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MIC distribution profile of newer antibiotics among gram-negative bacteria in a tertiary care centre

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Abstract : Worldwide, multi-drug resistant (MDR) gram-negative infections are a growing concern. Majority of the nosocomial infections including pneumonia, urinary tract infections, intra-abdominal infections and bloodstream infections are caused by gram-negative organisms such as Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii complex. These infections are responsible for significant mortality and morbidity. Currently limited numbers of antimicrobials are available for use to treat these multi-drug resistant organisms. Newer antimicrobials have been evaluated and approved for use in few countries. However the efficacy and baseline MIC values of the new antimicrobials against the gram-negative organisms have not been evaluated in other countries including India. Morover, no standard interpretative breakpoints such as CLSI or EUCAST criteria are available for interpretation of the susceptibility results of these antimicrobials. In this study we calculated the MIC50 and MIC90 values for five antibiotics to confirm their efficacy as probable treatment options for MDR gram-negative organisms. MIC50 and MIC90 are statistical percentiles which help surmise the MIC results and reflect the susceptibility patterns of bacteria to specific antimicrobials.

Keyword :Multidrug resistant gram-negative organisms, MIC50, MIC90

Introduction:

Multi-drug resistant gram-negative infections are an increasing problem in hospitals and healthcare facilities worldwide. Gram-negative organisms such as *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii complex* account for most nosocomial infections including pneumonia, urinary tract infections, intra-abdominal infections, and are an important cause of blodstream infections (1). These infections contribute to significant mortality and morbidity. Currently available antimicrobials to treat these multi-drug resistant organisms are limited. Newer antimicrobials are available and found to be effective in few countries. These have not been tested in India. However, they have been found to be useful and are in routine use in other countries like Japan, Canada and European countries.

Minimum Inhibitory Concentration (MIC) is defined as the minimum concentration of the antibiotic required to inhibit the visible growth of a micro-organism after overnight incubation. Clinically, it helps choose the appropriate antibiotic and the dose required for therapy (2). MIC50 and MIC90 are statistical

An Initiative of The Tamil Nadu Dr. M.G.R. Medical University University Journal of Pre and Para Clinical Sciences percentiles which help surmise the MIC results and reflect the susceptibility patterns of bacteria to specific antimicrobials. The MIC50 represents the MIC value at which >50% of the isolates in a test population are inhibited. It is equivalent to the median MIC value. The MIC90 represents the MIC value at which >90% of the strains within a test population are inhibited (90th percentile) (3). The objective of this study is to document the baseline MIC50 and MIC90 values of these antibiotics - ar bekacin, biapenem, cefminox and colistin to different organisms E.coli, K.pneumoniae, P.aeruginosa and A.baumannii Arbekacin is a semisynthetic aminoglycoside complex. antibiotic primarily effective against Gram-positive organisms such as Staphylococcus aureus. It is used for short term treatment for multi-resistant bacterial infections such as Methicillin Resistant Staphylococcus aureus (MRSA).(4) Biapenem is a second generation of carbapenem which inhibits cell wall synthesis. It is a broad antibacterial spectrum against aerobic Gram positive and Gram negative bacteria and anaerobic bacteria. Morover, the frequency of administration of biapenem is twice daily compared to Imipenem/Meropenem which have to be administered 3-4/day (5). Cefminox is a second generation cephalosporin antibiotic which acts by inhibiting bacterial cell wall synthesis. It shows broad spectrum of activity against Gram positive and Gram negative bacteria including anaerobes.(6) Colistin, also known as Polymyxin E, is an older antibiotic with significant in vitro activity against some multiresistant Gram-negative pathogens including *P.aeruginosa, A.baumannii* and *Klebsiella pneumoniae.* (7) However, the efficacy of these against the gram-negative organisms is unknown in many countries. Morover, no standard criteria such as CLSI or EUCAST criteria are available for interpretation of the susceptibility results of these

Aim and Objectives:

antimicrobials.

