Determination of Susceptibility Breakpoints of Elores in Extended Spectrum Beta-lactamases and Metallo Beta-lactamases Producing Acinetobacter Species

Madhav Prabhu[1], Mohit Arora[1], Zubin Ruttonji[2], Satish Patil[3], Vineeta Dhyani[4], Mohd Amin Mir[5]

ABSTRACT

Introduction: Infections caused by *Acinetobacter* species have now been becoming troublesome due to their ability to develop resistance. Current study was aimed and to find microbiological cut-off of Elores against *Acinetobacter* species.

Material and Methods: In this study, 457 clinical isolates of *Acinetobacter* species recovered from patients were involved. Breakpoint of Elores was established using the methods of pharmacokinetic and pharmacodynamic correlation.

Results: Among 457 isolates, 26.9% were ESBL (Extended Spectrum Beta-Lactamases) positive, 51.2% were MBL (Metallo Beta-Lactamases) positive and remaining 21.8% isolates were ceftriaxone susceptible. Out of identified ESBL and MBL producers 20 strains (10 ESBL and 10 MBL) were picked randomly for gene characterization and it was observed that 3 were TEM-1 positive, 1 was SHV-1 positive, 1 was AMP-C positive, 2 were OXA-1 positive, 3 was CTX-M positive, 3 were VIM-1 positive, 4 were IMP-1 positive, 3 were NDM-1 positive. In ceftriaxone, resistant *Acinetobacter* group, the MIC_{50} and MIC_{90} for ceftriaxone were 16 and 128 µg/ml and for Elores MIC_{50} and MIC_{90} were 4 and 8 µg/ml, respectively. The MIC cut off for Elores against ceftriaxone resistant *Acinetobacter* species was ≤8 µg/ml. Similarly, the AST cut off for Elores against ceftriaxone susceptible *Acinetobacter* species was ≥21 mm. The AST and MIC cut off for Elores against ceftriaxone resistant was same as of ceftriaxone susceptible.

Conclusion: Our data apparently indicates that Elores could be a better option to curb ESBL and MBL producing pathogens which synergistically provides clinically relevant concentration at higher susceptible MIC values.

Keywords: Adjuvant Therapy, Extended Spectrum Beta-Lactamases, *E. coli,* Pharmacodynamic, Pharmacokinetic

INTRODUCTION

Acinetobacter, an opportunistic pathogen, known as dominant cause of nosocomial infections such as meningitis, bacteraemia, wound infections, bloodstream infection, nosocomial-hospital acquired pneumonia or ventilator-associated pneumonia in ICU patients.[1-5]

Acinetobacter infections are commonly treated with cephalosporins, aminoglycosides, carbapenems, and tetracyclines.[4] However, infections caused by *Acinetobacter* species have now been becoming troublesome due to their ability to develop resistance against these drugs by acquiring various mechanisms predominantly extended spectrum beta lactamases (ESBLs) and metallo beta lactamses (MBLs), representing a major challenge in health care setting.[6-9] In India prevalence of resistance due to ESBLs and MBLs (70.9%) varies greatly.[10-12] Polymyxins and tigecycline have also been reported to be resistant to *A. baumannii*.[13-14]

Almost 35% *Acinetobacter* species have been reported to be resistant to carbapenem drugs.[1,15-16] According to a surveillance study conducted at 40 centers in 12 countries revealed a substantial increase in resistance rates in *Acinetobacter* species for meropenem (43.4%) and imipenem (42.5%).[17] The prevalence of imipenem resistance in *Acinetobacter baumannii* isolated from a burns unit of United State America was found to be 87%.[18] Similarly, according to a surveillance study conducted in several regions of Greece between 1996 and 2007. *A. baumannii* showed resistance to imipenem upto 85% (ICUs), 60% (medical wards), and 59% (surgical wards) [Greek System for Surveillance of Antimicrobial Resistance (GSSAR): http://www.mednet.gr/whonet/].

In light of the above background, the soaring rate of the antibiotic resistance and its impact on treatment failure, Elores, a combination of ceftriaxone, sulbactam and non-antibiotic adjuvant disodium EDTA (ethylene diamine tetraacetic acid) collectively called as antibiotic adjuvant entity (AAE) is a ray of new hope of clinicians.

Ceftriaxone, a third generation cephalosporin, has been used for the disposition of bounteous bacterial infections some of which are very severe such as endocarditis, meningitis, pneumonia, bone and joint infections, intra-abdominal infections, skin infections and urinary tract infection.[19-23] Sulbactam, well known ß-lactamase inhibitor, has been reported to have significant *in vitro* activity towards *Acinetobacter* species.[24] Its efficacy with combination of ampicillin was shown to have synergistic antibacterial activity.[24] Wood et al.[25] observed no differences in clinical outcomes between sulbactam-treated and imipenem-treated patients.

Elores exhibits antibacterial activity by inhibiting bacterial cell wall synthesis, irreversible inhibition of beta-lactamase enzymes. Moreover, EDTA synergizes the activity of ceftriaxone and sulbactam when administered along with these antibiotics.[26-28] Several recent published articles proved its in

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vitro and in vivo activity towards *Acinetobacter* species.[29-33] The aim of the current study is to provide the data to clinicians for their understanding and implementation of Elores (an antibiotic adjuvant entity (AAE) of ceftriaxone+sulbactam+EDTA) breakpoints for *Acinetobacter* species.

