The Battle Against Nosocomial Pathogens for Sepsis Control in Paediatric Burn Patients: Vancomycin and Carbapenems Serum Monitoring for Target Attainment by PK/PD Analysis

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Introduction: Optimizing antimicrobial prescription for severe infections is required to improve clinical outcome. Aim of the study was to evaluate drug effectiveness in septic paediatric burns with systemic vancomycin combined with carbapenems. Methods: patients (7F/19M) (95%CI): 5.6-9.0 yrs; 23.9-36.4 kg; 25.5-38.9% TBSA; SCr: 0.25-0.35 mg/dL; 23.7-40.5 days ICU. Results: Target attainment (TA) MIC 2 mg/L gram-positive strain was reached in 21.4% with vancomycin empiric dose, then, increase to 91.2 mg/kg/day for TA. Carbapenem effectiveness MIC <2 mg/L, strain occurred 10-20mg/kg tid; 20-40mg/kg tid was required MIC>2 mg/L aggressive pathogens. Conclusion: A carbapenem combined with vancomycin improves the effectiveness against infections in burn paediatric patients; while, dose adjustments must be done in real time based on PK/PD analysis permitting an earlier medical intervention to control nosocomial infection in pediatric burn patients and to avoid the bacterial resistance.

Keywords: vancomycin-carbapenems; septic pediatric burns; PK/PD analysis

1. Introduction: An Aim of the Study

Burn-related injuries are the main causes of morbidity and mortality in pediatrics and burning are one of the three main causes of injury-related to death as a consequence of the disruption of the normal skin barrier, immunocompromised status, and prolonged hospitalization. Burns make patients easy targets for microbial colonization and infections are responsible for poorer prognosis in these patients [1]. Then, appropriate antimicrobial therapy has proved to be quite important for the treatment of critically ill patients with suspicious or clinical and laboratorial documented sepsis. Long-term outcome of children surviving massive burns related to the major advances in treatment of burn injuries in the last 35 years have made it possible to save the lives of children with massive burns [2]. Then, appropriate antimicrobial therapy has proved to be quite important for the treatment of critically ill patients with blood stream, severe infections, and clinical suspicious or laboratorial documented sepsis. Over the last 50 years, vancomycin has remained the drug of choice for the treatment of nosocomial Gram-positive infections, including methicillin-resistant *Staphylococcus sp*, MRSA or MRSE [3]. Therefore, vancomycin plasma monitoring is quite important to improve the outcome and, consequently, to avoid multi-resistant bacterial infections in paediatric critically ill patients [4]. Considering vancomycin for control of nosocomial infections caused by MRSA/MRSE, the empirical paediatric dosage regimen for patients without renal dysfunction is 40–60 mg/kg/day, and the trough concentration at the steady-state is a guide for subsequent dosage adjustment. However, data indicate that dose requirements higher than 2g daily are recommended to adults to attain the 10–20 mg/mL target trough concentrations [5,6]. Recently, data reported in paediatric burn patients with normal renal function recommend 90 mg/kg as a loading dose, since the biological half-life is shorter than would be expected as a consequence of the reduction of volume of distribution at the steady state and plasma clearance decreased or even increased in the hypermetabolic phase after thermal accident that occur in paediatric burn patients in comparison to burn adults [7]. Current recommended dosing of vancomycin for paediatric patients with invasive methicilin-resistant *Staphylococcus aureus* MRSA infection at the lowest recommended dose resulted in subtherapeutical blood levels; therefore, bacterium resistance appears, and a poor outcome is expected [8]. Few studies were found concerning the empiric vancomycin dosage recommendations in burn patients related to the target attainment recommended to critically ill patients; consequently, vancomycin dose adjustment based on the predictive index estimated by pharmacokinetic-pharmacodynamic (PK/PD) analysis must improve the clinical outcome in burn adult patients and also in paediatrics even after massive burns [6,7,9].

Recently, a blood stream infection mortality risk score was available as an excellent tool to predict the prognosis of patients with gram-negative based on acute severity of illness, the primary source of infection and major host comorbidities. Since over half million individuals developed gram-negative blood stream infections annually in the USA, and 75,000 deaths occurred; then, appropriate empirical antimicrobial therapy is critical to improve the outcome, and the ability to predict the prognosis of patients with gram-negative blood stream infections have a significant impact on the morbility and mortality of critically ill patients [10]. Consequently, vancomycin plus carbapenem seems to be a rational association for patients in sepsis by gram-positive and also gram-negative/resistant strains to beta-lactams as

piperacyllin-tazobactam, ampicyllin-sulbactam or even to cephalosporins third/forty generation. Then, carbapenems as imipenem or meropenem are the newest available agents to be prescribed to patients with blood stream infections caused by beta-lactamase producers gram-negative pathogen susceptible.

Considering the control of nosocomial infections caused mainly by gram-negative strains susceptible to imipenem or meropenem, the empirical paediatric dosage regimen for a patient with normal renal function usually starts with 10-20 mg/kg time intradoses (tid), but the maintenance dose could increases up to 40mg/kg tid in those patients [11,12,13]. Drug effectiveness of these antimicrobial agents against isolated strains will be based on the fraction of the time dose interval that the free drug serum concentration at the steady-state is maintained above the minimum inhibitory concentration (MIC 90) required to kill 90% of colonies in blood cultures; then, the bactericidal free drug levels in the patient (host) must be attained above the MIC, only in a fraction of the time dose interval for the antimicrobial effectiveness guarantee. Consequently, the predictive index for carbapenem effectiveness (40%*f*T>MIC) will be a guide for subsequent dosage adjustment [14]. However, few data reported in burns indicate that dose requirements to attain the therapeutic target in pediatrics differs those recommended to adults. Therefore, optimizing antimicrobial prescription is required to improve clinical outcome from severe infections and to reduce the development of antimicrobial resistance, once it is well known that the pharmacokinetics is altered in critically ill patients, including burns. Additionally, it was reported in those patients, that in general, drug kinetic disposition changes in a different way in adults compared to paediatric patients, and also, few evidences are available on population dosing and dose adjustment requirements in burns [7,15,16].

It is well known that the thermal injury affects negatively the pharmacokinetics of antimicrobial agents, making it difficult to establish dose-regimen guidelines in critically ill paediatric burns. Then, drug serum monitoring and PK/PD analysis must be applied, since the predictive index of drug effectiveness must be estimated to guide the physician concerning dose adjustment for target attainment during the antimicrobial therapy [17].

The aim of the present study described in the Chapter was to evaluate dose adjustment requirements in septic burn paediatric patients receiving systemic antimicrobial therapy by the association of vancomycin with meropenem or imipenem in the Intensive Care Burn Unit (ICBU) by applying the PK/PD analysis for the treatment of nosocomial infection. Drug effectiveness was based on the predictive index recommended for vancomycin ($AUC^{ss}_{0\text{-}24}$/MIC) and for carbapenem agents (%*f*T>MIC) [6,14]. In this chapter, dose adjustment requirements are presented in critically ill pediatric burn patients for target attainment (TA) against gram-positive vancomycin susceptible strains MIC>1mg/L. In addition, dose adjustments for TA mainly against gram-negative carbapenem susceptible strains MIC>2mg/L are focused to make possible the control of severe infections in those patients. Differences on PK-data for both in pediatric and adult burn patients occur, and also changes of clinical relevance on PK of antimicrobial investigated will be discussed [7,15,16]. Many factors that affect drug pharmacokinetics related to renal function, total burn surface area, drug effectiveness in septic shock, and susceptibility of the nosocomial pathogens were included, considering always the application of the PK/PD tool focusing the improvement in clinical outcome. Finally, PK/PD analysis based on simulation studies or the traditional PK-calculation will be considered.

