Colistin, Polymyxin B and Tigecycline Susceptibility to Metallo Betalactamase Producing Acinetobacter Baumannii Isolated From Tertiary Health Care Hospital

Venkata sarada1,*, Ratna Rao1, Ratna mani1, K V Ramana2

1Department of microbiology, Apollo health city, Jubilee Hils, Hyderabad, Andhra Pradesh, India
2Department of microbiology, Prathima Institute of Medical Sciences, Karimnagar, Andhrapradesh, India
*Corresponding author: venkatasaradaa@yahoo.com

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Abstract Acinetobacter baumannii have emerged as important nosocomial pathogens especially in intensive care patients. Carbapenems are the choice of drug for their treatment but they started developing resistance to carbapenems predominantly by producing Metallo beta-lactamases (MBL). Treatment of such carbapenem resistant Acinetobacter has led to reintroduction of polymyxins like colistin, polymyxin B and newer tetracycline group of drugs like tigecycline. As there were limited studies in on Minimum inhibitory concentrations (MIC) of MBL producing Acinetobacter to polymyxins and tigecyclines from India, the present study was undertaken. MBL detection was done by combined disc test with imipenem and EDTA. MIC of the drugs was determined by Agar dilution method. MIC50 and MIC90 of tigecycline were found to be 0.5 μg/ml and 1 μg/ml respectively. Susceptibility MIC ranges for tigecycline were found to be 0.5 to 2 μg/ml. Resistance rates for tigecycline were found to be 6%. MIC50 and MIC90 of colistin was found to be 2 μg/ml. Susceptibility MIC ranges for colistin were found to be 0.25 to 1 μg/ml. MIC50 and MIC90 of polymyxinB were found to be 1 μg/ml and 2 μg/ml respectively. Susceptibility MIC ranges for polymyxin B were found to be between 0.5 to 2ug/ml. The susceptibility rates with colistin and polymyxin B were found to be 99.2% and 100% respectively. Polymyxin B and colistin are very good drugs of choice for treatment of MBL producing Acinetobacter baumannii with 0.8% and 0% resistance rates. Though good sensitivity was noticed with tigecycline, we found 6% resistance rate.

Keywords: Acinetobacter baumannii, Metallo beta-lactamases(MBL), colistin, polymyxin B, Minimum inhibitory concentrations (MIC), tigecycline

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1. Introduction

Acinetobacter species are opportunistic pathogens causing nosocomial infections especially among patients admitted to intensive care units (ICUs) associated with mortality and morbidity [1]. Carbapenems have remained as drugs of choice for treatment of Acinetobacter infection. Over usage of carbapenems has to development of Metallo beta lactamase (MBL) producing bacteria [4]. Treatment of infections caused by MBL producing Acinetobacter has become difficult as few antimicrobial agents remain active against these pathogens [2,3]. The increased incidence of nosocomial infections by multidrug-resistant Acinetobacter spp creates demand for reintroduction of older antimicrobials like polymyxins and newer tetracyclines like tigecycline [5,6].

Polymyxins, are a group of polypeptide antibiotics that consists of 5 chemically different compounds (polymyxins A–E) that mainly target cytoplasmic membrane of bacterial cell [8]. Polymyxins (colistin and polymyxin B) have not been used for many years because of availability of less toxic antibiotics. Gram-negative bacteria that are resistant to the aminoglycosides, beta-lactams, and fluoroquinolones are often susceptible to the polymyxins [7,11].

Tigecycline is a glycycline antibiotic having similar structure as tetracyclines. It inhibits protein synthesis by binding to 30s ribosomal subunit[9]. In comparison with tetracyclines, tigecycline binds to corresponding ribosomal sites with greater affinity, and irrespective of the presence of mutations that confer resistance to tetracycline’s [10]. Tigecycline shows greater activity against multidrug resistant gram-negative bacteria such as Acinetobacter baumannii, Stenotrophomonas maltophilia and Klebsiella pneumoniae [9].

Though much of data regarding Minimum inhibitory concentration of polymyxins and tigecyclines was available from studies of other countries, very less data is available from India. The objective of this study was to assess the in vitro susceptibilities of polymyxins and...
tigecycline to metallo beta lactamase producing *Acinetobacter baumannii*, and to know local susceptibility (MIC) ranges that can be important prerequisite for appropriate use of antibiotics.

2. Materials and Methods

2.1. Bacterial Strains

The present study was done in the Department of Microbiology at Apollo Health City; Hyderabad. A total of 102 strains of carbapenem resistant *Acinetobacter baumannii* were collected from various specimens of nosocomial infections from July 2008 to Jan 2010. Species confirmation was done by semi-automated system MINI API ID 32 GN(Biomerieux USA). All the isolates were stored at -20°C in glycerol trypticase soya broth.

2.2. MBL Detection

Phenotypic detection of MBLs were done by IPM-EDTA Disk Synergy test proposed by Young D et al [12].

2.3. Sensitivity Testing

Susceptibility testing to various antibiotics like Gentamicin, to Amikacin, Ceftazidine, Ciprofloxacin, Gatifloxacin, Cefepime, Piperacillin/ tazobactam, Aztreonem, Amphicillin/ sulbactam, Meropenum and Imipenum was done by disk diffusion test(Hi-media).