MATERIAL AND METHODS

In this study, a total of 457 clinical isolates of *Acinetobacter* family including *A. baumannii, A. iwoffii, A. junii, A. nosocomialis* and *A. pittii* were used. This study was conducted between year 2013 to 2015 in Jawaharlal Nehru Medical College-Belagavi, Karnataka. Re-identification of these isolates was carried out as described earlier.[34] These isolates were characterized into ceftriaxone susceptible and ceftriaxone resistant based on the CLSI breakpoints. Quality control (QC) was assured by concurrent testing of *E. coli* ATCC 25922 (ESBL-ve), *E. coli* ATCC 35218 (TEM+ve), *K. pneumoniae* ATCC 700603 (SHV+ve), *P. aeruginosa* ATCC 27853 (MBL-ve), *K. pneuminiae* ATCC BAA-2146 (NDM-1+ve), *K. pneumoniae* NCTC 13439 (VIM-1+ve) and *E. coli* NCTC 13476 (IMP+ve).

Reagents and media: SCD Broth Medium, Mueller-Hinton (MH) agar, Cation-adjusted MH broth (CAMHB), Barium chloride and Sulphuric acid were used for the study. All media and reagents were procured from Hi-Media, India.

Preparation of inoculum, McFarland standard and agar plates: The collected bacterial isolates of *Acinetobacter* species were spread onto SCDA plates and incubated at 35ºC to 37ºC for 18 to 24 hrs. Following incubation, 3 to 5 morphologically identical colonies of bacterial cells from SCDA plates were inoculated into 10 ml of MHB to achieve the turbidity of the 0.5 McFarland standard (18-24 hrs) and were used for further identification and analysis. The 0.5 McFarland Standard was prepared in MHB medium. Briefly, 0.5ml of 0.048 M $BaCl_2$ (1.172% w/v $BaCl_2.H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring and the absorbance was monitored at wavelength of 625 nm. SCDA and Mueller Hinton Agar (MHA) plates were prepared by pouring freshly prepared, sterilized, and hot (~50ºC) agar media (20-25 ml) into 90 mm flat-bottom sterilized petri dishes. These petri dishes were then allowed to solidify at room temperature. The SCDA and MHA plates were stored in sealed plastic bags for one week at 2 to 8 °C, when not in use. MHB was prepared according to instructions of manufacturer.

Drugs: The drugs used in the study were: ceftriaxone plus EDTA plus sulbactam; Elores (1.5 g) and ceftriaxone (1.0 g). Both the drugs were reconstituted in water for injection.

Screening for ESBL and MBL production: Ceftriaxone resistant isolates were further subjected for extended spectrum beta-lactamases (ESBL) and metallo beta-lactamases (MBL) characterization using the methods described earlier.[35-36] Randomly selected twenty isolates which were found to be ESBL and MBL positive (ten of ESBl and ten of MBL) were further processed for gene characterization using the previous reported method (Table 1).

Antimicrobial susceptibility testing (AST): The AST was determined using the method described earlier in CLSI.[35] The discs of ceftriaxone (30 µg) and Elores (ceftriaxone 30 µg; sulbactam 15µg) were used. Each disc of Elores contain 100 µg of EDTA and because EDTA is considered as non-antibiotic content hence not disclosed on the disc. Quality control (QC) was assured by concurrent testing of *E. coli* ATCC 25922 (ESBL-ve), *E. coli* ATCC 35218 (TEM+ve), *K. pneumoniae* ATCC 700603 (SHV+ve), *P. aeruginosa* ATCC 27853 (MBL-ve), *K. pneumoniae* NCTC 13439 (VIM-1+ve), *K. pneumoniae* ATCC BAA-2146 (NDM-1 +ve) and *E. coli* NCTC 13476 (IMP+ve).

Minimal inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) was determined using broth micro dilution assay following the CLSI guidelines.[35] MIC can be defined as the lowest concentration of antimicrobial agent that prevented turbidity after 16 to 20 hours of incubation at 35 ± 2°C.

MIC_{50} and MIC_{90} determination: The concentrations that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the strains were calculated for each of the antimicrobial agents as described earlier[37] using the formula of geometric.

MIC breakpoint determination: The MIC breakpoint for susceptible microorganisms was determined as per equation 1.[38]

Breakpoint concentration = C_{max} * f * s/ (e*t)

Where, C_{max} = maximum serum concentration; f is a protein binding factor, which is equal to 1 (or <70% protein binding), 0.5 (for 70-90% protein binding) and 0.2 (for >90% protein binding); s is a shift (or reproducibility) factor, which is usually equal to 1; e is factor by which the C_{max} should exceed the MIC and t is factor accounts for the serum elimination half-life, which is equal to 2 (for $T_{1/2}$ <1 hr), 1 (for $T_{1/2}$ = 1-3 hrs), and 0.5 (for $T_{1/2}$ >3 hrs). Values were taken from literature reference. The MIC breakpoint for resistant microorganisms was determined based on PKPD index %T>MIC (equation 2).[39]

%T>MIC = ln (Dose*/ (V_d*MIC)) *($T_{1/2}$/0.693) *(100/DI)

Where V_d(L) is the apparent volume of distribution in the central compartment, MIC is minimum inhibitory concentration (µg/mL) of Elores, $T_{1/2}$ (hr) is half-life of antibiotic, and DI is the dosing interval (hrs).