2. Methods

2.1 Study design, patient eligibility and the initial antimicrobial therapy

The clinical protocol was a prospective, open-label study approved by the Ethical Committee of Hospital of Clinics, Medical School of University of Sao Paulo. The study was conducted from July 2013 to December 2014, and informed written consent was obtained from all legally designated patient representatives. Paediatric patients from the Intensive Care Burn Unit (ICBU) up to 15 years old, presenting severe thermal injury and a sepsis diagnosis by the "American Burn Association consensus conference to define sepsis and infection in burns" in clinical evaluation and laboratorial data were eligible for inclusion [18]. On the other hand, patients with vancomycin, meropenem or imipenem intolerance or renal impairment were excluded. The study was based on the recommended antimicrobial treatment to suspected or documented gram-positive and gram-negative nosocomial infections. Thus, vancomycin combined with meropenem or imipenem were prescribed. Initially, patients received systemically the antimicrobials prescribed as empiric daily dose and the initial dose regimen was given four times a day by one hour pump infusion for vancomycin (10–15 mg/kg tid). In addition, imipenem or meropenem were administered systemically by 0.5-hour pump infusion at the dose regimen 10-20 mg/kg tid, according to the institutional protocol. Complete medical histories, physical examinations were obtained for each enrolled patient; laboratory data included strains documented in blood cultures and susceptibility testing done to obtain the minimum inhibitory concentration (MIC 90) for each antimicrobial agent against the pathogen isolated. Individual demographic and clinical characteristics at admission in the Intensive Care Burn Unit (ICBU) are shown in Table 1. Creatinine clearance was estimated by Schwartz's method, population laboratory data are shown in Table 1 [19].

Table 1 Demographic and clinical characteristics of individual data for paediatric patients.

Patient allocation Gender	Age (years)	Weight (kg)	BMI Kg/m2	CLcr ml/min	TBSA %	Inhalation injury	Endotracheal intubation	Vasoactive Drugs	ICBU Days	Outcome
#1 M	9	30	17.5	206	31	0	0	1	29	Survivor
#2 F	6	25	19.6	201	75	1	1	1	8	Nonsurvivor
#3 F	3	25	27.7	261	18	1	1	0	40	Survivor
#4 M	11	40	20.1	194	30	0	0	0	19	Survivor
#5 M	5	15	12.6	250	11	1	1	0	43	Survivor
#6 F	5	14	12,0	186	23	0	0	0	24	Survivor
#7 M	11	45	22.6	353	38	0	0	0	55	Survivor
#8 M	3	12	13.0	406	54	1	1	1	30	Survivor
#9 F	3	18	19.9	249	40	1	1	1	14	Nonsurvivor
#10 M	5	18	15.2	171	22	0	0	0	28	Survivor
#11 M	11	40	20.1	228	33.5	1	1	1	36	Survivor
#12 M	11	35	17.6	242	30	1	1	1	28	Survivor
#13 M	5	15	12.6	273	41.8	0	0	1	32	Survivor
#14 M	5	17	14.3	299	16	1	0	0	19	Survivor
#15 M	2	12	15.9	109	15	1	1	1	45	Survivor
#16 F	10	40	21.3	343	40	0	0	0	41	Survivor
#17 M	1	16	28.4	206	28.5	0	1	1	26	Survivor
#18 M	1	15	26.7	275	16.5	0	0	0	16	Survivor
#19 F	6	30	23.5	201	20	1	1	1	28	Survivor
#20 M	14	70	26.3	236	20.5	1	1	0	20	Survivor
#21 M	3	16	17.4	377	36	1	1	1	13	Survivor
#22 M	14	34	12.8	140	14	0	0	0	21	Survivor
#23 F	8	43	27.5	264	80	1	1	0	14	Nonsurvivor
#24 M	15	59	20,7	148	16.5	1	1	1	29	Nonsurvivor
#25 M	15	60	21,0	251	42	1	1	1	56	Survivor
#26 M	8	40	25,2	217	45	1	1	1	120	Survivor
95% CI (LL-UL)	5.6/9.0	23.9/36.4	18/22	214/269	26/39	16	16	14	23.7/40.5	22 /4

Abbreviations: M: male; F: female; 95% CI (LL-UL): Confidence interval (lower-upper limits).

Population laboratory data related to renal function and inflammation profile are shown in Table 2. Data was expressed by mean/standard deviation, 95% confidence interval, minimum/maximum values and medians/quartiles obtained in patients´ population.

Table 2 Population Laboratory data of paediatric burn patients during blood sampling sets.

N=26 19M/7F	Mean/SD Variability %	95% Confidence Interval CI Lower-Upper limits	Min/Max values	Median (Quartiles 25-75)
Serum creatinine (mg/dL)	0.30+/-0.13 42.5%	0.25 – 0.35	0.13/0.64	0.31 (0.21 – 0.35)
Serum urea (mg/dL)	25.96+/-8.81 33.94%	22.57- 29.35	11/48	25.96 (19.2-31.0)
C-reactive protein (mcg/L)	167+/-84 54.0%	135 – 199	26/355	165 (124 - 186)
White blood cells (WBC/mm3)	13,860+/- 7,037 50.6%	11,155 – 16,574	1890/26250	13,860 (8,058-17,043)
Platelets (cells/mm3)	456,230+/- 23,5960 52.0%	365,530-546,930	92,000/899,000	436,000 (238,750- 615,500)

2.2 Blood sampling

A set of blood samples was obtained by collection via a venous catheter at a steady-state level. Serial blood samples (2 mL/each) were collected at the 2^{nd}, 4^{th}, 6^{th} and if possible at the trough of drug administration, for dosing regimen every 8 hours. Three to a maximum of four samples were drawn from each patient from a venous catheter into BD Gel-vacuum tubes. Blood samples collected from each patient' set were centrifuged at 2800g for 20 minutes; the serum was added of the same volume of 1M 3-morpholinopropanesulphonicacid, MOPS (J.T.Baker, Phillipsburg, USA) buffer solution; then, transferred to labeled polyethylene vials and analyzed on the same day, or yet stored in a freezer at -35^{o}C until drug assay.