2.4. MIC Testing

The MIC of each drug were detected by Agar dilution method. Stocks of colistin, polymyxin and tigecycline were prepared. Serial two fold dilutions of each drug were prepared. The dilutions ranged from 32μg to 0.125μg. These dilutions were mixed into 20ml of Muller- Hinton medium and poured into Petri dishes. 3μl of inoculums was seeded onto the plate using a micropipette at specifically marked columns of different test organisms. The plates were kept for overnight incubation at 37°C and observed for growth [13,16,17]. ATCC controls were included in the study. MIC ranges for each drug were studied as MIC90 and MIC50.

3. Results

Out of 102 isolates of *Acinetobacter baumannii*, 56 were isolated from endotracheal secretions, 15 from purulent discharge, 12 from blood, 10 from body fluids, 8 from sputum, and 1 from urine. All the isolates were found to be MBL producers phenotypically by double disk synergy test (imipenum and EDTA synergy test). Of the 102 isolates of *Acinetobacter baumannii*, 83.3% were resistant to Gentamicin, 94% to Amikacin, 99% to Ceftazidine, 98% to Ciprofloxacin, 27% to Gatifloxacin, 93% to Cefepime, 91.% to Piperacillin/ tazobactam, 98% to Aztreonem 84% to Amphicillin/ sulbactam,100% to Meropenum and 95% to Imipenum.

| Antibiotics | No of isolates tested | 0.125 μg/ml | 0.25 μg/ml | 0.5 μg/ml | 1 μg/ml | 2 μg/ml | 4 μg/ml | 8 μg/ml | 16 μg/ml | 32 μg/ml |
|-------------|------------------------|-------------|------------|-----------|---------|---------|---------|---------|---------|---------|
| Colistin    | 102                    | 1           | 0          | 5         | 35      | 60      | 0       | 0       | 1       | 0       |
| Polymyxin B | 102                    | 1           | 1          | 8         | 52      | 40      | 0       | 0       | 0       | 0       |
| Tigecycline | 102                    | 6           | 7          | 62        | 14      | 7       | 1       | 2       | 1       | 2       |

| Antibiotics | MIC 50 μg/ml | MIC 90 μg/ml | % susceptibility | Break points |
|-------------|-------------|-------------|------------------|--------------|
| Colistin    | 2           | 2           | 99.2             | ≤2 μg/ml     |
| Polymyxin B | 1           | 2           | 100              | ≤2 μg/ml     |
| Tigecycline | 0.5         | 1           | 94.1%            | ≤2 μg/ml     |

Of the total isolates maximum number isolates were susceptible to Colistin, Polymyxin and Tigecycline at 2 μg/ml, 1 μg/ml and 0.5 μg/ml respectively.

MIC 50 and MIC 90 of colistin, polymyxin, tigecycline were found to be 2μg/ml and 2μg/ml, 1μg/ml and 2 μg/ml, 0.5 and 1 μg/ml respectively

4. Discussion

In our study high resistance was observed to aminoglycosides, third and fourth generation cephalosporins, penicillins and even to monobactums and Amphicillin / sulbactum. 73% of isolates were sensitive to Gatifloxacin but only 2% were found sensitive to Ciprofloxacin. Though all the isolates were resistant to meropenem, 5% of the isolates were found susceptible to imipenem.

In our study MIC 90 of colistin was found to be 2μg/ml similar to the studies from Singapore and EUCAST studies [18,19,20,22]. MIC 50 of colistin was also found to be 2μg/ml which was little higher than other studies 1μg/ml [18,19]. 99% of the isolates were found susceptible to colistin with MIC break points S ≤2 and R>2 [16]. Maximum isolates were found to be sensitive at 2μg/ml with sensitive MIC ranges 0.5-2μg/ml. Only 1 isolate was found resistant to colistin. Resistance rates were almost similar to studies from Turkey [6] and Thailand [11].

MIC 50 and MIC 90 of Polymyxin B were found to be 1μg/ml and 2 μg/ml similar to the sentry report USA [21,22]. Maximum isolates were found sensitive at 1μg/ml with susceptible MIC range 0.25-2 μg/ml. All the isolates were found sensitive to Polymyxin with MIC break points ≤2 μg/ml [23]. We noticed 0% resistance to Polymyxin B where as sentry report USA reported 2.5% resistance [21].

In our study MIC50 and MIC90 of tigecycline were found to be 0.5 and 1 respectively similar to the studies in Bangkok [14], lower than the MIC50 and MIC90 2μg/ml.
from the studies of Spain [15] by dilution methods. Maximum isolates were found susceptible at 0.5 µg/ml with sensitive MIC range .125 to 2 µg/ml. Only 6 isolates were found to be resistant to tigecycline. Resistance rates were similar to the from Spain 8% [15] and less when compared to other studies from Turkey 25.8% [6]. The lower resistance rates may be due to recent introduction of tigecycline in India. The susceptibility rates for tigecycline were found to be 94% with MIC break points ≤2 µg/ml [25]. As there are no approved standard MIC break points for Acinetobacter baumannii to tigecycline in the present study we took provisional MIC break points as ≤2 µg/ml (Wyeth Research, personal communication) [15,20].

5. Conclusion

In summary colistin and polymyxin B are very good drugs of choice for treatment of infections caused by Acinetobacter baumannii. Due to toxicity of polymyxins, tigecycline is the next better drug of choice with 94% susceptibility rates.

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