AST breakpoint determination: The AST breakpoints were obtained by drawing a perpendicular towards X axis which when intersected at horizontal line from the MIC breakpoints and the point of intersection of regression line, that value at x-axis, was considered as AST breakpoint corresponding to that MIC. Based on this AST was categorized as the vulnerability of the bacterial isolates into susceptible, intermediate, and resistant against the target antibiotic.[38,40]

STATISTICAL ANALYSIS

The scatter plots were drawn between MICs and corresponding AST values of the Elores and ceftriaxone against the bacterial isolates. Least square regression analysis was then performed to identify the best possible correlation between MIC and AST values of Elores and ceftriaxone.

RESULTS

Bacterial isolate characterization

Out of 457 isolates of *Acinetobacter* species (*A. baumannii, A. iwoffii, A. junii, A. nosocomialis, A. pittii*), 357 (78.1%) isolates were ceftriaxone resistant and outstanding 100 (21.8%)

isolates were ceftriaxone susceptible. Among ceftriaxone resistant isolates, 26.9% (123/457) were ESBL producers and 51.2% (234/457) of population were MBL producers. The distribution of ESBL and MBL are illustrated in Table 2. Out of identified ESBL and MBL producers 20 strains (10 ESBl and 10 MBL) were picked randomly for gene characterization and it was observed that 3 were TEM-1 positive (2 *A. baumannii;1 A. iwoffii*), 1 was SHV-1 positive (*1 A. baumannii*), 1 was AMP-C positive (*A. baumannii*), 2 were OXA-1 positive (1 *A. baumannii, 1 A. nosocomialis*), 3 were CTX-M positive (1 *A. junii and 2 Acinetobacter pittii*), 4 were VIM-1 positive (3 *A. baumannii, 1 Acinetobacter pittii*), 3 were IMP-1 positive (2 *A. baumannii, 1 A. junii*), 3 were NDM-1 (2 *A. baumannii, 1 A. iwoffii*) positive.

MIC_{50} and MIC_{90}

In ceftriaxone, resistant *Acinetobacter* group, the MIC_{50} and MIC_{90} for ceftriaxone were 16 and 128 µg/ml and for Elores MIC_{50} and MIC_{90} were 4 and 8 µg/ml respectively, indicating 16 folds' reduction in MIC_{90} of Elores compared to ceftriaxone. The ceftriaxone susceptible *Acinetobacter* isolates were susceptible to both ceftriaxone and Elores with MIC_{50} and MIC_{90} were ranging 2-4 µg/ml. The ceftriaxone susceptible *Acinetobacter* isolates, wild type strains and ESBLand MBL negative quality control strains were susceptible to both ceftriaxone and Elores with MIC_{50} values were ranging 0.25 to 0.5 and MIC_{50} and MIC_{90} values were identical for both drugs for ceftriaxone susceptible isolates that is 4 and 2 µg/ml (Table 3, Table 4, and Table 5).

MIC and AST breakpoint determination

As per PK-PD study, the MIC breakpoint values for ceftriaxone and sulbactam were 13.5 and 7.7 µg/ml respectively. Since, the MIC breakpoint values fall between the MIC of 8 and 4 µg/ml, hence MIC breakpoint value of 8 µg/ml was selected to categorize susceptible (Table 6). Ceftriaxone shows time dependent killing of bacteria, hence percentage of time above the MIC (%T>MIC) was used to set a breakpoint between resistant and intermediate pathogens. As depicted in (Table 7), %T>MIC of ceftriaxone was 68.58 and 43.58 at the MIC of 32 and 64 µg/ml, respectively. In Elores, %T>MIC was noted to be below 50%, pressing the synergy of all components in Elores (Table 8). Hence the MIC cut off 16-32 µg/ml and ≥64 µg/ml were used to categories intermediate and resistant.

AST percentage for isolates are depicted in Figure 1. To perform regression analysis, a graph was plotted between MIC and AST values of Elores against *Acinetobacter* species (Figure 2). Least square correlation analysis was performed to obtain regression line and correlation coefficients (Figure 3). The AST cut off for Elores against *Acinetobacter* species was ≥21 mm (susceptible), 14-20 mm (intermediate) and ≤13 mm (resistant). Detailed AST breakpoint of AAE, are depicted in Figure 4. AST breakpoint of AAE, are depicted in Figure 4. The AST and MIC breakpoints of all the bacterial species are shown in (Table 9). The AST and MIC cut off for Elores against ceftriaxone resistant was same as of ceftriaxone susceptible.