2.3 Analytical procedure for serum drug measurements

Drugs in blood samples were analyzed after purification procedure by the precipitation of serum proteins with acetonitrile for vancomycin and meropenem, while the ultrafiltration procedure was applied to imipenem. Then, purified extracts of vancomycin, meropenem and imipenem were analyzed by a liquid chromatograph LC10A instrument, Shimadzu Corporation (Kyoto, Japan) as reported previously by Lopez et al. for vancomycin, Santos et al for imipenem and meropenem [15,16,20]. Only 0.2 mL of each sample was required for drug serum measurements

using high performance liquid chromatography/ultraviolet detection (HPLC–UV) according to the simple and accurate bioanalytical methods with adequate linearity and sensitivity. The coefficient of determination (r2>0.99) for the drug assay over the standard curve concentrations based on eight serum calibrators (C1-C8: 0.2–100 mg/L) plus the zero (C0) were acceptable; additionally internal quality controls were included. Calibration daily curve was accepted on the basis on the systematic error lower than 15% for the quality internal controls (high, medium and low drug serum concentrations); then, drug serum levels in patients' samples were determined on the basis of the accepted daily curve using the internal standard method. Vancomycin was analyzed by a reversed phase column Supelcosil LC-18, 25cmx4.6mm, 5 µm (Supelco, Bellefonte, USA), mobile phase was 0.075M acetate buffer and acetonitrile (92:8, v/v) and peaks were monitored at 210nm, using cefuroxime as internal standard. Imipenem was analyzed by a reversed phase column Supelcosil LC-18, 25cmx4.6mm, 5 µm (Supelco, Bellefonte, USA), mobile phase was 0.01M phosphate buffer and acetonitrile (99:1, v/v) and peaks were monitored at 300 nm, ceftriaxone was chosen as internal standard. Meropenem was analyzed by a reversed phase column Shim-pack VP-ODS 150cm x 6.0mm, 5 µm Shimadzu Corporation (Kyoto, Japan), mobile phase was 0.01 M acetate buffer and acetonitrile (9:1, v/v) and peaks were monitored at 307 nm, cefepime as internal standard.

2.4 Pharmacokinetic analysis

Vancomycin, meropenem, and imipenem serum concentrations – time data were analyzed by the traditional approach based on one compartment model through a Noncompartmental PK Data Analysis, PK Solutions 2.0 (Summit, Montrose, CO, USA). The area under the curve, steady state levels, at the time dose interval ($AUC^{ss}\tau$) was obtained by the antimicrobial serum concentration-time curve over the time dosing; the elimination rate constant (kel) was the slope of a semilogarithmic plot, the biological half-life ($t_{(1/2)\beta}$) was based on the ratio 0.693/slope, total body clearance (CL_T) and the volume of distribution at the steady state (Vd^{ss}) were calculated for each patient investigated. Data were expressed by medians and quartiles (IQ25-75) and the variability was based on the standard deviation by the mean ratio, expressed as percentage.

2.5 PK/PD analysis

PK data are related to the *in vivo* parameter, while PD data related to the minimum inhibitory concentration (MIC) obtained by the antimicrobial susceptibility testing done for each pathogen in the Microbiology Laboratory after the strain documentation; MIC 90 is the minimum inhibitory concentration to kill 90% of pathogen colonies (*in vitro,* PD parameter. The index of antimicrobial effectiveness is estimated on the basis of vancomycin serum concentration over time. Thus, the predictive index of vancomycin effectiveness is expressed by $AUC^{ss}_{0\text{-}24}$/MIC ratio > 400; once ratio values above 400 were recommended for target attainment [6,7]. Since the drug effectiveness is time dependent for carbapenem agents investigated in this study protocol (meropenem or imipenem), the predictive index of drug effectiveness is estimated on the basis of the trough, the elimination rate constant (kel), time dose interval and MIC data. Thus, drug effectiveness expressed by the PK/PD analysis for target attainment was estimated by the fraction of the time dose interval that imipenem or meropenem serum concentration is maintained above the MIC, 40%*f*T>MIC strain [10,14,21].

2.6 Data analysis

Statistical data analysis was carried out using the Statistical Package for Social Sciences 13.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Version 4.0 (GraphPad Software Corporation, San Diego, CA, USA); p-Values lower than 0.05 were considered significant. Quantitative variables were described using central tendency and dispersion measures. The statistical model used for the evaluation of each variable was chosen based on the distribution pattern of each variable.

3. Results and Discussion

Antimicrobial therapy by association of vancomycin with a carbapenem agent was considered the best choice for nosocomial infections control; data obtained in paediatric burn patients, including daily dose, dose at time intradoses (tid) and the respective trough for each antimicrobial investigated are described in the Table 3.

Table 3 Antimicrobial therapy for septic paediatric burn patients.

Antimicrobial therapy	Mean/SD Variability (%)	95%CI LL- UL	Median (Quartiles 25-75)
VANCOMYCIN - 23 patients, pump infusion 1hr			
Daily dose (mg/kg/day)	62.06+/- 24.64 39.71%	55.01-69.10	50.00 (50.00- 66.67)
Trough levels (mg/L) Blood sampling 42 Sets	11.31+/-4.29 37.97%	10.08 – 12.53	10.25 (8.48-13.05)
MEROPENEM – 10 patients, pump infusion 0.5 hr			
Daily dose (mg/kg/day)	99.97+/- 48.98 48.99%	75.98-123.97	80.36 (64.29- 137.50)
Dose tid (mg/kg)	29.39+/- 16.67 56.71%	21.22-37.55	25.00 (16.07- 42.36)
Trough levels (mg/L) Blood sampling 16 Sets	4.01+/-2.94 73.23%	2.57 – 5.45	3.10 (1.60-5.90)
IMIPENEM – 12 patients, pump infusion, 0.5 hr			
Daily dose (mg/kg/day)	81.09+/- 36.68 45.23%	64.60 -97.58	75.00 (50.00- 108.82)
Dose tid (mg/kg)	20.82+/- 9.07 43.55%	16.74-24.90	20.00 (14.58- 27.21)
Trough levels (mg/L) Blood sampling 19 Sets	3.27+/-1.66 50.89%	2.52 – 4.02	3.00 (2.20-4.00)

Abbreviations: SD: standard deviation; CI (LL-UL): confidence interval (lower-upper limits).

Initially, the empirical doses recommended were administered systemically by pump infusion; significant dose adjustment requirements for target attainment were necessary for vancomycin against MIC >1 mg/L susceptible strains; while for carbapenems, doses were increased against MIC>2mg/L susceptible gram-negative strains, Table 3.

3.1 Vancomycin dosing stratification for drug effectiveness

Based on drug requirements for target attainment, vancomycin daily dose was stratified for paediatric burn patients investigated; data related to the empiric dose against adjusted dose were compared, once it was shown the proportionality between dosing increases and trough levels obtained, Table 4.

Table 4 Stratification of vancomycin empiric *vs* adjusted daily dosing in paediatrics.

Vancomycin 23 Patients	Empiric daily dose (mg/kg.day) 30 sets	Trough levels (mg/L) 30 sets	Adjusted daily dose (mg/kg.day) 12 sets	Trough levels (mg/L) 12 sets
Mean/SD	50.4+/-7.9	9.6+/-2.5	91.2+/-28.3*	15.8+/- 4.6**
Variability (%)	15.72%	26.10%	31.08%	29.30%
95%CI (LL-UL)	47.58-53.25	8.64 -10.45	83.07 - 99.28	13.31 – 18.35
Min/max values	32.0/66.7	5.00/13.50	70.9/142.9	9.0/23.0

Abbreviations: CI (LL-UL): confidence interval (lower-upper limits); min/Max: minimum/maximum. Statistics: vancomycin dosing (*) $p < 0.0001$; trough levels (**) $p<0.02$ unpaired sample statistics.

