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DOSE OPTIMIZATION OF CEFTRIAXONE-SULBACTAM COMBINATION IN PEDIATRIC USING *IN-VITRO* SYSTEMS AND STOCHASTIC SIMULATIONS APPROACHES

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ABSTRACT: Increase in complexity and cost associated with pediatric safety and efficacy studies have reduced the interest of pharmaceutical companies to study bio-therapeutics in pediatric population. Dose recommendations are being made as per patient body weight or body surface area, with the assumption of linearity in body size dose adjustments. In the present study, newer approach i.e. flat fixed age-based dosing is explored. Stochastic simulations were performed on once-a-day (OD) and twice-a-day (BD) dosage regimens of a fixed dose combination (FDC) of ceftriaxone/sulbactam (2/1) to identify a promising dose range that can effectively evaluate exposure-response (E-R) relationships of the FDC against bacterial infection in pediatric population. Selected dose range of 60-120 mg/kg of FDC was evaluated against ESBL infection using in-vitro systems (dilution method and hollow fiber method). Effect of dose, dose frequency, severity of infection and age groups were analyzed in age-based subgroups i.e. neonates, infant, child and toddler. Recommended dose for infants, child and toddler were 75-120 mg/kg based on severity of infection. However, physiologically-induced reduction in drug's clearance in neonates had resulted in lower dose recommendation against bacterial infection. Neonate dose of 120 mg/kg OD was best among all the exposures tested and its potential dose-related toxicity can be reduced either by reducing the doses (in mild infection) or by fractionating the dose into BD regimen (in severe infection). The E-R relationships of 120 mg/kg OD and BD dosage regimens in neonates were confirmed using *in-vitro* hollow fiber system.

INTRODUCTION: Dose optimization of a drug depends on the characterization of pharmacokinetic (PK) and pharmacodynamic (PD) relationships. The investigation of these relationships for anti-infective, particularly antibacterial agents is very critical in case of pediatric population. Physiological development during childhood can produce significant effect on drug absorption, distribution, metabolism and excretion [1-3] (**Table 1**).

In spite of these physiological changes and its altered PK, physicians are forced to make empirical assumptions to treat children on trial-and-error basis [4]. Pediatric doses are generally recommended based on two dosing strategies i.e. flat fixed dosing, and body size-based dosing. The most common approach is to adjust dose based on body weight/body surface area, with the assumption of linear maturation relationship between weight and drug clearance.

This approach obviously simplifies the impacts of developmental factors and clinical conditions on PK parameters, plus increases the complexity in terms of dose preparation and administration; and increases risk of medical error. Alternative (newer) approach is to provide a fixed dose (mg/kg) for a certain age or certain body size age group. This will

ease the preparation of drug and its administration, reduces the risk of medical error, better compliance and cost effectiveness.

TABLE 1: PHYSIOLOGICAL DEVELOPMENT DURING CHILDHOOD AND ITS IMPACT ON ADSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) PROCESS [2]

Process	Physiological changes after birth
Adsorption	Gastrointestinal absorption, secretion, motility, metabolism and transport
Distribution	Body composition, tissue perfusion and plasma protein binding
Metabolism	Maturation in cytochrome P450 enzyme-mediated metabolism, phase II metabolism
Excretion	Maturation of glomerular filtration and renal tubular function

The above-mentioned alternative approach was employed for the dose optimization of a FDC of ceftriaxone/sulbactam (2/1), where paediatric population was divided into four age groups i.e. neonates (0-30 days), infants (1-24 months), toddler (2-6 years) and child (6-12 years) [5]. These age groups differ in their important body functions, which directly affect the adsorption, distribution and clearance of all drugs [2, 3]. Focusing on few crucial body functions, three age groups i.e. infants, toddlers and child do not differ significantly in their total body water (TBW), extracellular fluids (EF), intracellular fluids (IF), glomerular filtration rate (GFR) and renal plasma flow (RPF) (**Table 2**).

However, neonates are immature child [3]. They have higher TBW, higher EF and lower IF, which results in higher distribution of drugs. Similarly, lower GFR and RPF of neonates results in decreased clearance of drugs as compared to other age groups [2, 6]. These different physiological conditions of pediatric age groups (especially neonates) alter the PK of drugs and thus, necessitates dose optimization based on exposure-response relationship of FDC to avoid dose-related toxicities [3].