DISCUSSION

Acinetobacter species are a common nosocomial infection and is one of the most challenging healthcare-associated burden. Infections due to this bug often lead to significant mortality and morbidity and is a serious concern including in intensive care units.[41] Based on substantial studies it has been found that resistance for *Acinetobacter* species is increasing worldwide, not only to third generation cephalosporins but also

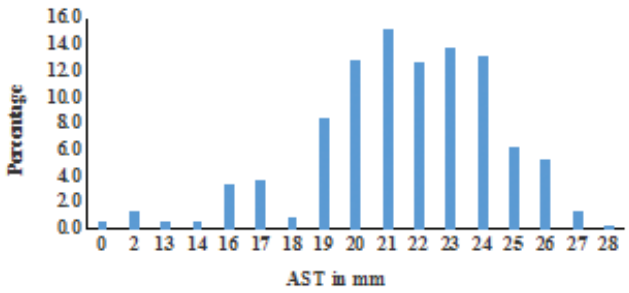

Figure-1: Elores AST percentage in ceftriaxone resistant *Acinetobacter* strains.

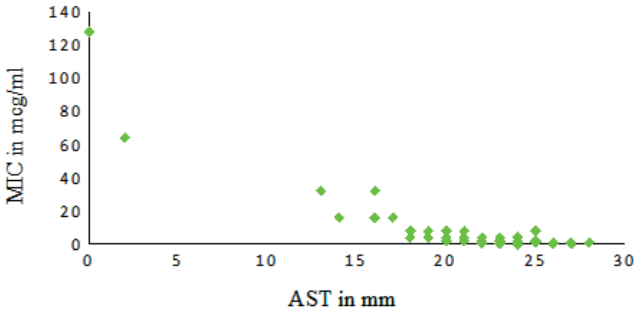

Figure-2: Scatter plot for Elores in ceftriaxone resistant *Acinetobacter* strains.

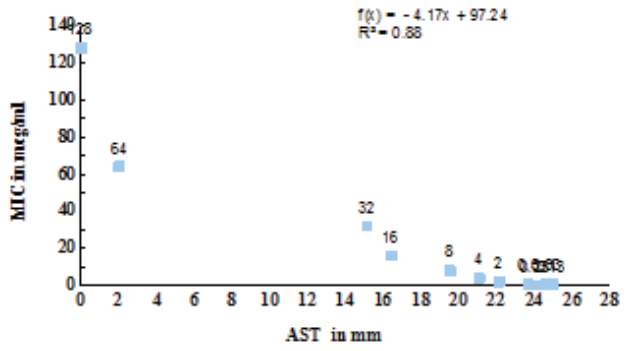

Figure-3: Regression plot for Elores in ceftriaxone resistant *Acinetobacter* strains.

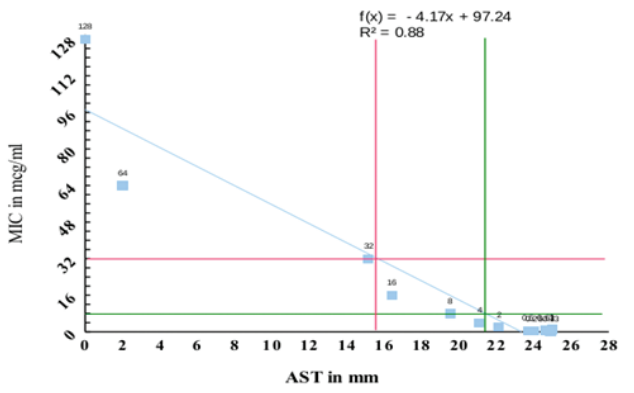

Figure-4: Breakpoints for Elores in ceftriaxone resistant *Acinetobacter* strains.

to multidrug categories of antibiotics restricting the treatment options.[42] *Acinetobacter* species has been seen to be resistant to beta-lactams in both ESBL and MBL producers.[43] The present scenario of increasing resistance due to ESBL and MBL amongst *Acinetobacter* species lead us to restrict our study in details in terms of resistance profile with ceftriaxone and Elores. Even though, addition of beta-lactamase inhibitor to parent beta-lactam can counter some of the ESBL infections, but for MBL infections, there is no effective alternative available. Therefore, the objective of current study was not only to evaluate the breakpoint sin ESBL producing Ceftriaxone resistant *Acinetobacter* spp but also in MBL producing resistnat strains isolated from clinical settings along with known positive controls.

Primer	Primer sequences (5'-3')	Amplicon (base pair)	References
TEM-1	F-5' CTGGGAAACGGAACTGAATG-3' R-5' GGGGTATCCCGCAGATAAAT-3'	858	*52*
SHV-1	F-5'ATGAGTATTCAACATTTCCG-3' R-5' CCAATGCTTATTCAGTGAGG-3'	308	*52*
AmpC	F-5CCC CGCTTA TAGAGC AAC AA-3 R-5TCA ATG GTC GAC TTC ACA CC-3	634	*53*
OXA-2	5'- GCCAAAGGCACGATAGTTGT-3' 5'- GCGTCCGAGTTGACTGCCGG-3'	701	54
CTX-M-1	F:5' GACGATGTCACTGGCTGAGC 3' R: 5'AGCCGCCGACGCTAATACA 3'	499	54
NDM-1	F- 5'GGTTTGGCGATCTGGTTTTC-3' R- 5'CGGAATGGCTCATCACGATC-3'	621	55
IMP-1	F- 5'GGAATAGAGTGGCTTAAYTCTC- 3' R -5'CCAAACYACTASGTTATCT- 3'	188	56
VIM-1	F- 5'TTATGGAGCAGCAACGATGT-3' R- 5'CAAAAGTCCCGCTCCAACGA-3'	920	57