Considering the trough target recommended (10-20mg/L), when the empiric vancomycin daily dose recommended in our Institution (40-60mg/kg) was administered and trough at the steady state higher than 12 mg/L was achieved only in 8/30 blood sampling sets (26.7%) for paediatric burn patients at dosing (50.4 +/- 7.9 mg/kg, mean+/-SD) described in Table 4. Thus, the daily dose was increased to 91.2 +/- 28.3 mg/kg ($p <0.02$) and consequently, vancomycin trough was increased significantly from 9.5 +/- 2.5 mg/L to 15.7+/- 4.8 mg/L ($p < 0.001$), Figure 1.

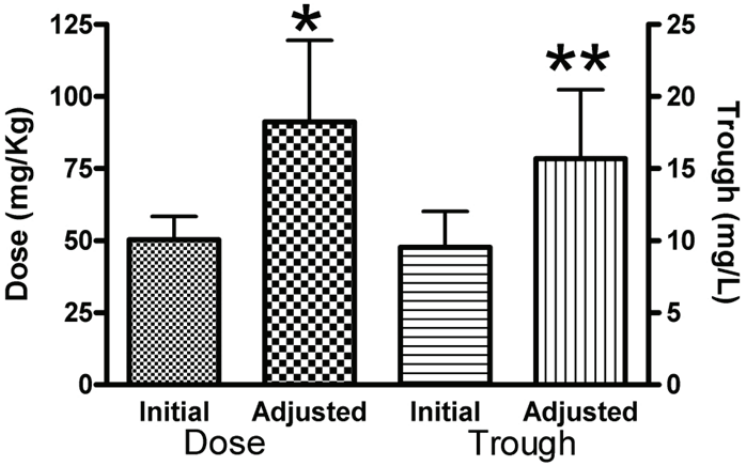

Fig. 1 Vancomycin administered to 23 patients to reach the recommended target trough serum concentration (10–20 mg/L). Significant differences between vancomycin dosing (*) $p < 0.001$ and trough levels (**): $p<0.02$ were demonstrated by unpaired sample statistics.

In the present study, the initial daily dose recommended for children (50mg/kg) was not sufficient to achieve the trough target between 10-20mg/L in 26.7% of burn patients investigated; consequently, dose required to reach the therapeutic target was significantly increased, Figure 1, Table 4.

Alternatively, for non-burn paediatric patients, vancomycin daily dose of 60.6 mg/kg resulted in trough plasma levels (7.8 mg/L), which is below 10 mg/L considering the desired target between 10-20mg/L [22]. In addition, Eiland et al. recommended to paediatric critically ill patients daily doses of 70-85 mg/kg to maintain the target trough level (15 mg/L) during the antimicrobial therapy with vancomycin [23]. More recently, it was recommended by Gomez et al, a loading dose of 90 mg/kg at the first day of therapy (D0) for septic paediatric burns to achieve the steady state faster than the conventional empiric dose (40-60 mg/kg/day), permitting an earlier clinical intervention by dose adjustment if necessary for severe nosocomial infection control in those patients [7]. It is well known that data from the susceptibility testing done after the confirmation of strain will be available later than required by the physicians; therefore, drug plasma monitoring for pharmacokinetic study and PK/PD analysis in a real time will be quite useful and can guide them for an earlier medical intervention to guarantee the control of septic shock by PK/PD analysis. Consequently, adequate antimicrobial therapy including the optimal dosage based on drug plasma monitoring contributes significantly to a desired outcome and obviously to reduce the risk of bacterial resistance in patients with life-threatening infections, such as those that occur in critically ill, including burn patients and pre-term infants [7,24,25,26]. The need for increasing daily doses to reach adequate trough levels has been documented in adult burn and non-burn patients. It was reported previously that vancomycin dosing 2g daily must be prescribed to adult critically ill patients with creatinine clearance lower than 60 mL/min; while for patients with better kidney function, corresponding doses must be increased to 3 g daily for aged higher than 65 years or to 3.5 mg a day for young adult patients, serum creatinine 1mg/dL [6,9,27]. Based on reduced biological half-life reported for paediatrics, a dosing interval of 6qh was chosen for 95.2% (40/42) of sets investigated against, a dosing interval of 8qh that occurred in only 2/42 sets, when vancomycin was administered 1g three times daily in paediatric burns investigated in the present study. Finally, increases on daily dose were required to achieve the trough target and the desired outcome in those patients.

3.2 Vancomycin Pharmacokinetics

Based on one compartment PK-model, a first order kinetic disposition may occur for vancomycin at a therapeutic dose regimen in paediatric patients with normal renal function, once PK-parameters estimated are dose independent; therefore, the constants like kel, $t_{(1/2)\beta}$, CL_T and Vd^{SS} must remain unchanged, since a PK dose independently would be expected. Vancomycin kinetic disposition was investigated in 23 paediatric burns, which were receiving different dose regimens; PK-parameters were estimated in all sets of blood sampling as described in Table 5.

Table 5 Pharmacokinetics of vancomycin in Paediatric Burn Patients.

Vancomycin 23 Patients 42sets Blood sampling	kel h-1	$t_{(1/2)\beta}$ h	CL_T ml/min/kg	Vd^{ss} L/kg	Vd^{ss} L
Medians	0.228	3.04	1.57	0.43	12.89
Interquartils	0.193-0.275	2.51-3.59	1.32-1.95	0.30-0.61	7.34-16.15
Mean/SD	0.238/0.071	3.26/1.44	1.66/0.75	0.52/0.32	13.37/7.58
95% IC	0.218-0.259	2.85-3.67	1.56-1.99	0.42-0.61	11.20-15.54
Variability %	29.71%	44.12%	42.29%	61.5%	56.7%
PK-parameter	increases	decreases	no change	decreases	decreases
Healthy adults[29]	0.151-0.189	3.65-5.31	1.21 – 1.70	0.45 -0.65	29.8 - 42.4

Abbreviations: kel: elimination rate constant; $t_{(1/2)\beta}$ biological half-life; CL_T total body clearance; Vd^{ss} volume of distribution at the steady state