To determine the *in vitro* activity of three antibiotics that are yet to be available for the use in India -arbekacin, biapenem, cefminox and an old but drug of renewed interest - colistin against clinicalisolates of *E.coli*, *K.pneumoniae*, *P.aeruginosa* and *A. baumannii* complex.To calculate the MIC50 and MIC90 of four antimicrobials –arbekacin, biapenem, cefminox and colistinfor these organisms as no standard interpretive criteria are available.

Materials and Methods:

This was a prospective study done over a period eight months from March 2012 to October 2012. Consecutive isolates of blood, urine and sputum positive for *E.coli, K.pneumoniae, P.aeruginosa* and *A.baumannii* complex were included in the study. These are identified as pathogens from these samples and the organisms which are suspected to be commensal were excluded from the study. MIC by broth

microdilution (Meiji Co., Japan) using cation-adjusted Muller-Hinton broth was determined for four antimicrobials - arbekacin, biapenem, cefminox and colistin. MIC50 and MIC90 were calculated to determine the MIC distribution.

Results:

Totally 925 samples were included in the study - the isolates from blood (n=282), sputum (n=241) and urine (n=86) sections which grew one of the following organisms - E.coli (n=211), K.pneumoniae (n=207), P.aeruginosa (n=153) and Acinetobacter baumannii complex (n=354). Ref.figure 1. The MIC50 and MIC90 values determined for the different antibiotics are as follows: For arbekacin, the MIC50 (g/ml) values were 2, 1, 1 and 128 for E.coli, K.pneumoniae, P.aeruginosa and A.baumannii complex respectively and the MIC90 (g/ml) values were 128 for E.coli, K.pneumoniae and A.baumannii complex and 64 for P.aeruginosa. Ref.figure 2. However, the MIC50 (g/ ml) values for biapenem for E.coli, K.pneumoniae, P.aeruginosa and A.baumannii complex respectively were 0.06, 0.25, 2 and 32. The MIC90 (g/ml) values for biapenem were 16, 64, 128 and 128 respectively for E.coli, K.pneumoniae, P.aeruginosa and A.baumannii complex. Ref.figure 3. Cefminox had higher MIC values - the MIC50 (g/ml) values being 1, 8, 128, 64 for E.coli, K.pneumoniae, P.aeruginosa and A.baumannii complex respectively whereas the MIC90 (g/ml) was 128 for all the four organisms. Ref.figure 4. For colistin, the MIC50 (g/ml) values were 0.25 for E.coli and 0.50 for K.pneumoniae, P.aeruginosa and A.baumannii complex respectively. The MIC90 (g/ml)

values were 0.5, 1, 2 and 32 respectively for *E.coli*, *K.pneumoniae*, *P.aeruginosa* and *A.baumannii*complex. Ref.figure 5.The comparison of MIC breakpoints recommended by standard committees is depicted in Table 1.



Figure 1: Pie chart showing the distribution of isolates included the study



Figure 2: Bar diagram showing MIC50 and MIC90 values of Arbekacin

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Figure 3: Bar diagram showing MIC50 and MIC90 values of Biapenem



Figure 4: Bar diagram showing MIC50 and MIC90 values for Cefminox



Figure 5: Bar diagram showing MIC50 and MIC90 values for Colistin

Discussion:

The continuously increasing problem of multidrug-resistant (extended-spectrum beta-lactamase and/or metallo-beta -lactamase producing) bacteria in recent years has created the need to explore newer options for therapy of these infections. Arbekacin is known to be an effective antibiotic for treatment of gram-positive infections including MRSA and is widely used in Japan. It is also known to be effective against gentamicin resistant strains of MRSA and hence is a good alternative to vancomycin (4). It is well known that arbekacin can be effective in cases of mixed infection with gram-negative organisms and MRSA. A study by Hamada et al., showed that, in patients with MRSA and gram-negative mixed infections, gram-negative bacteria (GNB) that had low minimal inhibitory concentrations (MICs) for amikacin or gentamicin were eradicated by arbekacin monotherapy. However, it was not effective in patients who were infected with gram-negative organisms with high level MICs for both amikacin and gentamicin (8). In this study we found that MIC50 and MIC90 against arbekacin were high against all four organisms tested. Although very effective for treatment of MDR gram-positive organism, arbekacin may not be very useful for the treatment of gram-negative infections in our setting

