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# PHARMACOKINETICS AND PHARMACODYNAMICS OF ELORES IN COMPLICATED URINARY TRACT INFECTIONS CAUSED BY EXTENDED SPECTRUM BETA-LACTAMASE STRAINS.

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**Keywords:**

Beta-lactamase, Ceftriaxone, Elores, Pharmacodynamic, Pharmacokinetic, Sulbactam

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**ABSTRACT:** The pharmacokinetic/pharmacodynamic indices generally predict the efficacy of antibiotics when used alone. The information related to these indices for the combination products and that too in cases of resistant bacterial infections is limited. The present study involved treatment of 12 subjects with severe complicated urinary tract infections, caused by resistant extended spectrum beta lactamase bacterial strains, with Elores; ceftriaxone and sulbactam combination with non-antibiotic adjuvant ethylenediamine tetracetate. In this study Elores 3g bd dose was administered and the pharmacokinetic/pharmacodynamic indices were evaluated. To see the impact of lower Elores dose on these indices, 3g od data was simulated using pharmacokinetic parameters from 3g bd dose. The pharmacokinetic/pharmacodynamic indices suggest that Elores 3g od can treat mild to moderate infection wherein minimum inhibitory concentration requirement is <16 µg/mL; however for severe complicated urinary tract infections with resistant strains wherein the requirement is 16 32 µg/mL, Elores 3g bd should be the choice.

**INTRODUCTION:** Dose optimization of any drug depends on the characterization of pharmacokinetic (PK) and pharmacodynamic (PD) relationships. The dosing regimens of antibacterial agents are based on estimates of minimum inhibitory concentration (MIC; a PD marker for characterizing antibacterial response). The MIC alone cannot address changes in bacterial count with time and their relationships to PK parameters.

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The time factor incorporation for quantifying relationship between the reduction of bacterial count and the changes in concentration of antibacterial agents provides information in the form of PK/PD indices (derived values which combine PK with MIC values of the antibacterial agents).

The PK parameters commonly used for such transformations are area under the plasma concentration–time curve (AUC) or maximum plasma concentration ($C_{max}$). Most frequently used PK/PD indices of antibiotics include Cmax/MIC ratio, T>MIC (the time duration in percentage [%T>MIC] for which concentration of drug in serum/plasma is above MIC for 24 h) and $AUC_{0-}$

$_{24h}$/MIC ratio. The PK/PD indices for single antibacterial agents are well characterized but information of these indices regarding antibacterial combinations is limited. Identification of PK/PD indices of antibacterial agent combinations is becoming extremely important as these are becoming the mainstay for treating infections for combating antibiotic resistance [1-5].

Among the infections, one of the main reasons for hospital admissions are complicated urinary tract infections (cUTIs). The two major pathogens (gram-negative bacteria) routinely encountered in treating cUTIs are *Escherichia coli* and *Klebsiella pneumoniae*. The major concerns for treating cUTI are increasing prevalence of antimicrobial resistance due to extended spectrum beta-lactamase (ESBL) strains. Though the recommendations to treat cUTI include penems and aminoglycosides, but their activities against ESBLs are limited [6-12].

Elores is a novel fixed dose combination (FDC) of ceftriaxone and sulbactam along with non-antibiotic adjuvant ethylenediamine tetra acetate (EDTA) which provides the extension of antibacterial activity against ESBLs and Metallo-beta-lactamases (MBLs) strains, in addition to routinely susceptible strains to ceftriaxone. The mechanistic superiority of Elores comes from various mechanisms by virtue of synergistic action apart from those, which are known for ceftriaxone and sulbactam. These include breaking antibiotic-resistance by eradicating bacterial biofilm, inhibition of curli formations and decreasing the expression of efflux transporters. Targeting different bacterial substrates result in lowering of MICs for Elores, which is several folds lower than various broad spectrum antibiotics like meropenem, piperacillin + tazobactam, ceftriaxone and cefoperazone plus sulbactam [13-15].

The efficacy and safety of Elores is proven for UTI.[16] However, the PK/PD of this FDC needs to be characterized for further optimization of dose taking into consideration the severity of infection and antimicrobial resistance. Various studies show that class-specific PK/PD parameters are good predictors of antibiotic efficacy, like %T>MIC for cephalosporin [1, 17], AUC/MIC ratio for aminoglycosides [18], but for FDCs, the information for predictive capacity of PK/PD indices in eliminating resistant strains is limited. Therefore, the present study was undertaken to evaluate the PK/PD indices for the FDC Elores in severe cUTI (caused by ESBL gram negative bacteria) cases and to further assess the predictive capacity of these PK/PD indices.