Concerning kinetic disposition of vancomycin in adult healthy volunteers described by Boeckh et al, it was shown in paediatric burns with normal renal function, that the reduction of biological half-life (3.0 hrs) *versus* 3.65-5.31 hrs in healthy adults could be justified by volume of distribution at the steady state decreased (12.89 L) in these patients by comparison to data in healthy adults (29.8 - 42.4 L). It is important to highlight that the total body clearance remained unchanged since several factors could alter this PK-parameter in patients investigated, even with normal renal function in all of them; consequently the total body clearance remains unchanged 1.57 (1.32-1.95) ml/min/kg in our patients by comparison with data of healthy adults (1.21-1.70 mL/min/kg), Table 5. Thus, vancomycin pharmacokinetic data obtained in the present study (23 patients, 42 sets) are according to the results reported for critically ill paediatric patients, burns and non-burns, once a high PK-variability occurs in those patients [7,28,29]. Many causes of intrapatient variability (haemodynamic status, fluid administration, surgery and inflammatory processes) may influence PK changes during a patient's stay in the ICU, making it difficult to reach and maintain the desired target; while, interpatients PK-variability also occurs. Differences between patients related to changes on PK-data could justify the minimum/maximum dose adjustment requirements for vancomycin effectiveness as described in Table 4 and discussed afterwards. Four paediatric burn patients of this study required dose adjustment, and the minimum dose adjusted 70.9mg/kg/day was given to three patients that resulting in different vancomycin troughs (19 mg/L, 18 mg/L and 11mg/L), whom target attainment MIC 0.5-2 mg/L, susceptible strains were reached for all of them. Considering dose

adjustment for the fourth patient, whom the highest vancomycin dose required 142.9 mg/kg was given, the resultant trough was 12 mg/L and target achievement MIC 0.5-1 mg/L, susceptible strains was reached.

At the present time, vancomycin remains the drug of choice for the treatment of invasive MRSA infections; however, an increased MIC, even within the susceptible range, is a risk factor for vancomycin treatment failure in critically ill patients, adults and paediatrics [3,5,7,8,31]. Additionally, it was justified by Onishi et al that the vancomycin kinetics disposition are different in adults and children due to differences in body size, fluid compartments and urinary excretion [30]. Therefore, the pharmacokinetics of vancomycin in pediatric burns investigated in the present study varied greatly from the pharmacokinetics previously reported in septic adult burn patients, since the shorter half-life and lower trough resulted from Vd^{ss} reduced in these patients [9,24,27,31,32,33].

In contrast, the pharmacokinetics of vancomycin in adult critically ill patients, burns compared to non-burns, has been described previously by Garrels & Peterie. PK changes in burns were related to an increased volume of distribution and drug plasma clearance; while the biological half-life was related to alterations in those both PK-parameters [27]. Revilla et al, reported in adult ICU patients' increases on the volume distribution of vancomycin higher than serum creatinine data (greater than 1mg/dL); therefore, age and creatinine clearance were identified as the main covariates explaining the pharmacokinetic variability in vancomycin plasma clearance in critically ill adult ICU patients [6].

3.3 Vancomycin PK/PD analysis

In the present study, vancomycin target attainment against gram-positive strains related to dosing stratification done for paediatric burns was based on the area under the serum concentration over time (AUC^{ss}_{0-24}) and the MIC 90 ratio, Table 6.

Table 6 Vancomycin Target attainment (PTA) against gram-positive strains based on dosing stratification for paediatric burns (n=23 patients, 42 sets blood sampling).

Vancomycin Initial Dose mg/kg tid	Gram-positive pathogens isolated bloodstream infections	MIC mg/L	Vancomycin Dose adjusted mg/kg tid	Gram-positive pathogens isolated bloodstream infections	MIC mg/L
Vancomycin 8.0-11.9 mg/kg tid	*Streptococcus sp* *Staphylococcus aureus*	*0.5* *1*	Vancomycin 13.3-16.7 mg/kg tid	*Staphylococcus.epidermidis* *Staphyloc. coagulase negative*	*1* *1*
MIC 90 < 1mg/L	**Suceptible strains documented** **PTA 85.7%**		**MIC 90: 1mg/L**	**Suceptible strains documented** **PTA 100%**	
Vancomycin 17.5-24.8 mg/kg tid	*Staphylococcus aureus MRSA* *Staphylococcus.epidermidis* *Staphyloc. coagulase negative*	*2* *2* *2*	Vancomycin 25.0-35.7 mg/kg tid	*Staphyloc..epidermidis MRSE* *Enterococcus faecalis* *Enterococcus faecium*	*4* *4* *4*
MIC 90: 2 mg/L	**Suceptible strains documented** **PTA 21.4%**		**MIC 90> 2 mg/L**	**Suceptible strains documented** **PTA 0%**	

Abbreviations: MIC 90: minimum inhibitory concentration 90%; PTA: percentage of target attainment.

A ratio higher than 400 is related to a trough levels above 10-15 mg/mL, assuming 1 mg/L MIC or less [5,7,34]. Despite the fact that the trough-based on dose adjustment data described in other studies, the results from the present study have revealed the need of dosing increases from 50.4 to 91.2 mg/kg daily to reach the therapeutic target by PK/PD analysis, since the parameter AUC^{ss}_{0-24}/MIC 90 ratio was above 400 in 100% of the sets for pathogens with 0.5 mg/L MIC, but the PTA decreases to 85.7% at 1 mg/L MIC strains, and to 21.4% for 2 mg/L MIC. Unfortunately, any patient investigated wasn't protected against *Staphylococcus epidermidis MRSE, Enterococcus faecalis Enterococcus faecium* MIC 4 mg/L. It is important to highlight that more aggressive gram-positive strains documented, MIC 4 mg/L for vancomycin, were eradicated in all pediatric burn patients by imipenem or meropenem therapy prescribed in the combined with vancomycin. Consequently, the combined antimicrobial therapy chosen was quite effective and a desired outcome related to the control of nosocomial infections occurred in all of them.

The vancomycin PK/PD correlation was plotted by AUC^{ss}_{0-24}/MIC ratio obtained for paediatric burn patients against MIC data vancomycin susceptible strains, Figure 2. Considering a target AUC^{ss}_{0-24}/MIC ratio above 400, the percentage of target attainment (PTA) was achieved at the empiric dose recommended in 100% (42/42) sets for MIC 0.5 mg/L, 85.7% (36/42) for MIC 1 mg/L, and only 21.4% (9/42) for MIC 2 mg/L; consequently, for MIC 1-2 mg/L vancomycin susceptible strains, dose adjustments were required for target attainment and the severe infection in paediatric burns caused by MIC 1-2mg/L strains susceptible were eradicated.

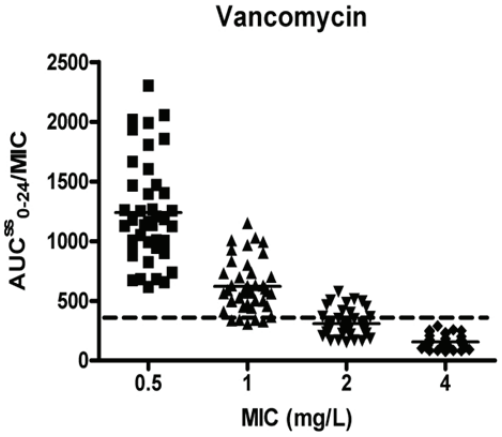

Fig. 2 Vancomycin empiric dose and the subsequent dose adjustment requirements for target attainment in 23 septic patients. PTA was distributed by $AUC^{ss}_{0\text{-}24}$/MIC ratio *versus* MIC data (0.5-4 mg/L); susceptibility testing done in the hospital' central laboratory. Data distribution and means were considered for 42 sets of blood sampling during antimicrobial therapy. Drug effectiveness index considered was $AUC^{ss}_{0\text{-}24}$/MIC > 400 (- - -).

