 isolate was found to be completely resistant and showed Zero MIC value. The comparison between the Colistin



Colistin disk diffusion test



Etest – resistant



## DISCUSSION

50 isolates were studied from the various samples processed in the Central Laboratory of the Victoria Hospital, Bangalore Medical College & Research Institute, Bangalore. These samples were found to be carbapenem resistant cases of *Pseudomonasaeruginosa*.

Further, these isolates were tested for Colistin disks and the E-strip. In this study most cases were found to be sensitive by both methods (86%). Nevertheless, 7 cases (14%) were resistant to the Colistin disk method. These were further tested for the Etest and 6/7 were found to be sensitive by the Etest. On the contrary just 1/50 (2%) was resistant to both the above methods. Colistin had good activity against MDR-PA isolates (98% susceptibility rate with low MIC50 and MIC90). Tam *et al* (2010) found 95% of MDR-PA isolates were susceptible to colistin with a MIC50 and MIC90 for colistin of 1.5 and 2 µg/ml, respectively (Tam, 2010). There have been reports of *P. aeruginosa* resistant to colistin. The emergence of colistin resistant *P. aeruginosa* has increased due to the use of colistin in the treatment of *P. aeruginosa* being on the rise (Johansen *et al*, 2008; Tam *et al*, 2010). The Etest is a simple and accurate alternative method for the susceptibility testing of colistin (Behera *et al*. 2010). In our study, we deduced an excellent concordance between disk and Etest methods. There were no false-resistant results by Etest detected.

## Conclusion

The above study showed the disk diffusion method to be equally efficacious as the Etest with 86% of the isolates showing susceptibility by both the methods in detecting the carbapenem resistant cases of *Pseudomonasaeruginosa*. Hence disk diffusion method can be used for Antibiotic susceptibility testing routinely in the labs.

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