## MATERIALS AND METHODS:

**Study population**: The study protocol was approved by the ethics committee and was registered in clinical trial registry of India (CTRI/2012/08/002899). Briefly, inclusion criteria included: 1) Subjects of either gender; 18 to 65 years of age with diagnosis of UTI on the basis of clinical signs and symptoms; 2) UTI caused by ESBL confirmed by urine cultures and the treatment required intravenous therapy; 3) subjects with indwelling catheters have had catheter removed or replaced and if removal had not been clinically acceptable, then catheter was not indwelled longer than 12 h, after randomization; 4) in cases of obstructive uropathy, the obstruction to be relieved by stent or nephrostomy tube no later than 24 hours after randomization; and 5) subjects receiving antibiotics for the current infection and the duration of therapy was $\leq 24$ hours before screening visit and there was no improvement or stabilization of clinical condition. Informed consent was obtained from each subject wherein purpose of the study, the process and the risk/benefit of the study was thoroughly explained.

**Study design**:

This was an open-labeled PK/PD study of Elores (Ceftriaxone + Sulbactam + non-antibiotic adjuvant EDTA) in subjects infected with ESBL (gram-negative bacteria). The study included screening phase, treatment phase and follow-up phase (if required). In the treatment phase, subjects were administered study drug twice daily by 90 min intravenous (IV) infusions.

**Microbiological investigations, time kill curve estimations and MIC determinations**:

The specimens (serum) for routine culture and gene characterization for resistant strains were obtained within 24h prior to start of treatment. The causative organisms responsible for cUTI were identified according to previously reported methods and

antimicrobial susceptibility studies for time kill curve (TKC) estimations and MIC determinations were conducted according to Clinical Laboratory Standard Institute guidelines [19-21].

**Pharmacokinetic analysis**:
Blood samples were collected over a period of 24 h after the administration of 5$^{th}$ dose (Day 3). The samples were taken from forearm vein by an intravenous catheter before the dose (0.00 h) and from 0.25 to 24.00 h post-dose. A validated liquid chromatography (LC) mass spectrophotometry (MS)/MS [LC-MS/MS] bio-analytical method was used for estimation of analytes in plasma. The PK parameters were analyzed (WINNONLIN, Ver. 5.1; Pharsight Corporation, USA) and included area under the plasma concentration-time curve (AUC), highest concentration reached (the peak; $C_{max}$), elimination rate constant ($K_e$), half-life ($t_{1/2}$), volume of distribution ($V_d$), and clearance (Cl). These parameters were calculated for 3g bd dose of Elores. In addition, simulations for dosage regimen of Elores 3g od were performed for each individual subject, using the identified Vd and Cl from 3g bd data; $C_{max}$ and AUC were calculated.

**Pharmacodynamic analysis**:
Clinical responses were evaluated on the basis of changes in clinical sign/symptoms from screening to end of therapy. The responses were categorized as 'cure'; 'failure'; and 'improved'. Bacteriological responses were evaluated on the basis of presence or absence of pathogens in cultures from the collected biological specimens (urine/blood) at screening (baseline), end of therapy and at follow-up stages. The responses were categorized as 'eradication'; 'failure', 'super-infection'; and 'resolved'. The duration of Elores treatment required to complete the therapy was also assessed for each subject [16.]

**DNA isolation and genetic characterization of ESBL strains:**
The clinical isolates were used to isolate the DNA as per alkaline lysis method. [22] A polymerase chain reaction (PCR) assay was used to identify the ESBL encoding genes using the specific primers, mainly TEM, SHV, Amp-C, CTX-M, QnrA, and QnRB[16, 23-27].

**Pharmacokinetic/Pharmacodynamic indices**:
The three PK/PD indices calculated were AUC/MIC ratio, $C_{max}$/MIC ratio and %T>MIC. The calculations were done for both the antibiotic components of Elores; ceftriaxone and sulbactam.

**Safety evaluations:**
All subjects, who received at least one dose of Elores, were evaluated for safety analysis. Adverse events (AEs) were monitored throughout the study and investigators categorized AEs as per causality assessment and also by the intensity of AEs (mild, moderate, or severe). Any abnormal laboratory parameter (biochemical, hematological, and/or urine analysis) finding was also included in safety analysis.