A study on the *in vitro* activity of biapenem, imipenem and meropenem against gram-negative and gram-positive pathogens in more than 6,000 clinical isolates worldwide revealed that the activity of biapenem was comparable to imipenem and meropenem.(9) However, in the present study, the MIC50 and MIC90 values for biapenem for the four organisms were high. The CLSI susceptibility breakpoint for Imipenem/ Meropenem for *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp.

is 1, 2 and 4 g/ml) respectively. Similarly the susceptibility breakpoint by EUCAST is 2 g/ml for *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp. respectively with the exception of Imipenem breakpoint for Pseudomonas spp. which is 4 g/ml. Compared to these breakpoint values, the MIC50 and MIC90 of the four organisms was high, thus indicating biapenem to be an ineffective choice for therapy of MDR gram-negative organisms.

Cefminox which is a second-generation cephalosporin antibiotic is known to have good efficacy against both gram-positive and gram-negative infections. Damaso *et al.* observed that cefminox had better *in vitro* activity against gram-negative organisms and cefoxitin against gram-positive organisms (6). On the contrary, we observed that the MIC50 values were low for *E.coli* and *K.pneumoniae*, the MIC90 values being high for all four organisms. From this finding, we infer that Cefminox may not be a good alternative choice for treatment of MDR gram-negative infections.

Colistin, an old antibiotic also known as polymyxin E, has attracted more interest recently because of its significant activity against MDR P.aeruginosa, A.baumannii complex and K.pneumoniae, with low resistance. The CLSI susceptible breakpoint criteria of colistin is 2 g/ml for E.coli, K.pneumoniae and A.baumannii complex; and 4 g/ml for P.aeruginosa. Among the isolates tested, colistin was effective against E.coli, K.pneumoniae and P.aeruginosa with low MIC50 and MIC90 values. On the other hand, MIC90 of colistin for A.baumannii complex was very high - this finding indicates that caution is required when prescribing against MDR A.baumannii complex. Many newer antimicrobials are available for treatment of MDR gram-negative organisms, but the clinical efficacy is unknown. MIC50 and MIC90 are statistical percentiles which help infer the MIC results and reflect the susceptibility patterns of bacteria to specific antimicrobials. Among the four antimicrobials evaluated in this study, arbekacin, biapenem and cefminox appear to be ineffective in vitro against gram-negative organisms. Whereas colistin, a drug of renewed interest appears a good option for MDR gram-negative infections such as E.coli, K.pneumoniae and P.aeruginosa with the exception of Acinetobacter spp.

Table 1: Comparison of MIC breakpoints recommended by various Antimicrobial Susceptibility Breakpoint Committees

Committee Interpretative criteria 2012	Enterobacteriaceae <u>P.aeruginosa</u> <u>A.baumanii</u> complex MIC breakpoints for <u>Arbekacin, Biapenem</u> and <u>Cefminox</u>								
	S	I	R	S	I	R	S	I	R
CLSI	NR	NR	NR	NR	NR	NR	NR	NR	NR
EUCAST	NR	NR	NR	NR	NR	NR	NR	NR	NR
	MIC breakpoints for colistin								
	S	1	R	S	1	R	S	1	R
CLSI	NR	NR	NR	2	-	8	2	-	4
EUCAST	2	-	>2	4		> 4	2		>2

NR – No breakpoint recommendations available; CLSI - Clinical and laboratory standards institute, USA; EUCAST - European Committee for Antimicrobial Susceptibility Testing

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Interpretative criteria 2012 MIC breakpoints for Arbekacin, Biapenem and Cefminox S I R S I R S I R CLSI NR NR NR NR NR NR NR NR NR EUCAST NR NR NR NR NR NR NR NR NR MIC breakpoints for colistin S I R S I R S I R CLSI NR NR NR 2 - 8 2 - 4 EUCAST 2 - > 2 4 - > 4 2 - > 2 Jul;48 Suppl 1:5–16.

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