TABLE 2: COMPARISON OF TOTAL BODY WATER (TBW), EXTRACELLULAR FLUIDS (EF), INTRACELLULAR FLUIDS (IF), GLOMERULAR FILTRATION RATE (GFR) AND RENAL PLASMA FLOW (RPF) OF DIFFERENT AGE GROUPS (NEONATES, INFANTS, TODDLER, AND CHILD) [2]

	TBW (%)	EF (%)	IF (%)	GFR (mL/min)	RPF (mL/min)
Neonates	85	50	35	30-60	20-400
Infants	60	23	37	50-120	400-600
Toddler	60	20	40	80-140	500-700
Child	60	20	40	80-140	500-700

Dose adjustment in pediatric should lead to consistent exposure as compared with adults and should decreases inter-subject variability in the drug exposure. The recommended dose of ceftriaxone in pediatric is 50-75 mg/kg with a maximum of 100 mg/kg in severe cases like bacterial meningitis[7]. The dose will be equivalent to Elores dose of 75 -112.5 mg/kg with a maximum dose of 150 mg/kg in severe cases, after accounting for sulbactam presence in the FDC at 2/1 (w/w) ratio. However, the above extrapolation does not account for the synergistic possibility between the active components of FDC in terms of antibacterial efficacy and possible dose reduction.

In the present study, stochastic simulations of antibacterial effect of the FDC were performed at different exposures and promising dose ranges were selected. The selected dose range were evaluated *in-vitro* (dilution and hollow fiber system) for their PD effect in pediatric patients; and dosage regimens were recommended based on the severity of infection and age group of pediatric patients.

MATERIAL AND METHODS:
Compounds, microorganism and determination of MIC: The FDC of ceftriaxone/sulbactam (2/1) vials (Elores) were obtained from Venus Remedies Ltd, H.P., India. Characterized ESBL clinical isolates with reduced susceptibility to ceftriaxone were taken from clinical isolate bank of Venus Medicine Research Centre, Baddi. The media Mueller-Hinton broth (Becton Dickinson, Sparks, MD) was used to perform all *in-vitro* studies involving MIC determination, bacterial kinetics, dose ranging and fractionation studies. Fiber Cell hollow-fiber C2011 cartridges were obtained from Fiber Cell Systems, Inc. (Frederick, MD).

Bacterial density, minimum inhibitory concentration and fractional inhibitory concentration index determinations:
The bacterial density (CFU [colony forming units]) and MIC determinations were conducted according to Clinical Laboratory Standard Institute guidelines [8]. The FIC index was determined using checkerboard method [9].

***In-vitro* system:**
One-compartment dilution system:
The *in-vitro* modeling of the FDC was done using a single-compartment chemostat infection system as described previously [10, 11]. Briefly, the chemostat system assembly consisted of a 500 mL glass central reservoir chamber with ports for the addition and removal of media via silicone tubes connected to peristaltic pumps, injection of drug (antibiotic combination) solution, and removal of medium samples. Prior to each experiment, ESBL colonies were grown overnight to obtain a starting inoculum of 10^8 CFU/mL in 500 mL central reservoir flask containing media. An aqueous solution of FDC was prepared. The "in" and "out" flow rates from the central reservoir were adjusted as per the half-life of active components [12, 13]. Samples from the central reservoir were collected at different time points from 0 to 24 h post-FDC addition and were analysed for bacterial densities.

Hollow fiber system:
The selected drug exposures were also evaluated in hollow fiber model, as described previously [14]. Initial inocula of 10^8 CFU/mL was achieved by injecting 10 mL of ESBL producing *E. Coli* into extra-capillary space, which was separated from central reservoir by semi-permeable hollow fibers. After 2 h of incubation in media, FDC was injected into central reservoir. Drug can freely cross back and forth between the extra-capillary space and central reservoir, so that ESBL *E. coli* were exposed to same drug concentration as those in the central compartment. Central compartment was connected to inlet and outlet reservoir through a pump whose flow rates were adjusted as per the half life of active components. Samples were collected from extra-capillary space for three days and were analyzed for drug concentration and bacterial densities.

Pharmacokinetic / pharmacodynamic driver analysis: The FIC curves for ceftriaxone and sulbactam combinations were generated from the exposure-time curves. Briefly, the concentrations of each antibiotic at every time-point were divided by their respective MIC contributions towards MIC_{comb} to obtain $FIC_{ceftriaxone}$ and $FIC_{sulbactam}$ values. These values were added (FIC_{comb}) for each time points and plotted against time. The resultant FIC-time profile was fitted to one compartment model and $\%T>MIC_{comb}$ (percentage of time during which FIC concentration is above MIC_{comb}) was calculated [15, 16].

Stochastic simulations:
Simulations for 1000 adult subjects were performed to determine how likely the FDC dose of 30, 60, 75, 90 and 120 mg/kg would achieve $70\%T>MIC_{comb}$ at different values of MIC i.e. 1, 4, 8, 16, 32, 64 µg/mL. In case of infants, toddler and child, the clearance and half life of 0.25 L/h of 6.93 hr, respectively were used for ceftriaxone, whereas the same parameters were 9.01 L/h and 0.45 h respectively for sulbactam [17]. In case of neonates, ceftriaxone's clearance and half life of 0.12 L/h and 15.4 hr, respectively were used; whereas sulbactam has 0.4 L/h and 9.24 hr of clearance and half life, respectively [18, 19]. The concentration-profiles of once-a-day and twice-a-day dosing regimens were generated using CL and k_e of each simulated subjects and $\%T>MIC_{comb}$ was calculated for all simulated subjects for different exposures at all MIC values. The PTA was then defined as percentage of simulated subjects showing $\%T>MIC_{comb}$ of more than 70.