Table-1: Oligonucleotides used in the study for each tested genes

Characterization	Types	No of isolate/total	Percentage
Ceftriaxone resistant	ESBL	123/457	26.9
	MBL	234/457	51.2
Ceftriaxone susceptible	Non-ESBL+MBL	100/457	21.8

Table-2: Prevalence of ESBL, MBL, and non-ESBL and MBL among *Acinetobacter* clinical isolates

	No of pathogens	Ceftriaxone		Elores	
		MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}
Acinetobacter (Ceftriaxone resistant strains)	357	128	16	8	4
Acinetobacter (Ceftriaxone susceptible strains)	100	4	2	4	2

Table 3: MIC_{50} and MIC_{90} values of ceftriaxone and Elores against *Acinetobacter* isolates.

	Number of occurrence at MIC (µg/ml) of Elores												
Organisms	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
E. coli (ATCC 25922) (ESBL-)	6	-	-	-	-	-	-	-	-	-	-	-	-
E. coli (ATCC35218) (TEM+)	-	1	5	-	-	-	-	-	-	-	-	-	-
K. pneumoniae (ATCC700603) (SHV+)	-	1	5	-	-	-	-	-	-	-	-	-	-
K. pneumoniae ATCC BAA-2146 (NDM-1)	-	-	-	-	-	-	-	6	-	-	-	-	-
K. pneumoniae NCTC 13439 (VIM-1)	-	-	-	-	-	-	-	6	-	-	-	-	-
E. coli NCTC 13476 (IMP)	-	-	-	-	-	-	-	6	-	-	-	-	-
P. aeruginosa (ATCC27853) (MBL-)	-	-	-	-	6	-	-	-	-	-	-	-	-
Number of occurrence at MIC (µg/ml) of Ceftriaxone													
Organisms	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
E. coli (ATCC 25922) (ESBL-)	5	1	-	-	-	-	-	-	-	-	-	-	-
E. coli (ATCC35218) (TEM+)	-	-	-	-	1	5	-	-	-	-	-	-	-
K. pneumoniae (ATCC700603) (SHV+)	-	-	-	-	-	1	5	-	-	-	-	-	-
K. pneumoniae ATCC BAA-2146 (NDM-1)	-	-	-	-	-	-	-	-	-	-	5	1	-
K. pneumoniae NCTC 13439 (VIM-1)	-	-	-	-	-	-	-	-	-	-	3	3	-
E. coli NCTC 13476 (IMP)	-	-	-	-	-	-	-	-	-	-	4	2	-
P. aeruginosa (ATCC27853) (MBL-)	-	-	-	-	-	-	-	-	3	3	-	-	-

Table-4: MIC values for quality control strains.

In the current investigation, 457 isolates of *Acinetobacter* species (*A. baumannii, A. iwoffii, A. junii, A. nosocomialis, A. pittii*) were used and susceptibility behaviour of Elores and ceftriaxone on these clinical isolates were evaluated in terms of MIC_{50} and MIC_{90} as both are important parameters of presenting susceptibility. These data indicate that >90% of the organisms were inhibited at MIC 8 µg/ml with Elores which is indicative of its susceptibility towards *Acinetobacter* resistant isolates. This is also in agreement with the "90-60 rule" which means 90-95% of the time therapy would respond,[44-45] hence showing a good outcome for Elores. Earlier studies also demonstrate a higher susceptibility of Elores against resistant *Acinetobacter* isolates.[30,31-32]

MIC breakpoints have been used to categorize susceptibility and resistant behaviour of any drugs.[46] It relies on pharmacokinetic-pharmacodynamic (PK/PD) variables such as protein binding, half-life, volume of distribution. The PK/PD data for AAE was calculated using earlier published paper.[47] The PK/PD data for AAE was calculated using earlier published paper.[47,48] The regression analysis, a graph was plotted between MIC and AST values, showed equal to or near to 0.9 regression coefficients (R2) suggesting strong relationship between MIC values and AST distribution.

***A. baumannii* (MTCC1425) (MIC µg/ml)**
≤1

Table-5: MIC value of Elores in MTCC strain

PK-PD indices define the activity of an antibiotic is of great importance not only in treating infections but also to combat antibiotic resistance. For cephalosporin, %T>MIC PK/PD parameters are good predictors of antibiotic efficacy,[49] but scanty knowledge is available for AAEs.[50-51] In Elores, ceftriaxone shows time dependent killing of bacteria, hence percentage of time above the MIC (%T>MIC) was used to set a breakpoint between resistant and intermediate pathogens. The %T>MIC of ceftriaxone was 68.58 and 43.58 at the MIC of 32 and 64 µg/ml respectively. The MIC 32 µg/ml is the highest MIC value at which antibiotic concentration remains above MIC for more than 50% of the time, thus selected as a MIC breakpoint to categorize intermediate and resistance isolates. Elores has shifted the MIC distribution values, suggesting higher efficacy of the it at lower concentrations. Sulbactam is disposed along with ceftriaxone and EDTA to inhibit beta-lactamase enzyme.