PK/PD data obtained indicate that the daily dose must be increased almost by twice (50.4 to 91.2 mg/kg) for target achievement and strains with MIC values higher than 1 mg/L. These results are according to data reported previously by Gomez et al for pediatric burn patients. In contrast, considering pediatric patients (burns compared to non-burns), in whom vancomycin daily dose increases in a different manner as reported by Frymoyer et al, by 66% against 100% necessary to paediatric burns described by Gomez et al [7,8]. Optimizing vancomycin doses according to PK/PD principles showed in the present study, the potential to maximize the drug efficacy against MIC strains higher than 1 mg/L. Recommended trough range (10–20 mg/L) was proposed to guarantee PK/PD target achievement $AUC^{ss}_{0\text{-}24}$/MIC ratio higher than 400 and to increase the probability of the clinical cure of MRSA [5,7,34]. However, the efficacy and safety of the recommended target trough concentration and the PK/PD parameter have not been evaluated by any randomized or interventional studies [5]. More recently, a prospective multicentre trial to investigate the effectiveness and safety was conducted; and it was reported by authors that vancomycin trough concentrations above 15 mg/L with a threefold increased risk of nephrotoxicity [35]. Otherwise, it was suggested by Rybak et al that for complicated infections like bloodstream infection, endocarditis, osteomyelitis, meningitis and hospital-acquired pneumonia caused by S. aureus, a vancomycin trough of 15–20 mg/L range is needed to achieve $AUC^{ss}_{0\text{-}24}$/MIC > 400. While, higher troughs are usually associated with daily dose and highest AUCs; a more deep discussion related to this problem is necessary, since a single vancomycin plasma concentration like the trough does not directly reflect the AUC estimated by drug plasma concentration over time; once the trough is highly dependent on the dosing interval, i.e., daily doses with longer time dosing resulted in lower troughs. Therefore, dose adjustments based solely on trough values may be clinically misleading depending on the MIC data for strain documented, and vancomycin effectiveness depends on drug plasma concentrations over time dosing higher than the MIC 90. Thus, it is essential to highlight the importance of vancomycin exposure related to the dose regimen, the area under the curve and also the MIC concerned to the strain's microbiological profile, especially for MRSA, to provide the clinical team with a safe and reliable dose adjustment tool [7,8,36]. Finally, it was not the purpose of this study to compare patients' mortality among those who reached or did not reach the ideal antimicrobial's trough level and PK/PD target. However, the desired outcome for paediatric burn patients was reached; since it was resulted 22/26 releases against 4/26 deaths that occurred as a function of the high extension of burning (>70% TBSA) in two children, or was related to a higher gastrointestinal tract hemorrhage after an explosion occurred in the accident, and for the last one (40% TBSA) death was a consequence of the pulmonary atelectasis complicated. In summary, based on high PK-variability of vancomycin in pediatric burn patients, routine drug plasma monitoring and PK/PD analysis must be considered as an important tool to guarantee adequate vancomycin dosing during antimicrobial therapy for ICU´ patients. The current empiric daily dose of approximately 40–60 mg/kg recommended to hospitalized children is inadequate for achieving target trough levels and desirable AUCs in paediatric burns, and an increase in daily doses of 80–100 mg/kg are needed to achieve the desired targets in terms of drug effectiveness/safety and for achieving pharmacodynamic goals.

3.4 Carbapenems dosing and their Pharmacokinetics in critically ill patients

Meropenem and also imipenem are the broad-spectrum β-lactam antimicrobials that have been used in the therapy of gram-positive and gram-negative infections that occur in critically ill patients after higher surgeries, burns and non-burns patients. Those critically ill patients with serious infections frequently have an altered volume of distribution (Vd^{ss}) for these antimicrobials [12,14,21,37,38,39,40]. Therefore, the clinical significance for the dosing of these agents, such as current dosage guidelines for imipenem and meropenem (0.5–1.0 g every 6 hours) are derived from pharmacokinetics studies in healthy volunteers; these studies suggest at steady-state, PK-data related to volume of distribution and biological half-life for meropenem and imipenem, respectively were: 9.2-10.3L (0.15-0.17 L/kg) and 14.8 – 21.9 L (0.18-0.36 L/kg) for the volume of distribution expressed by volume or even body weight volume normalized; 0.50-0.58hrs biological half-life of meropenem and for imipenem 0.84-1.12hrs in young healthy adults. Additionally, the total body clearance was quite similar for these carbapenems in healthy volunteers 0.21 L/h.kg (12.0 L/h) for meropenem against 0.16-0.19 L/kg (12.0 L/h) for imipenem [41,42].

Initially, it was reported by Belzberg et al, in adult hospitalized patients with severe infections that imipenem was considered the first choice frequently used in critically ill patients with serious infections, mainly for gram-negative bacterium resistant to other antimicrobials; once an adequate minimum inhibitory concentrations (MIC 90) must be

achieved. Thus, imipenem was investigated in patients without critical illness and any changes on PK data occurred. It was demonstrated by the authors that the plasma levels of imipenem in severely ill and injured patients are lower than those expected in normal subject populations, once 50% of patients investigated had levels below the breakpoint at 6th hour postdosing. Consequently, in critically ill ICU patients, the systemic inflammatory distress syndrome response impacts the volume of distribution and higher doses of imipenem were required for those patients, once unpredictable PK occurs in critically ill patients [37]. Concerning imipenem PK in critically ill burn patients with normal renal function Blanchet et al described in a revision study that there are conflicting data in the literature regarding the investigators related to the pharmacokinetics of imipenem in burn patients [31]. Any significant PK-changes were described by Boucher et al. in severe burn adult patients; in contrast, Gomez at al., in a recent study reported that an important change on imipenem PK occurs in burn adult patients to a prolongation of biological half-life as a consequence of Vd^{ss} increased; also a reduction on Kel e decreases on plasma clearance occurred [40,43]. In addition, Ikawa et al described a reduction only in total body clearance, once any change in Vd^{ss}, kel and $t_{(1/2)\beta}$ was observed in critically ill adult patients with intra-abdominal infections. Similar results were related by Watanabe et al., which no changes on PK-parameters were obtained in adult patients with severe respiratory infection. It is important to observe that high variability on PK of imipenem was registered for patients in the investigations described by both authors [14,21].

3.5 Carbapenems dosing stratification for drug effectiveness

Concerning antimicrobial therapy with carbapenem agents for children, the empiric dose recommended were 10 mg/kg (tid) or 20mg/kg tid for both agents; then, the present study conducted in paediatric burns, as described above by vancomycin dose were also stratified for imipenem and meropenem based on available MIC data against susceptible strains documented as follows: MIC 0.5-1 mg/L (10 mg tid) or MIC 2 mg/L (20 mg tid). Considering MIC >2 mg/L (MIC 4-8 mg/L strains), doses higher than 20mg/kg tid were required for both carbapenem agents, Table 7.

Table 7 Stratification of carbapenems dosing for target attainment (PTA).