**Statistical analysis:**
Descriptive statistics were used for reporting all PK variables. The reduction in bacterial counts in *in-vitro* TKC data was compared after 12 h with the initial count by paired t-test (alpha=0.05). Log transformed data was used wherever applicable. The PK/PD indices were compared for Elores 3g bd vs od by one way ANOVA followed by Tukey test. The statistical analysis was done using SPSS version 20, IBM, USA.

**RESULTS:**
**Study population**:
All 12 subjects (7 males, 5 females) in the study completed the study. All subjects were positive for severe cUTI caused by ESBL gram-negative bacteria and were evaluated for pharmacokinetic, pharmacodynamic and safety analyses (**Table 1**). The vital parameters (blood pressure, electrocardiogram [ECG], heart rate) were normal.

**Microbiological investigations, time kill curve estimations, and MIC determinations:**
The ESBL gram-negative bacteria isolated from serum specimen were *E. coli* and *K. pneumoniae* (5 subjects) (**Table 1**). Th(7 subjects), e *in-vitro* TKC of ESBL pathogen present in serum collected at various time points in 12 subjects showed significant reduction in counts of colony forming units at 12$^{th}$ hour compared to initial count (data not shown). The $MIC_{Elores}$ for these pathogens were in the range of 16 µg/mL to 64 µg /mL (16 µg/mL [*E.coli* positive-6 subjects]; 32 µg/mL

[*K. pneumoniae* positive-5 subjects]; 64 µg /mL [*E.coli* positive-1 subject]; **Table 1**).

**TABLE 1: DISPOSITION OF SUBJECTS ALONG WITH DEMOGRAPHICS/BASELINE CHARACTERISTICS**

| Characteristics | Measurements |
|---|---|
| Planned/ Enrolled/ Completed the study/ Follow-up | N=12 |
| Pharmacokinetic analysis set | N=12 |
| Safety analysis set | N=12 |
| Age (years) | 42 ± 8.86 |
| Weight (Kg) | 61.5 ± 34.08 |
| Height (cm) | 155.75 ± 48.02 |
| Gender (Male/Female [%]) | Male (58.3%)/Female (41.7%) |
| Culture positive (name of pathogen [%]) | *Escherichia coli* (n=7; 58.3%) |
| | *Klebsiella pneumoniae* (n=5; 41.7%) |
| Severity of infection (mild/moderate/severe [%]) | Severe (100%) |

Wherever applicable, Mean (±Standard deviation [SD]) values are reported.

**Pharmacokinetic analysis**:

The plasma concentrations of ceftriaxone, sulbactam and EDTA at various time points after Elores 3 g bd dose regimen for each subject were evaluated using validated LC-MS/MS method. The plasma profile plots for ceftriaxone, sulbactam and EDTA were used to calculate PK parameters (**Table 2**). The $C_{max}$ 263.3 µg/mL; after an intravenous dose of Elores 3 g was reached in 0.25 h (1.75h after start of 90 min IV infusion).

Similarly, maximum plasma concentrations of sulbactam 29.2 µg/mL and EDTA 23.6 µg/mL were achieved in 0.25 h **(Table 2).** The average $AUC_{0-24}$ for ceftriaxone and sulbactam were 4322.7 and 229.3µg/mL*h, respectively **(Table 2)**. The PK parameters $V_d$, CL, $K_e$, $t_{1/2}$ for ceftriaxone and sulbactam (**Table 2**) were within the range of reported values [28, 29].

**TABLE 2: PHARMACOKINETIC PARAMETERS OF ACTIVE COMPONENTS OF ELORES (CEFTRIAXONE AND SULBACTAM), ALONG WITH NON-ANTIBIOTIC ADJUVANT EDTA IN SUBJECTS WITH COMPLICATED URINARY TRACT INFECTION AFTER ELORES 3g bd DOSE.**

| Pharmacokinetic parameters (N=12) | Elores (3g bd) Ceftriaxone (2 g) | Sulbactam (1 g) | EDTA (0.074 g) |
|---|---|---|---|
| $C_{max}$ (µg/mL) | 263.3 ±4.14 | 29.2±1.87 | 23.6±3.70 |
| $AUC_{0-24}$(µg/mL*h) | 4322.7±81.20 | 229.3±7.65 | 184.6±24.40 |
| $AUC_{0-\infty}$ (µg/mL*h) | 5180.2±107.76 | 229.3±7.65 | 184.7±24.43 |
| $V_d$ (L) | 7.47±0.21 | 23.9±0.90 | 5.4±1.23 |
| Cl ((L/h) | 0.84±0.02 | 8.6±0.30 | 3.49±0.11 |
| $t_{max}$ (h) | 1.75±0.00 | 1.75±0.00 | 1.75±0.0 |
| $K_{el}$ ($h^{-1}$) | 0.1±0.01 | 0.4±0.07 | 0.67±.016 |
| $t_{1/2}$ (h) | 5.6±1.80 | 1.8±0.25 | 1.08±0.24 |