CONCLUSION

Elores shows higher susceptibility towards ceftriaxone resistant *Acinetobacter* isolates while ceftriaxone failed to respond such strains because of degradation. The MIC cut off for Elores against ceftriaxone resistant *Acinetobacter* was ≤8 µg/ml (susceptible), 16-32 µg/ml (intermediate) and ≥64 µg/ml (resistant). From this study, it appears that Elores could be a better alternative than ceftriaxone alone for combating ESBL and MBL producing resistant pathogens which synergistically provides clinically relevant concentration at higher susceptible

MIC breakpoint for susceptible and intermediate = C_{max} * f * s/e*t							
Elores component	**C_{max}**	**F**	**s**	**e**	**T**	**Breakpoint value**	**Cut off**
Ceftriaxone	135	0.2	1	4	0.5	13.5	>8
Sulbactam	31.1	1	1	4	1	7.77	>4

Table-6: Calculation of MIC breakpoint of Elores 1.5 g for susceptible organisms.

%T> MIC = ln (Dose/(Vd*MIC)) *(t1/2/0.693) *(100/DI)						
MIC_ Elores (µg/ml)	**MIC_of corresponding Ceftriaxone content (µg/ml)**	**Dose (mg)**	**V_d (L)**	**T1/2 (hrs)**	**DI (hrs)**	**%T> MIC**
4	2.67	1000	7	6	24	143.60
8	5.34	1000	7	6	24	118.59
16	10.68	1000	7	6	24	93.59
32	21.34	1000	7	6	24	68.58
64	42.69	1000	7	6	24	43.58

Table-7: Calculation of MIC breakpoint for resistant isolates as per ceftriaxone component of Elores 1.5 g OD.

%T> MIC = ln (Dose/(Vd*MIC)) *(t1/2/0.693) *(100/DI)						
MIC_ Elores (µg/ml)	**MIC_of corresponding Sulbactam content (µg/ml)**	**Dose (mg)**	**V_d (L)**	**T1/2 (hrs)**	**DI (hrs)**	**%T> MIC**
4	1.33	500	20	1.5	24	26.44
8	2.67	500	20	1.5	24	20.19
16	5.34	500	20	1.5	24	13.94
32	10.68	500	20	1.5	24	7.77
64	21.34	500	20	1.5	24	1.52

Table-8: Calculation of MIC breakpoint for resistant strains as per sulbactam component of Elores 1.5 g OD.

Bacterial Strain	**AST breakpoints (mm)**			**MIC breakpoints (µg/ml)**		
	Susceptible	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
Acinetobacter (ceftriaxone susceptible)	≥21	14-20	≤13	≤8	16-32	≥64
Acinetobacter (ceftriaxone resistant)	≥21	14-20	≤13	≤8	16-32	≥64

Table-9: MIC and AST breakpoints of Elores used to segregate different bacterial isolates in susceptible, intermediate, and resistant

MIC values as observed through current study.