Data analysis:
Descriptive statistics were used for reporting all PK variables and summary tables were prepared using mean, standard deviation (SD), median, and range (whichever appropriate). Log transformed data was used wherever applicable. The statistical analysis was done using GraphPad Prism (version 4.01, GraphPad software, San Diego, CA).

RESULT AND DISCUSSION:
The pharmacokinetic and pharmacodynamic (PK/PD) relationship of a drug dictates the selection of dose regimen in adult as well as special populations. Focusing on a special population i.e.

pediatric, physiological development during childhood can significantly impact drug absorption, distribution, metabolism and excretion [1, 2]. The objective of dose adjustment in pediatric population was to give consistent drug exposure as compared with adults and reduces inter-subject variability in the drug exposure. In the present study, dose recommendation of the FDC of ceftriaxone and sulbactam was done for pediatric population. The recommended dose of ceftriaxone alone in pediatric is 50-75 mg/kg with a maximum of 100 mg/kg in severe cases like bacterial meningitis [7]. The dose would be equivalent to FDC dose of 75 -112.5 mg/kg with a maximum dose of 150 mg/kg in severe cases, after accounting for sulbactam presence in the FDC at 2/1 (w/w) ratio. However, this extrapolation undermines synergistic effect of sulbactam and ceftriaxone, which should potentially reduce the dose required to attain the target exposure.

To begin with, the pediatric population was divided into four groups based on their age, i.e. neonates (0-30 days), infants (1-24 months), toddler (2-6 years) and child (6-12 years) [5]. The stochastic simulations were then performed to identify a MIC value and dose range, which can be employed to evaluate E-R relationship of FDC *in-vitro*. The pop-PK parameters of all four groups were obtained from literature [18,19] and employed in carrying out stochastic simulations (For pop-PK parameters, refer to "stochastic simulations" sub-section of "Materials and methods" section). The simulations were performed for OD and BD dose range of 30- 120 mg/kg at MICs of 1, 4, 8, 16, 32, and 64 μg/mL for all four age groups (**Fig. 1**). As shown in the **Fig. 1**, neonates have shown higher probability of attainment (PTA) at higher MICs (especially at MIC of 8μg/mL) as compared to infants, toddler and child.

Higher PTA in neonates was the result of physiologically- induced reduction in clearance of active components of the FDC. In other three age groups i.e. infants, toddler and childs, the effect of selected doses were seen. As dose is administered on weight basis (mg/kg) and weight is generally proportional to age (at least in pediatric group), amount of dose generally increases from infants to

child, resulting in corresponding increase in PTA. For instance, dose of 90 mg/kg OD has the PTA of ~30%, which rises to 60% in toddler and 80% in child (**Fig. 1**). The lower therapeutic effect can be rescued by BD dosing, which generally increases the drug exposure as compared to OD regimen (compare PTA of OD vs. BD regimen for all four age groups).

Focusing on selecting MIC value, ceftriaxone alone have shown MIC of 128 μg/mL against resistant ESBL infection. However, incorporation of sulbactam in ceftriaxone-sulbactam combination drastically reduces the MIC to 8 μg/mL. Additionally, figure 1 has shown maximum fluctuation in PTA with respect to increased doses in all four age groups at MIC of 8 μg/mL. Thus, ESBL producing *E. coli* strain of 8 μg/mL MIC was selected to evaluate exposure-response relationship of ceftriaxone-sulbactam combination. In term of selecting dose range, 60 mg/kg is the lowest dose (both in OD or BD regimen) where the appreciable PTAs at MIC of 8μg/mL have been achieved in all four age groups. Dose of 120 mg/kg was the highest dose analyzed in the stochastic simulation for evaluating its antibacterial potential in terms of PTA. Dose of 150 mg/kg (equivalent to 100 mg/kg of ceftriaxone alone) was not incorporated in **Fig. 1** as maximum PTA was already achieved at lower doses (<150 mg/kg), and thus higher PTA for 150 mg/kg dose was more than obvious. Therefore, dose range of 60-120 mg/kg was selected to evaluate the E-R relationships of ceftriaxone-sulbactam combination *in-vitro* against ESBL producing *E. coli* infection.