$AUC_{0-24}$ = Area under plasma concentration curve for 0-24 h; $AUC_{0-\infty}$ = Area under plasma concentration curve for 0-infininity; Cl = Clearance; $C_{max}$ = Peak plasma concentration of drug achieved after first dose of twice daily regimen; $K_{el}$ = Elimination rate constant of the drug; $V_d$ = Volume of distribution; $t_{1/2}$ = Half-life of the drug; $t_{max}$ = Time required to achieve peak plasma concentration of the drug; All values are reported as Mean (±SD).

**Pharmacodynamic analysis:**

Clinical and bacteriological response was observed for all 12 subjects. The treatment duration was in the range of 7 to 8 days. Out of 12 subjects, complete clinical cure in terms of total relief and no-disease symptoms was shown by 11 (91.66%) subjects and improved response was shown by one (8.33%) subject. Similarly out of 12 subjects, bacterial eradication was shown by 11 (91.66%) subjects and persistence in culture was shown by only one (8.33%) subject (**Table 3).**

**TABLE 3: PHARMACOKINETIC PARAMETERS OF ACTIVE COMPONENTS OF ELORES (CEFTRIAXONE AND SULBACTAM) AT 3g bd DOSE IN SUBJECTS WITH COMPLICATED URINARY TRACT INFECTIONS**

| Subject No. | Pathogen Isolate (Gene Characterization) | MICElores (µg/mL) | Clinical Response | Bacteriological Response | Individual MIC (µg/ml) | | Pharmacokinetic/Pharmacodynamic indices | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | %T>MIC | | $AUC_{0-24}$/MIC | | $C_{max}$/MIC | |
| | | | | | Cef | Sul | Cef | Sul | Cef | Sul | Cef | Sul |
| 1 | *E. Coli* (TEM, SHV) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 44.8 | 405.7 | 42.6 | 24.8 | 5.5 |
| 2 | *E. Coli* (TEM, AmpC) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 44.8 | 406.7 | 44.4 | 24.9 | 5.7 |
| 3 | *K. Pneumoniae* (AmpC, TEM) | 32 | Cured | Eradicated | 21.3 | 10.7 | 100 | 34.4 | 200.6 | 22.2 | 12.6 | 3.1 |
| 4 | *E. Coli* (AmpC, TEM) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 44.8 | 400.7 | 44.5 | 25.4 | 5.0 |
| 5 | *K. Pneumoniae* (SHV, AmpC) | 32 | Cured | Eradicated | 21.3 | 10.7 | 100 | 34.4 | 202.9 | 20.8 | 12.3 | 2.7 |
| 6 | *E. Coli* (SHV, CTX-M) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 42.7 | 389.7 | 43.5 | 24.2 | 5.8 |
| 7 | *K. Pneumoniae (*TEM, CTX-M) | 32 | Cured | Eradicated | 21.3 | 10.7 | 100 | 33.3 | 202.5 | 21.2 | 12.2 | 2.7 |
| 8 | *E. Coli* (TEM, SHV) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 43.3 | 402.1 | 43.4 | 24.4 | 5.2 |
| 9 | *K. Pneumoniae* (SHV, AmpC) | 32 | Cured | Eradicated | 21.3 | 10.7 | 100 | 33.3 | 204.1 | 21.4 | 12.2 | 2.8 |
| 10 | *E. Coli* (AmpC, CTX-M) | 16 | Cured | Eradicated | 10.7 | 5.3 | 100 | 44.8 | 403.5 | 45.5 | 24.9 | 5.1 |
| 11 | *E. Coli* (QnrA, QnrB) | 64 | Improved | Persistence | 42.7 | 21.3 | 100 | 15.4 | 106.1 | 10.0 | 6.1 | 1.4 |
| 12 | *K. Pneumoniae* (CTX-M, TEM) | 32 | Cured | Eradicated | 21.3 | 10.7 | 100 | 39.6 | 202.7 | 20.8 | 12.3 | 2.7 |