REFERENCE

1. Kilic A, Li H, Mellmann A, Basustaaoglu CA, Kul M, Senses S, et al. *Acinetobacter* septicus sp. nov. association with a nosocomial outbreak of bacterimia in neonatal intensive care unit. Journal of Clinical Microbiology. 2008;46:902-908.
2. Peck KR, Kim MJ, Choi JY, Kim HS, Kang CI, Cho YK, Park DW, Lee HJ, Lee MS, Ko KS. In vitro time-kill studies of antimicrobial agents against blood isolates of imipenem-resistant *Acinetobacter* baumannii, including colistin- or tigecycline-resistant isolates. Journal of Medical Microbiology. 2012;61:353–360.
3. Nahar A, Anwar S, Saleh AA et al. isolation of *Acinetobacter* species and their antimicrobial resistance pattern in an intenside care unit of a tertiary care hospital in Dhaka, Bangladesh. Bangladesh Journal of Medical Microbiology. 2012;6;3-6.
4. Howard A, O'Donoghue M, Feeney A, Sleator RD. *Acinetobacter* baumannii. Virulence. 2012;3:243-250.
5. Peleg AT, Seifert H, Paterson DL. *Acinetobacter* baumannii: emergence of a successful pathogen. Clinical Microbiology and Review. 2008;21:538–582.
6. Metan G, Sariguzal F, Sumerkan B. Factors influencing survival in patients with multidrug resistant *Acinetobacter* baumanii bacterimia. EFIM. 2009;20:540-544.
7. Coelho J, N Woodford, J Turton, D M Livermore . Multiresistant *Acinetobacter* in the UK: how big a threat. Journal of Hospital Infections. 2004;58:167–169.
8. Prashanth K, Badrinath S, Nosocomial infections due to *Acinetobacter* spp: clinical findings, risk, and prognostic factors. Indian Journal of Medical Microbiology. 2006;24:39- 44.
9. Safari M, Nejad ASM, Bahador A et al., Prevalence of ESBL and MBL encoding genes in *Acinetobacter* baumannii strains isolated from patients of intensive care units (ICU) Saidi Journal of Biological science. 2015;22; 424-429.
10. Karthika RU, Rao S, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, Prashanth K. Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacter* baumannii from a South Indian tertiary care hospital. Journal of Medical Microbiology. 2009;58:43035.
11. Kumar M, Chaudhary S, Makkar DK, et al., Comparative antimicrobial efficacy evaluation of a new product Elores against meropenem on gram-negative isolates. Asian Journal of Pharmaceutical and Clinical Research. 2013;8:1-4.
12. Shweta S, Hans C, Makhija LK et al., Prevalence of Extended Spectrum ß -lactamases and Metallo B-lactamases in bacterial isolates from burn patients. International Journal of Current Microbiology and Applied Sciences. 2014;3:529-535.
13. Doi Y, Husain S, Potoski BA, McCurry KR, Paterson DL. Extensively drug-resistant *Acinetobacter* baumannii. Emergence of Infectious Disease. 2009;15:980–982.
14. Park YK, Peck KR, Cheong HS, Chung DR, Song JH, Ko KS. Extreme drug resistance in *Acinetobacter* baumannii infections in intensive care units, South Korea. Emerging Infectious Diseases. 2009;15:1325–1327.
15. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA, Global challenge of multidrug-resistant *Acinetobacter* baumannii. Antimicrobial Agents Chemotherapy. 2007;51:3471–3484.
16. Sinha, M, Srinivasa H, Macaden R. Antibiotic resistance profile and extended spectrum beta-lactamase (ESBL) production in *Acinetobacter* species. Indian Journal of Medical Research. 2007;126:637.
17. Turner PJ. Meropenem activity against European isolates: report on the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) 2006 results. Diagnostic Microbiology and Infectious Disease. 2008;60:1852.
18. Sareek PS, Sureshkumar D, Ramgopalakrishnan, Ramasubramanian V, Gaphur KA, Thirunarayanan MA. Antibiotic sensitivity pattern of blood isolates of *Acinetobacter* species in a tertiary care hospital: a retrospective analysis. American journal of Infectious Disease. 2012;8:65-69.
19. Lamb HM, Ormrod D, Scott LJ, Figgitt DP. Ceftriaxone: an update of its use in the management of community-acquired and nosocomial infections. Drugs. 2002;62:1041-89.
20. Tunkel AR, Barry J. Hartman, Sheldon L. Kaplan, Bruce A. Kaufman, Karen L. Roos, W. Michael Scheld, and Richard J. Whitley. Practice guidelines for the management of bacterial meningitis. Clinical Infectious Diseases. 2004;39:1267–84.
21. Wieland BW, Marcantoni JR, Bommarito KM, warren DK, Marschall J. A Retrospective comparison of ceftriaxone versus oxacillin for osteoarticular infections due to methicillin- susceptible Staphylococcus aureus. Clinical Infectious Disease. 2012;54:585–590.
22. Sweet RL. Treatment of acute pelvic inflammatory disease. Infectious Diseases in Obstetrics and Gynecology, 2011; 2011.
23. Wells WG, Woods GL, Jiang Qi, Gesser RM. Treatment of complicated urinary tract infection in adults: combined analysis of two randomized, double-blind, multicentre trials comparing ertapenem and ceftriaxone followed by appropriate oral therapy. Journal of Antimicrobial Chemotherapy, 2004;53: Suppl. S2, ii67–ii74.
24. Corbella X, Ariza J, Ardanuy C et al.: Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multiresistant *Acinetobacter* baumanni. Journal of Antimicrobial and Chemotherapy. 1998;42:793-802.
25. Wood GC, Hanes SD, Croce MA, Fabian TC, Boucher BA, Comparison of ampicillin- sulbactam and imipenem-cilastatin for the treatment of *Acinetobacter* ventilator-associated pneumonia. Clinical Infectious Disease, 2002;34:1425-1430.
26. Rajpurohit H, Kumar VBM, Sharadamma KC, Radhakrishna PM. Comparative study of antimicrobial activity of ceftriaxone in combination with sulbactam and tazobactam using disc diffusion method. International Research Journal of Pharmacy. 2012;3:331-334.
27. Totir MA, Helfand MS, Carey MP, et al. Sulbactam forms only minimal amounts of irreversible acrylate -enzyme with SHV-1 beta-lactamase. Biochemistry. 2007;46:8980-7.
28. Chaudhary M, Sudaroli M, Kumar S, Krishnaraju. V. Catering ESBL resistance challenge through strategic combination of Ceftriaxone, Sulbactam and Ethylenediaminetetra acetic Acid. International Journal of Drug Development and Research. 2012;4:72-81.
29. Sahu M, Sanjith S, Bhalekar P, Keny D. Waging war against extended spectrum beta lactamase and metallo-