The E-R relationship of 60, 90 and 120 mg/kg were evaluated using *in-vitro* dilution and hollow fiber system. Preliminary studies were performed in in-vitro dilution system because of its simple design, high time efficiency and great potential to predict direct E-R relationships of a drugs. The final results were then tested in hollow fiber system to confirm the preliminary results of *in-vitro* dilution system and also to evaluate the recommended dose in more realistic condition. **Table 3** has shown the PK parameters employed in the *in-vitro* studies of the FDC of ceftriaxone/sulbactam (2/1) against ESBL bacterial infection.
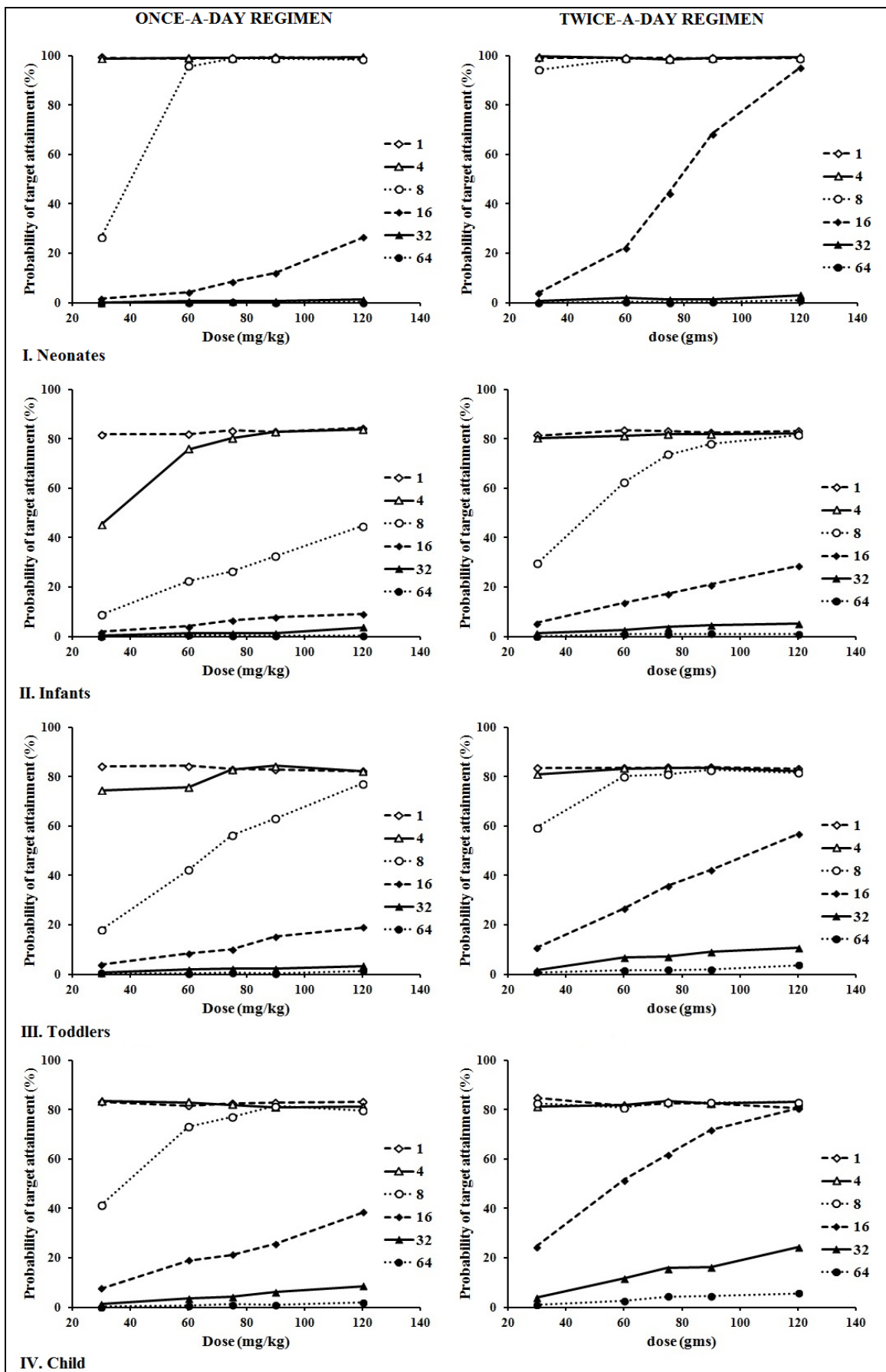
FIG. 1: STOCHASTIC SIMULATIONS OF ONCE-A-DAY(OD) AND TWICE-A-DAY(BD) DOSAGE REGIMEN OF 30 - 120 mg/kg DOSE RANGE AGAINST MIC RANGE OF 1-64 mcg/mL IN NEONATES (I), INFANTS (II), TODDLERS (III) AND CHILD (IV)

TABLE 3: PK PARAMETERS EMPLOYED IN *IN-VITRO* STUDIES TO MIMIC PK PROFILE OF CEFTRIAXONE AND SULBACTAM IN NEONATE, INFANT, CHILD AND TODDLERS [20, 21]