Blood sampling 35sets N=22 Patients	**Imipenem tid 10-20 mg/kg N=12 Patients 19 sets**	**Imipenem tid >20 mg/kg**	**Meropenem tid 10-20 mg/kg N=10 Patients 16 sets**	**Meropenem tid >20 mg/kg**
Mean/SD	14.4+/-4.8	30.2+/-4.2*	13.2+/-2.8	35.3+/-11.6*
Variability %	33.41%	13.99%	21.32%	32.77%
MIC< 2mg/L	Susceptible strains isolated	MIC ≤2 mg/L	Susceptible strains isolated	MIC≤2mg/L
Carbapenem dosing 10-20mg/kg tid	*Streptococcus pneumoniae* *Streptococcus pyogenes* *Staphylococcus coagulase negative* *Staphylo.epidermidis/S.haemolyticus* *Streptococcus agalactiae* *Enterobacter aerogenes/E.cloacae* *Escherichia coli* *Acinetobacter baumanii* *Klebsiella pneumoniae/K.oxytoca* *Haemophylus influenza*	*0.016* *0.064* *0.125* *0.125* *0.25* *0.5* *0.5* *1* *2* *2*	*Streptococcus pneumoniae* *Haemophylus influenza* *Proteus mirabilis* *Klebsiella pneumoniae* *Streptococcus C-G group* *Enterobacter cloacae* *Enterob aerogenes* *Escherichia coli* *Acinetobacter baumanii* *Pseudomonas aeruginosa*	*0.016* *0.25* *0.25* *0.25* *0.5* *0.5* *0.5* *0,5* *2* *2*
MIC> 2mg/L	Suceptible strains isolated	MIC >2 mg/L	Suceptible strains isolated	MIC>2mg/L
Carbapenem dosing >20mg/kg tid	*Proteus mirabilis; Proteus vulgaris Pseudomonas aeruginosa;* *Morganella morganii;* *Enterococcus faecalis/E. faecium*	*4* *4* *4* *4*	*Enterococcus faecalis* *Enterococcus faecium*	*8* *8*

Abbreviations: tdi: time intradose

Initially, imipenem dose regimen recommended for children (10-20mg/kg tid) was administered to septic burn patients for target attainment against MIC ≤ 2mg/L strains, but, if MIC data was higher than 2 mg/L, dose must be higher than 20mg/kg tid. Data expressed by means/SD (95%CI) were recommended initially 14.4+/-4.8 (12.2-16.5) mg/kg tid *versu*s 30.2+/- 4.2 (28.3-32.0) mg/kg tid obtained by dose adjustment, since dose was increased to reach the target against MIC>2 mg/L strains.

In addition, similar procedure was done for paediatrics under meropenem therapy. Data obtained by comparison of empiric dosing *versus* adjusted dose, respectively were 13.2+/-2.8 (12.0-14.5) mg/kg tid for the initial dosing (MIC ≤ 2mg/ strains) and 35.3+/- 11.6 (30.1- 40.5) mg/kg tid after meropenem dosing increases needed to combact strains MIC>2mg/L, Table 7.

A dosing interval of 6 qh was chosen respectively for imipenem and meropenem in 89.5% (17/19) and in 31.3% (5/16) of the sets investigated. In addition, a dosing interval of 8qh administered 1g three times daily was chosen in only 2/19 (10.5%) sets for imipenem against 68.7% (11/16) sets for meropenem.

3.6 Pharmacokinetics of imipenem and meropenem in paediatric critically ill patients

Although, PK-parameters have been reported in adult healthy subjects, patients without critical illness, and also in critically ill patients, total body clearance were poorly described for carbapenem agents; few PK-data were found in children critically ill patients related to meropenem, and any data were found for imipenem PK. A first order kinetic disposition may occur at therapeutic dose regimen for carbapenems in paediatric patients with normal renal function, since they are excreted by glomerular filtration and tubular secretion in the urine. PK-parameters (kel, $t_{(1/2)\beta}$, CL_T and Vd^{ss}) are dose independently at therapeutic doses recommended for adults and children. Meropenem and imipenem kinetic disposition study were conducted in burn patients, whom were receiving different dose regimens; thus, PK-parameters, daily dose and trough were estimated in all sets of blood sampling. Daily doses of 80.4 (64.3- 137.5) mg/kg of meropenem resulted in trough 3.1 (1.6-5.9) mg/L; while daily dose 75.0 (50.0-108.8) mg/kg of imipenem achieved through 3.0 (2.2-4.0)mg/L, Table 8.

Table 8 Comparative Pharmacokinetics of Carbapenems in paediatric burn patients.

Carbapenems 22 Patients 35 sets Blood sampling	Dose mg/kg.day	Trough mg/L	Kel h-1	$t_{(1/2)\beta}$ h	CL_T L/h	Vd^{ss} L/kg	Vd^{ss} L
Meropenem, 10 Pat.	80.4	3.1	0.379	1.84	5.43	0.45	16.67
Medians (Quartiles)	(64.3- 137.5)	(1.6-5.9)	0.288-0.440	1.57-2.41	4.27-6.94	0.38-0.68	14.85-18.51
Variability	49,9%	73.2%	28.0%	36.1%	31.2%		
Healthy adults [41]	50	0.1-0.3	1.18-1.34	0.50-0.58	12.0-12.6	0.15-0.17	9.19-10.33
Imipenem, 12 Pat Medians (Quartiles)	75.0	3.0	0.378		5.27	0.56	15.46
	(50.0- 108.8)	(2.2-4.0)	0.321-0.435	1.83 1.59-2.16	4.79-6.63	0.50-0.66	10.90-20.42
Variability	45.2%	50.9%	20.6%	22.2%	25.9%	41.6%	36.6%
Healthy adults [42]	60	0.5-1.1	0.679-0.825	0.84-1.02	12.3-14.7	0.23-0.28	16.5-19.8

Abbreviations: Kel: elimination rate constant; $t_{(1/2)\beta}$ biological half-life; CL_T total body clearance; Vd^{ss} volume of distribution at the steady state.

The present study that was conducted in paediatric burn patients compares carbapenem dosing, serum concentrations and drug effectiveness by PK/PD analysis in critically ill patients, and any comparison PK-data for imipenem *versus* meropenem for hospitalized paediatric patients was found. PK data obtained in this study for meropenem and imipenem in paediatrics were compared to data reported in healthy adults reported previously [41,42]. Meropenem PK in paediatric burns seem to be altered by increases on Vd^{ss} 0.45 L/kg by threefold by comparison with healthy adults (015-0,17L/kg), while the biological half-life 1.84hrs (0.50-0.58hrs, healthy adults) were 3-4 times prolonged. Total body clearance in patients was 5.43 L/h, 2 times reduction by comparison with healthy adults (12.0-12.6 L/h); while a reduction on kel by 3 times was observed [41]. Concerning imipenem PK data, similar results were obtained by comparison also to the results reported in healthy adults [42].