%T>MIC: percentage of time in 24 h the concentration of antibiotic remained above MIC; $AUC_{0-24}$ = Area under plasma concentration curve for 0-24 h; $C_{max}$ = Peak plasma concentration of drug achieved after first dose of twice daily regimen; Cef = Ceftriaxone; MIC = Minimum inhibitory concentration; Sul = Sulbactam

Individual subject's pharmacokinetic/pharmacodynamic indices for ceftriaxone and sulbactam, using individual MICs for calculation. Subject wise genetic characterization of pathogen isolated from cultures and the bacteriological and clinical response after Elores 3g twice daily administration for 7.1±0.28 days

**PK/PD indices**:

The indices %T>MIC, $AUC_{0-24h}$/MIC and $C_{max}$/MIC were calculated for each subject **(Table 3)** for both ceftriaxone and sulbactam after Elores 3g bd administration. The index %T>MIC was 100% for all subjects for ceftriaxone, however, for sulbactam it ranged from 15.4 to 44.8%. The index $AUC_{0-24\ h}$/MIC for ceftriaxone ranged from 106.1 to 406.7 and for sulbactam it ranged from 10.0 to 45.5. The index $C_{max}$/MIC for ceftriaxone ranged from 6.1 to 24.9 and for sulbactam it ranged from 1.4 to 5.7. The desired level of PK/PD indices %T>MIC, $AUC_{0-24h}$/MIC, and $C_{max}$/MIC are ≥70%, 125 and 10 respectively (**Table 3, Fig. 1**). As a monotherapy, antibacterial action of ceftriaxone is best explained by index %T>MIC and thus the antibacterial effect is considered to be time dependent. However in combination with sulbactam, ceftriaxone achieved desired levels of all three PK/PD indices in all those subjects who were clinically and bacteriological cured from cUTI.

In order to see the effect of change in dose of Elores on these three PK/PD indices, individual subjects PK data was simulated for Elores 3g od dose (data not shown). The individual PK parameters $V_d$, Ke obtained from Elores 3g bd data were used for simulation. The peak plasma concentration obtained from simulated data was 248.2±6.29µg/mL for ceftriaxone and 31.9±1.92µg/mL for sulbactam. Similarly, $AUC_{0-24h}$ for ceftriaxone was 2264.9±33.69 µg/mL*h and for sulbactam it was 110.5±14.65 µg/mL*h. The $AUC_{0-24h}$ values for ceftriaxone and sulbactam from Elores 3g od

simulated data and 3g bd observed data show ≈2-fold change (ceftriaxone: 4322.7/2264.9; sulbactam: 229.3/110.5), suggesting simulation of 3g od did not show any deviation from dose proportionality, as far as exposure of drug is concerned.

The three PK/PD indices from the simulated data of Elores 3g od administration were calculated. The index %T>MIC was ranging from 66.7 to 100.0% for ceftriaxone, however, for sulbactam it ranged from 4.2 to 28.9%. The index $AUC_{0\text{-}24\ h}$/MIC for ceftriaxone ranged from 52.1 to 214.6 and for sulbactam it ranged from 3.3 to 22.5. The index $C_{max}$/MIC for ceftriaxone ranged from 5.7 to 24.9 and for sulbactam it ranged from 1.3 to 6.4. The PK/PD index $AUC_{0\text{-}24\ h}$/MIC for ceftriaxone and sulbactam for Elores 3g od is significantly lower than Elores 3g bd dose for MICs 16 and 32 µg/mL (**Table 4**). In addition, PK/PD index %T>MIC for sulbactam for Elores 3g od is also significantly lower than Elores 3g bd dose for MICs 16 and 32 µg/mL (**Table 4**).