Age group	Dose (mg/kg)	Ceftriaxone			Sulbactam		
		V_D (L/kg)	K_e (h^{-1})	C_{max}	V_D (L/kg)	K_e (h^{-1})	C_{max}
Neonate	60	3.48	0.05	16.67	2.47	0.09	29.35
	90	3.48	0.05	25	2.47	0.09	44.03
	120	3.48	0.05	33.33	2.47	0.09	58.7
Infant	60	3.9	0.11	20.5	3.4	0.90	29.41
	90	3.9	0.11	30.75	3.4	0.90	44.12
	120	3.9	0.11	41.01	3.4	0.90	58.52
Child	60	14.43	0.15	17.78	12.58	0.93	11.65
	90	14.43	0.15	26.67	12.58	0.93	17.48
	120	14.43	0.15	35.56	12.58	0.93	23.31
Toddler	60	8.5	0.11	23.53	5.61	0.91	15.7
	90	8.5	0.11	35.29	5.61	0.91	23.55
	120	8.5	0.11	47.06	5.61	0.91	31.4

V_D: volume of distribution; K_e: elimination constant; C_{max}: maximum concentration

In one-compartment *in-vitro* infection model, ESBL- *E. coli* strain of 10^{10} CFU/mL were added to central compartment (500 mL) and allowed to incubate for 3-4 h. Drug was added as well as eliminated to/from central compartment as per the clearance of the drugs tested. In order to mimic the severe infection, second inoculum of *E. coli* bacteria were added post-9.5 h after drug exposure, which will compensate bacterial loss and increase bacterial load. Bacterial growth control experiments (without drug) were performed for both mild and severe infection The PD effects (drug induced-bacterial killing) of all experiments were measured over 9.5 h and 24 h; and was adjusted in reference to bacterial growth controls (i.e. normal rate of bacterial killing without drug treatment). The study design consist of 4 age groups (neonates, infant, toddler, and child), 3 doses (60, 90 and 120 mg/kg), 2 dose frequency (OD and BD) and 2 stages of infection severity (mild and severe).

Effect of dose in severe infection:
To start with, exposure of two age groups i.e. infant and toddlers were evaluated for bacterial killing against severe ESBL infections at dose of 60, 90 and 120 mg/kg OD (**Fig.2**). The objective was to observe the impact of amount of dose on bacterial killing efficiency. As expected, increase in dose increases the drug exposure, thus better therapeutic effect was observed in both age groups. Focusing on infants, there was an increase of 2.15 fold log reduction in bacterial count when dose was increased from 60 to 120 mg/kg after 9.5 h post-drug-exposure. The same trend was observed in

toddler where the bacterial killing was increased from 2.41 to 3.63 on doubling dose of 60 mg/kg post-9.5 h of drug exposure. The appreciable log reductions of 5.74 and 3.63 at dose of 120 mg/kg were observed in infant and toddler, respectively (**Fig. 2**). However, the net bacterial reduction of all doses of FDC over 24 h was very low (close to zero) in both infant and toddler. This suggest that post-24 h of drug addition, the PD effect subsides as bacterial growth rate dominates over its killing kinetics.
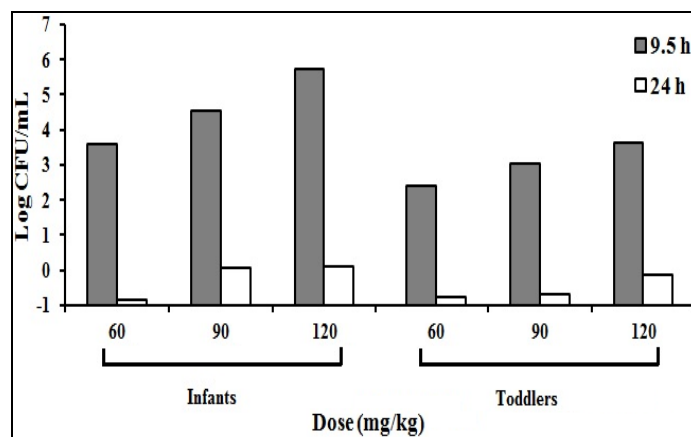

FIG. 2: IMPACT OF INCREASE IN DOSE OF CEFTRIAXONE-SULBACTAM ON *IN-VITRO* DRUG-INDUCED BACTERIAL KILLING OVER 9.5 h AND 24 h IN INFANTS AND TODDLERS AGAINST ESBL INFECTION

In other words, initial reduction in bacterial count occurred due to initial concentrations; but after ~9.5 h (equivalent to 1½ half life of ceftriaxone and 8 half life of sulbactam), the active concentration of the FDC was not enough to sustain bacterial killing kinetics above the bacterial growth kinetics. The

observations were in concordance with the results of "dose optimization of ceftriaxone-sulbactam in adults" (in-house study), where the given exposures have shown similar bacterial killing against ESBL infections.