betalactamase producing pathogens- novel adjuvant antimicrobial agent CSE1034- an extended hope. J Clin Diagn Res. 2014;8:DC20-3.
30. Chaudhary M, Payasi A. Molecular Characterization, and Antimicrobial Susceptibility Study of *Acinetobacter* baumannii Clinical Isolates from Middle East, African and Indian Patients. Journal of Proteomics and Bioinformatics. 2012;5:265-269.
31. Chaudhary M and Payasi A. Incidence, prevalence, and control of multidrug resistant (MDR) carbapenemase producing *Acinetobacter* baumanii in Indian intensive care units. Journal of Pharmacy Research. 2013a;7;175-180.
32. Chaudhary M, Payasi A. Clinical, microbial efficacy and tolerability of Elores, a novel antibiotic adjuvant entity in ESBL producing pathogens: Prospective randomized controlled clinical trial. Journal of Pharmacy Research. 2013b;7:275- 280.
33. Bagga R. Retrospective analysis of antibiotic susceptibility and resistance patterns against nosocomial gram negative pathogens in fortis memorial research institute Gurgaon. International Journal of Current Advanced Research. 2015;4:347-351.
34. Bouvet PJ1, Grimont PA. Identification and biotyping of clinical isolates of *Acinetobacter*. Annales de l'Institut Pasteur Microbiology. 1987;138:569-78.
35. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI document M100-S23. 2013; Wayne, PA 19087 USA.
36. Yong D, Lee K, Yum JH, et al. Imipenem EDTA disc method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and *Acinetobacter* spp. 2002;40:3798e3801.
37. Smith JA, Henry D, Ngui-Yen J,Castell A, Coderre S. Comparison of agar dilution, microdilution, and disk elution methods for measuring the synergy of cefotaxime and its metabolite against anaerobes. Journal of Clinical Microbiology. 1986;23:1104-1108.
38. MacGowan AP, Wise R. Establishing MIC breakpoints and interpretation of in vitro susceptibility tests. Journal of Antimicrobial and Chemotherapy. 2001;48:Suppl-1, 17–28.
39. Narawadeeniamhun, Panomvana D, Pongpech P, Athavudhdeesomchok. Pharmacodynamic target associated with clinical outcome of hospital-acquired pneumonia treatment with cefoperazone/sulbactam. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4:584-589.
40. Turnidge JD, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. Clinical Microbiology and Review. 2007;20:391-408.
41. Lee BY, Mcglone SM, Doi Y, Bailey RR, Harrison LH. Economic value of *Acinetobacter* baumannii screening in the intensive care unit. Clinical Microbiology and Infection. 2011;17:1691–1697.
42. Shahcheraghi F, Abbasalipour M, Feizabadi MM, Ebrahimipour GH, Akbari N, Isolation and genetic characterization of metallo-beta-lactamase and carbapenamase producing strains of *Acinetobacter* baumannii from patients at tehran hospitals. Iranian Journal of Microbiology. 2011;3:68–74.
43. Owlia P, Azimi L, Gholami A, Asghari B, Lari AR. ESBL and MBL mediated resistance in *Acinetobacter* baumannii: a global threat to burn patients," Infezioni in Medicina. 2012;20:182–187.
44. Kuper KM, Boles DM, Mohr JF, Wanger A. Antimicrobial susceptibility testing: a primer for clinicians. Pharmacotherapy. 2009;29:1326-43.
45. Gumbo T. New susceptibility breakpoints for first-line antituberculosis drugs based on antimicrobial pharmacokinetic/pharmacodynamic science and population pharmacokinetic variability. Antimicrobial Agents and Chemotherapy. 2010;54:1484–1491.
46. Jenkins SG, Jerris RC. Critical assessment of issues applicable to development of antimicrobial susceptibility testing breakpoints. Journal of Clinical Microbiology. 2011;49:S5–S14.
47. Attili VSS, Chaudhary M. Pharmacokinetics and pharmacodynamics of elores in complicated urinary tract infections caused by extended spectrum beta-lactamase strains. International Journal of Pharmaceutical Sciences and Research. 2015;6:2569-2578.
48. Foulds G, Stankewich JP, Marshall DC, O'Brien MM, Hayes SL, Weidler DJ, and McMahon FG, Pharmacokinetics of sulbactam in humans. Antimicrobial Agents and Chemotherapy. 1983;23:692–699.
49. Turnidge JD. The pharmacodynamics of beta-lactams. Clinical Infectious Disease. 1998;27:10-22.
50. Frimodt-Moller N. How predictive is PK/PD for antibacterial agents? International Journal of Antimicrobial Agents. 2002;19:333-339.
51. Li RC, Zhu ZY. The integration of four major determinants of antibiotic action: bactericidal activity, post-antibiotic effect, susceptibility, and pharmacokinetics. Journal of Chemotherapy. 2002;14:579-583.
52. Dashti AA, Jadaon MM et. al., Heat treatement of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait medical journal. 2009;41:117-122.
53. Feria C, Ferreira E, Correia JD, Goncalves J, Canica M. Patterns, and mechanisms of resistance to beta-lactams and beta-lactamase inhibitors in uropathogenic Escherichia coli isolated from dogs in Portugal. Journal of Antimicrobial Chemotherapy. 2002;49:77-85.
54. Meyer L, Labuschagne CDJ, Ehlers MM, Dove MG, Weldhagen GF. Diversity of bla-type genes in extended-spectrum β-Lactamase- producing Klebsiella pneumoniae isolated during 2003-2004 at pretoria academic Hospital. The Southern African Journal of Infectious Diseases. 2007;22:5-7.
55. Nordmann P, Poirel L, Carrer A, Toleman MA,Walsh TR, How to detect NDM-1 producers. Journal of Clinical Microbiology. 2011;49:718–721.
56. Ellington MJ et. al. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. Journal of Antimicrobial Chemotherapy. 2007;59:321-2.
57. Yan J-J et. al., Metallo-beta-lactamases in clinical Pseudomonas isolates in Taiwan and identification of vim-3, a novel variant of the VIM-2 enzyme. Antimicrobial Agents and Chemotherapy. 2001:2224–2228.

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