**TABLE 4: PHARMACOKINETIC/PHARMACODYNAMIC INDICES COMPARISON OF ELORES 3 G BD VS OD DOSE. FOR COMPARISONS ANOVA FOLLOWED BY TUKEY TEST WAS PERFORMED USING DOSAGE REGIMEN AS "FACTOR".**

| $MIC_{Elores}$ (µg/ml) | Individual MIC (µg/mL) | | N | Dosage regimen (Elores) | %T>MIC | | $AUC_{0\text{-}24}$/MIC | | $C_{max}$/MIC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cef | Sul | | | Cef | Sul | Cef | Sul | Cef | Sul |
| 16 | 10.7 | 5.3 | 6 | 3g bd | 100 | 42.8# | 372.7* | 40.9$ | 23 | 5.1 |
| 32 | 21.3 | 10.7 | 5 | 3g bd | 100 | 35.0## | 202.6** | 21.3$$ | 12.3 | 2.8 |
| 64 | 42.7 | 21.3 | 1 | 3g bd | 100 | 15.4 | 106.1 | 10.0 | 6.1 | 1.4 |
| 16 | 10.7 | 5.3 | 6 | 3g od | 99.5 | 26.4 | 196.5 | 20.5 | 21.7 | 5.7 |
| 32 | 21.3 | 10.7 | 5 | 3g od | 99.1 | 18.8 | 106.9 | 10.5 | 11.6 | 3.0 |
| 64 | 42.7 | 21.3 | 1 | 3g od | 66.7 | 4.2 | 52.1 | 3.3 | 5.7 | 1.3 |

%T>MIC: percentage of time in 24 h, the concentration of antibiotic remained above MIC; $AUC_{0\text{-}24}$ = Area under plasma concentration curve for 0-24 h; $C_{max}$ = Peak plasma concentration of drug achieved after first dose of twice daily regimen; Cef = Ceftriaxone; MIC = Minimum inhibitory concentration; Sul = Sulbactam

# P<0.005 % T>MIC for sulbactam (individual MIC: 5.3 µg/mL) Elores 3g bd vs Elores 3 g od

## P<0.005 % T>MIC for sulbactam (individual MIC: 10.7 µg/mL) Elores 3g bd vs Elores 3 g od

* P<0.005 $AUC_{0\text{-}24}$/MIC ratio for ceftriaxone (individual MIC: 10.7 µg/mL) Elores 3g bd vs Elores 3 g od

** P<0.005 $AUC_{0\text{-}24}$/MIC ratio for ceftriaxone (individual MIC: 21.3 µg/mL) Elores 3g bd vs Elores 3 g od

$ P<0.005 $AUC_{0\text{-}24}$/MIC ratio for sulbactam (individual MIC: 5.3 µg/mL) Elores 3g bd vs Elores 3 g od

$$ P<0.005 $AUC_{0\text{-}24}$/MIC ratio for sulbactam (individual MIC: 10.7 µg/mL) Elores 3g bd vs Elores 3 g od

**Genetic characterization of ESBL strains**: Among 12 subjects, one subject did not show clinical cure, however, there was improvement in the condition. The bacteriological response also showed persistence. The genetic characterization revealed that ESBL *E.coli* for which $MIC_{Elores}$ was 64 µg/mL had QnrA and QnrB genes (quinolone resistant genes) (**Table 3**). It has been reported that isolates carrying Qnr genes are multidrug-resistant including cephalosporin [30, 31].

For all other subjects showing clinical and bacteriological cure against ESBL strains were positive for resistant genes viz; AmpC, CTX-M, SHV, and/or TEM (**Table 3**). The ESBL strains with specific genetic characterization were resistant to ceftriaxone, and Elores has shown activity against ESBL *E.coli* and *K. pneumoniae* except for the one strain positive for QnrA and QnrB genes[16, 32, 33].

**Safety analyses:**

There were no serious AEs reported in the study. Vitals and laboratory parameters measured were normal throughout the study for all study subjects.

**DISCUSSION:** The difficulty in targeting bacterial resistance mechanisms has limited our choices for therapy in combating antimicrobial resistance. Some of the proposed solutions include usage of optimized antibiotic dose and/or use of antibiotic combinations to lower the individual MIC and/or extend the spectrum of previously used antibiotics by combining them with drug or adjuvant that may not have antibacterial activity of their own. Among various infections encountered, severe cUTI are becoming one of the main reasons for hospital

administration and the antimicrobial resistant cases in these infections are rising. The choice to treat these infections is limited and even the available antibiotic choices are not able to provide the desired response [34], and in some cases it is as low as 6.5% (imipenem) and 1.3% (meropenem) [35].