Effect of age group in severe infection:
The rationale of sub-optimal effect of infants and toddlers was not valid in neonates as drugs have longer half life in neonate population due to different physiological conditions [3]. Therefore, impact of age groups on PD effect of the FDC was identified by comparing the therapeutic response of neonates, infant, toddler, and child against severe ESBL infection. The promising therapeutic responses of 120 mg/kg OD dose have made itself an ideal candidate for further investigation. The reduction in bacterial count was observed at 9.5 h and 24 hr after the drug exposure (**Fig. 3**). Similar **Fig. 2**, dose of 120 mg/kg OD have shown similar log reduction of 3.5-5.2 across all age groups after 9.5 h of drug exposure; and the therapeutic response of infants, toddler and child was diminished post-24 h of drug exposure. However, neonates have shown four-fold bacterial reduction against severe ESBL infection after 24 h of drug exposure. The therapeutic effect was a result of the maintenance of higher drug exposure in neonates, due to their longer half lives, i.e. 13.86 h and 7.7 h of ceftriaxone and sulbactam, respectively. In case of other age groups i.e. infants, toddler and child, both drug concentrations dropped after 12 h to such an extent that bacterial growth rate dominates over bacterial killing rate, making the net therapeutic effect sub-optimal.
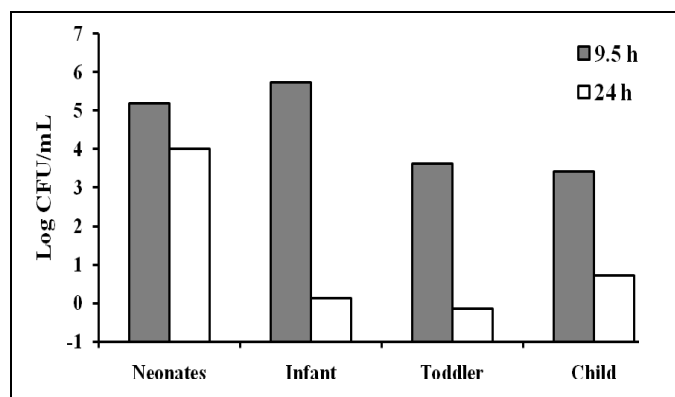

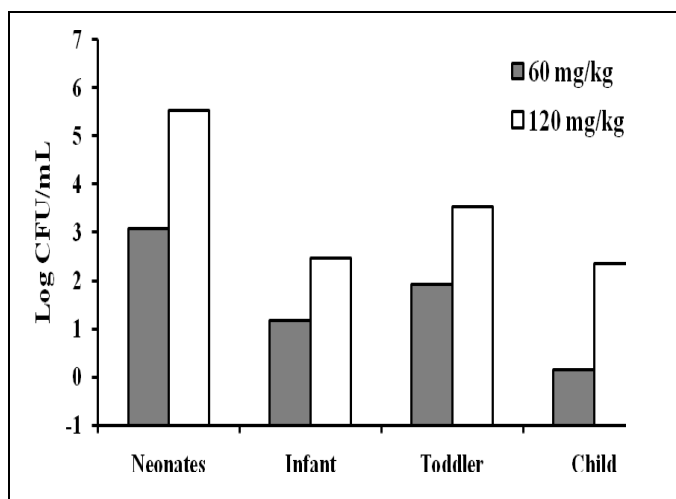**FIG. 3: IMPACT OF 120 MG/KG DOSE OF CEFTRIAXONE-SULBACTAM COMBINATION ON *IN-VITRO* DRUG-INDUCED BACTERIAL KILLING OVER 9.5 h AND 24 h ACROSS ALL FOUR AGE GROUPS (NEONATES, INFANTS, TODDLERS AND CHILD) AGAINST ESBL INFECTION**

Effect of dose and age group in mild infection:
The dismal therapeutic effect of FDC in infant, toddler and child age groups could be rescued either by increasing the drug exposure or changing the severity of infection. Both approaches were already shown in stochastic simulations to boost the PD effect of the drug against ESBL infection (**Fig. 1**). The first approach basically highlights the importance of increased drug exposure for attaining better PD effect. The higher PD effect was recorded in terms of higher PTA (refer **Fig.**1, compare BD vs. OD regimen) or higher *in-vitro* bacterial killing (refer **Fig. 2**, compare 120 mg/kg vs. 60 mg/kg). Focusing on second approach employing *in-vitro* studies, it was observed that all of the age groups have shown promising results up to 9.5 h after drug exposure (**Fig.3**), which infer that the selected dose should be effective against mild bacterial load. To test the validity of this statement, an hypothesis was generated which states that the selected dosage regimen would be effective against mild infections due to low bacterial load and enough drug exposure (that can maintain the bacterial killing rate higher than its growth rate).

The above-mentioned hypothesis was tested by carrying out *in-vitro* E-R studies of selected dosage regimen of FDC against mild infection. In order to mimic mild infection, the second inoculum of bacteria was not added to the central compartment of *in-vitro* chemostat assembly. After 3 h of incubation with ESBL bacteria, dose of 60 and 120 mg/kg was added and bacterial killing was recorded post-24 h of drug exposure (Figure 4). It was observed that all of the four age groups have shown significant bacterial killing as per the drug exposure to the infection loaded *in-vitro* system. Focusing on the dose of 60 mg/kg, log bacterial reduction of 1.93 and 1.18 in toddlers and infants respectively were observed. However, the same dose have shown 3.07 fold log reduction in neonates, stressing the high PK/PD variability among pediatric age group and advocating the need of dose individualization for pediatric patients.

On escalating the dose exposure to 120 mg/kg, significant improvements in bacterial killing were recorded. The log reduction in bacterial count was 5.54, 2.47, 3.53 and 2.36 for neonates, infants,

toddler and child respectively (**Fig. 4**). The observed PD effect could be explained based on drug concentration of FDC, bacterial load and PK characteristics of the pediatric age group. For instance, toddler have 50 mg/L and 35 mg/L of ceftriaxone and sulbactam, respectively; whereas, child have 36 mg/L and 24 mg/L of ceftriaxone and sulbactam. The relative lower concentration of active components of FDC in child results in lower therapeutic as compared to toddler PD response [compare bacterial reduction of 2.36 (child) vs. 3.53 (toddler)].

FIG. 4: IMPACT OF 60 mg/kg AND 120 mg/kg DOSE OF CEFTRIAXONE-SULBACTAM COMBINATION ON *IN-VITRO* DRUG-INDUCED BACTERIAL KILLING OVER 24 h ACROSS ALL FOUR AGE GROUPS (NEONATES, INFANTS, TODDLERS AND CHILD) AGAINST ESBL INFECTION

Recommendation for infants, toddler and child dosing:
Infants, toddler and child do not differ significantly in their PK characteristics, thus same dose scale (mg/kg) could be utilized as per their body weight or body surface area. In case of mild infection, the optimal therapeutic effect was seen in all three age groups at 120 mg/kg OD (**Fig. 4**). Therefore, 120 mg/kg OD of FDC (equivalent to 80 mg/kg of ceftriaxone) is recommended for all the three age groups in mild infection. In case of severe infection (or resistant bacteria), adequate therapeutic response was not seen at 120 mg/kg OD in *in-vitro* system, which is in accordance with literature recommending higher dosing (100 mg/kg ceftriaxone equivalent to 150 mg/kg of the FDC) for severe infection [7]. The drug exposure should be decided based on severity of infection, characteristic of resistant microbes and tolerability

of host. If higher exposure is needed, dose should be fractionated to maintain the required drug exposure while keeping its toxicity low.

Recommendation for Neonates dosing:
As explained before, neonates are physiologically different from other age groups and needs a special attention in terms of dose recommendation [3]. In the present study, FDC dose of 120 mg/kg OD have shown bacterial log reduction of 5.54 and 4 against mild and severe infection, respectively. The good PD effect is the result of reduced clearance in neonates, which maintain high drug exposure for a longer time.

However, the same high exposure also raises the concern of systemic toxicity in neonates. The objective was to minimize the adverse events while maintaining the required drug exposure in patients. In case of mild infection, the bacterial killing was very high (almost at saturation) at 120 mg/kg and thus lowering of dose can be done conveniently without compromising the therapeutic response of the FDC. In other words, neonates' dose should not exceed 120 mg/kg OD in mild infection to avoid any possibilities of adverse events. For the selection of the lower limit of dose, dose range of 60-120 mg/kg was evaluated *in-vitro* for E-R relationship against ESBL infection (data not shown).

It was observed that dose of 75 mg/kg have shown reasonable reduction in bacterial count (3.22) against *E. coli* induced ESBL infection The results were in concordance with literature [7], where 50 mg/kg of ceftriaxone (equivalent to 75 mg/kg of ceftriaxone-sulbactam combination) was recommended as a lower limit of dose in pediatric patients. To sum up, dose of 75 -120 mg/kg OD was recommended to neonates in case of mild bacterial infection.