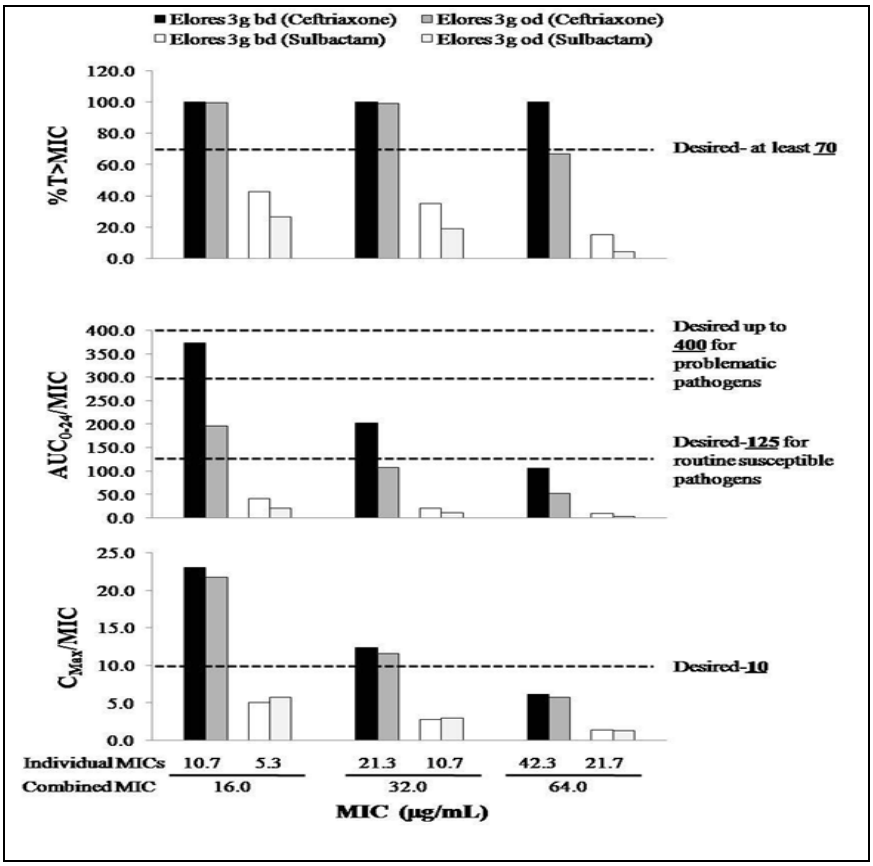


**FIG. 1: PHARMACOKINETIC/PHARMACODYNAMIC INDICES COMPARISON OF ELORES 3G BD VS OD DOSE WITH DESIRED LEVEL OF ATTAINMENT OF THESE INDICES**

The common pathogen showing resistances against routinely employed antibiotics (e.g., ceftriaxone, penems) include ESBL *E.coli* and *K. Pneumonia* [6, 9-12, 33]. One of the previously conducted study have shown that Elores (a combination of ceftriaxone and sulbactam along with non-antibiotic adjuvant) is effective and safe for treating mild, moderate, and severe UTI (both susceptible and resistant) [16]. The present study was undertaken to optimize Elores dose on the basis of PK/PD indices that can best explain the pharmacodynamic response (clinical/bacteriological cure) in cUTIs. The Elores 3g bd regimen was chosen on the basis of efficacy and safety achieved in previous study [16] involving treatment of various bacterial infections.

Twelve subjects positive for ESBL *E.coli* and *K. pneumoniae* completed the study and eleven of them were completely cured (clinically/ bacteriologically). The treatment duration was 7-8 days. All of the subjects were given Elores 3g bd dose and no SAEs were observed during the study. From literature it is evident that the ceftriaxone component of Elores shows time-dependent antibacterial effect [1, 17]. The % T>MIC calculated for these eleven subjects showed that the time for which the concentration of ceftriaxone remained above $MIC_{ceftriaxone}$ was 100% for a period of 24 h and for sulbactam it was ≈35-43% above $MIC_{sulbactam}$ (**Table 3, 4,** and **Fig. 1**). Though sulbactam has very mild antibacterial activity, it is the maintenance of sulbactam (40% time of 24 hr ≈ 5-6 h suggestive of 2-3 h maintenance after every Elores 3g infusion) concentration for first few hours of infusion that extends the activity of ceftriaxone.

In addition, EDTA added as an adjuvant, further improves the penetrability of ceftriaxone/sulbactam combination in bacterial colonies and assists the

antibacterial effect by catalytic action through chelation and by reduction in efflux transporter expression, bacterial biofilm eradication and inhibition of curli formation [13-15, 36]. The presence of sulbactam and EDTA for a period of 2-3 h around their $C_{max}$ extends the antibacterial spectrum of ceftriaxone. Both sulbactam and EDTA were unable to achieve the 70%T>MIC level for time-dependent antibacterial activity, this further suggests that sulbactam and non-antibiotic adjuvant has an initial role to target beta-lactamases and bacterial colonization formations for achieving enhanced anti-bacterial action attained by ceftriaxone.