Focusing on severe bacterial infection, the bacterial reduction is already at its lower limit (4 log reduction in bacterial count). Further decrease in dose might compromise the therapeutic response of the FDC. To address the concern of exposure-toxicity relationship while maintaining the same drug exposure, the PK/PD driver of efficacy of ceftriaxone/sulbactam was first analyzed. As per

literature [22, 23], both FDC components i.e. ceftriaxone and sulbactam exhibit time-dependent bacterial killing. Thus, plot of $\%T>MIC_{comb}$ (percentage of time during which fractional inhibitory concentration remained above MIC_{comb} i.e. 8) of different FDC exposure versus corresponding change in bacterial counts (measured as Δ CFU/mL) was drawn (**Fig.5**). Correlation coefficient value of 0.88 was observed which confirms that $\%T>MIC_{comb}$ is highly correlated with the antibacterial efficacies of the FDC; and thus can be employed as a tool to estimate the pharmacodynamic effect of the FDC.
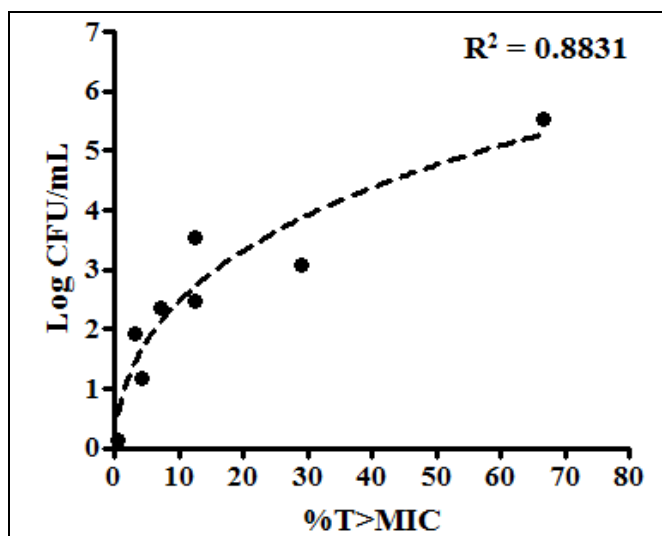
FIG.5: THE PLOT BETWEEN $\%T>MIC_{comb}$ AND CORRESPONDING CHANGE IN BACTERIAL COUNTS (MEASURED AS CFU/mL)

Dose of 120 mg/kg was then fractionated into twice-a-day and thrice-a-day dose frequencies and $\%T>MIC_{comb}$ values were calculated. It was observed that $\%T>MIC_{comb}$ values of once-a-day and twice-a-day regimens were 66.67 % and 65.63 %, respectively. Similar values of PK/PD driver indicate similar therapeutic responses for both dosage regimens in *in-vitro* system. Thus, 120 mg/kg OD was then fractionated into twice-a-day dosing and corresponding change in CFU was observed in both one compartment *in-vitro* dilution system and hollow fiber system. In case of *in-vitro* dilution system, both OD and BD dose frequency had displayed similar bacterial log reduction of 4 and 3.78, respectively. Similar results were obtained from hollow fiber system, where the bacterial killing of 2.47 and 2.04 log reductions were observed for once-a-day and twice-a-day regimen, respectively. The relatively lower

bacterial reduction in case of hollow fiber system as compared to *in-vitro* dilution system was a result of more rigorous and closer-to-reality set-up of hollow fiber system; where the bacterial infection was kept in extracellular matrix secluded from central compartment through a semi-permeable membrane.

The hollow fiber set-up elevates the severity of bacterial infection, manifested as lower CFU reduction, by preventing bacterial loss and providing optimal condition for resistance development. The results of both *in-vitro* systems have shown similar antibacterial efficacy for 120 mg/kg OD and BD dosage regimens in neonates. To summarize, in case of severe bacterial infection, dose of 120 mg/kg should be fractionated into twice-a-day dosage regimen to maintain the same therapeutic efficacy while keeping the possibilities of adverse events low.

CONCLUSION: The PK parameters of drugs in pediatric populations are different from adult population, which results in variability in drug exposure and its response. This necessitates the optimization of the dose as per the PK characteristics of the pediatric patients. Pediatric population was divided into four groups based on their age, i.e. neonates, infants, toddler and child. Based on pop-PK parameters of the active component of FDCs in pediatric populations, stochastic simulations were performed and promising dose range and dose frequency were selected to evaluate dose-exposure-response relationship. Exposure-response relationships of FDC were evaluated against mild and moderate infection using *in-vitro* dilution and hollow fiber system. Impact of dose, dose frequency, severity of infection and age of the patients were evaluated for dose recommendation.

In case of three age groups i.e. infant, toddler and child, FDC dosage regimen of 120 mg OD was recommended for mild infections; whereas its BD dosing was recommended in severe bacterial infection to increase the drug exposure while keeping a check on dose-related side effects. Neonates have different PK parameters as compared to other three age groups due to different

physiological conditions. For mild infection in neonates, 75-120 mg/kg OD of ceftriaxone/ sulbactam (2/1) was recommended. In case of severe infection, dose fractionation (twice-a-day dosage regimen) was recommended to maintain the required dose exposure while reducing the exposure-induced toxicity in neonates.

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