The other PK/PD indices that characterize the concentration-dependent antibacterial activity include $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC ratios. The unique feature of Elores 3g bd regimen is that ceftriaxone achieved desired $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC ratios of 125 and 10, respectively which prevents further development of resistance In addition, for $MIC_{Elores}$ of 16µg/mL, ceftriaxone even reached to $AUC_{0\text{-}24\ h}$/MIC ratio of approximately 400 (**Table 4** and **Fig.1**), which can provide therapeutic efficacy against problematic pathogens.

The $AUC_{0\text{-}24\ h}$/MIC ratio of 400 has been reported in studies to treat severe cases of sepsis and prevent associated mortalities. Ceftriaxone which is considered to have time dependent antibacterial action has also attained the desired levels of $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC for routine and problematic pathogens, probably by virtue of synergistic action provided by other components of Elores (**Fig.1**). The $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC ratios for sulbactam and EDTA were very low which further strengthens their initial role in providing boost to the activity of ceftriaxone [2, 4, 5, 18, 37]. The additional attainment of ceftriaxone for these PK/PD indices warrants further studies that can specifically address the concentration dependent action of ceftriaxone, when given in combination as Elores.

Only one subject did not show any clinical/bacteriological cure; however, Elores did provide some benefit. The $MIC_{Elores}$ requirement for the subject was 64µg/mL. Among the PK/PD indices only %T>MIC was 100 %, which was above the the desired level; however, $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC ratios were much below the desired levels (**Fig.1).** The gene characterization further suggests that ESBL *E. coli* strain was positive for quinolone resistant genes; QnrA and QnrB. Various broad spectrum cephalosporins alone are resistant to bacterial strains carrying QnrA and QnrB, and also Elores in previous clinical studies have not been shown to be active against this strain [16, 31, 33, 37].

This further suggests that strains requiring MIC of 64 µg/mL and having QnrA and QnrB resistant genes are quite difficult to be treated by Elores 3g bd dose. Secondly, it might be possible that for ESBL strains attainment of $AUC_{0\text{-}24\ h}$/MIC and $C_{max}$/MIC ratios might be required.

Ceftriaxone in Elores 3gbd dose provided 100%T>MIC, but if Elores 3g od would have been given then will the %T>MIC attained be enough to provide the clinical/bacteriological cure? To understand the effect on PK/PD indices by changing the dosage regimen, Elores 3g od data was simulated from same 12 subjects using their individual $V_d$ and $K_e$ values obtained form 3 g bd data. For eleven subjects that previously showed clinical and bacteriological cure, the %T>MIC for ceftriaxone was ≥99% for both $MIC_{Elores}$ 16 and 32 µg/mL and in addition $C_{max}$/MIC ratios were also close to desired level of 10 for both $MIC_{Elores}$ 16 and 32 µg/mL. However, $AUC_{0\text{-}24h}$/MIC ratio was below 125 at MIC of 32 µg /mL The PK/PD indices for sulbactam after 3g od simulation followed the same pattern as it had followed for 3g bd dose (**Table 4** and **Fig. 1)**.

From the PK/PD indices obtained for 3g od dose, it appears that this dose can treat mild to moderate UTIs wherein requirement for $MIC_{Elores}$ is less than 16 µg/mL, however for severe cUTI resistant infections wherein $MIC_{Elores}$ 16-32 µg/mL is required, Elores 3g bd should be the choice.

**CONCLUSION:** The PK/PD indices are good predictors for efficacy of antibiotics when used alone. The PK/PD indices of combination therapy like Elores should be seen in entirety rather than explaining the efficacy o on the basis of single PKPD index (i.e. %T>MIC). The situation

becomes more complex when the activity against resistant bacteria is to be explained in terms of single PK/PD index. In the present study involving treatment of cUTIs caused by ESBL producing strains with Elores, the composite of all PK/PD indices should be considered to achieve the best antibacterial action. From the PK/PD indices achieved, and the MIC requirement, it is recommended that Elores 3g od dose can treat mild to moderate UTIs, and Elores 3g bd can treat severe cUTI caused by resistant strains.

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