PK-data for both carbapenems were also compared; and it was shown that the total body clearance and the elimination rate constant that characterizes the drug elimination was 2 times reduced for both in this study. In addition, the volume of distribution was 3 times increased for meropenem against 2 times for imipenem with a consequent prolongation of biological half-life (4 times) for meropenem higher than for imipenem increased by 2 times. Therefore, PK-data obtained in the present study for paediatric burns could justify the variability on Vd^{ss} and the trough for meropenem, both higher than data obtained for imipenem.

Related to previous PK investigation in paediatric critically ill patients treated with meropenem few studies were found, while, for imipenem just one study was found [11,12,13,16,44].

Blumer et al investigated in a single-dose meropenem PK study in hospitalized infants and children, and change on pharmacokinetics occurred by increases on volume of distribution with a proportional prolongation of biological half-life in these patients [44]. In addition, three PK-studies with meropenem at steady state were reported [11,12,13].

Du et al, investigated 99 paediatric patients with meningitides, whom received the recommended dosing for children; authors describe an important change in all PK-parameters by the reduction on plasma clearance, increases on volume of distribution and a prolongation of biological half-life in those patients by comparison with data in healthy adults [11]. Concordantly, similar results were obtained by Ikava at al 2010 (n=40) and by Ohata, 2011 (n=50) critically ill patients. Therefore, meropenem PK-data obtained in the present study in paediatric burns are according to the results reported previously in children and also in infants [11,12,13,44].

Thus, it is considered that the pharmacokinetics of carbapenem agents, Table 8 are altered in paediatric burn patients with normal renal function when data were compared to the results reported in healthy adults [41,42]. These changes seem to be quite similar for both agents as the prolongation of biological half-life as a consequence of increases on volume of distribution, and the reduction on total body clearance described in healthy subjects. Additionally, it is important to highlight that the difference on pharmacokinetics obtained by comparison of meropenem and imipenem in

paediatric burns are strictly related to the volume of distribution increased three folds for meropenem against twice for imipenem; while the total body clearance was twice reduced for both agents by comparison to PK data in healthy volunteers. Finally, the variability on PK-parameters registered by comparison of meropenem and imipenem is mainly related to the volume of distribution of meropenem (83.2%), which variability was higher than data registered for imipenem (41.6%), Table 8. Consequently, based on interpatients variability PK-changes for meropenem were higher than for imipenem in our paediatric burns investigated.

Concerning adult critically ill patients, Cheatham et al described that any changes occurred in hospitalized patients with normal renal function after meropenem 0.5g every 6 hours (CLcr>60ml/min). Additionally, patients were allocated in three groups, whom received 0.5 g of meropenem every 6, 8, and 12 hours on the basis on creatinine clearances greater than 60, 40–60, or 10–39 mL/min, respectively. It was shown high variability on data obtained for each group of patients, and changes on PK-parameters were strictly related to the degree of renal impairment for plasma clearances and half-lives [38]. On the other hand, concerning imipenem PK study in critically ill burn patients with normal renal function, Blanchet et al described a revision study that there are conflicting data in the literature regarding the recommended dose and pharmacokinetics of imipenem in burns, once any significant change in pharmacokinetics was found by Boucher et al. in severe burn adult patients. In contrast, Gomez at al. reported in a recent study in burn adults patients that important change on imipenem PK occurs in these patients with normal renal function related to a prolongation of biological half-life as a consequence of Vd^{ss} increased; also a reduction on Kel related to decreases on plasma clearance; high variability was obtained for PK-parameters in those critically ill burn patients [31,40,43]. Total body clearance reduction, and Vd, kel and t1/2 unchanged were reported by Ikawa et al in critically ill adult patients with intra-abdominal infections; while, all PK-parameters remained unchanged for imipenem as described by Watanabe et al in adult hospitalized patients with severe respiratory infection. It is important to highlight that a large variability on imipenem PK-data was registered for both groups of investigators [14,21]. Related to Imipenem PK-data, it was considered that there are conflicting data in the literature until now regarding the changes on its pharmacokinetics in critically ill adult patients.

3.7 PK/PD Analysis of Carbapenems in paediatrics

Recommended PK/PD parameter to correlate dosing, PK-data and drug effectiveness for these antimicrobials were based on the fraction of time intradoses (tid) that drug serum concentration is maintained above the MIC (%*f*T>MIC); MIC is the *in vitro* measurement, that is the minimum inhibitory concentration required to kill 90% of culture' colonies for each pathogen isolated. Thus, PK/PD analysis for carbapenems was applied by plotting the drug effectiveness index 40%*f*T>MIC against MIC data susceptible strains documented in blood cultures, Figure 3.

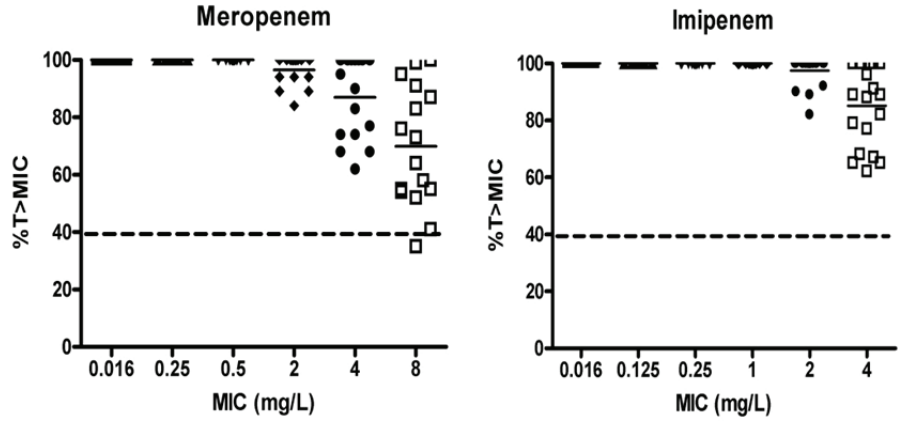

Fig. 3 Carbapenems empiric dose and dose adjustment requirements for target attainment in 22 patients with septic shock. PTA was distributed by %*f*T>MIC, MIC data (0.016-8 mg/L) considering the pathogens isolated and susceptibility testing done in the hospital' central laboratory. Data distribution and means were considered for 35 sets of blood sampling from ICBU patients during antimicrobial therapy. Drug effectiveness index recommended was 40%*f*T>MIC (**- - -**).

Carbapenem target attainment in paediatric burn patients was investigated by comparison of the empiric dose and dosing adjusted, described in Table 9. It was observed that PTA was improved by dose adjustment proportionally to increases on MIC data, considering the increases of dose, body weight normalized for both carbapenem agents. It was verified that imipenem susceptible strains need dose higher than 15mg/kg tid for sepsis caused by *Klebsiella pneumoniae, Kebsiella oxytoca;* Haemophylus influenza (MIC 2mg/L); while dosing higher than 30mg/kg tid was necessary for severe infections caused by *Proteus mirabilis; Pseudomonas aeruginosa; Proteus vulgaris; Morganella morganii; Enterococcus faecalis and Enterococcus faecium* (MIC 4 mg/L), Table 9. Imipenem PTA was attained in 63% of patients against strains susceptible (MIC 0.016-2 mg/L) and in 38% of patients for strains MIC higher than 2 mg/L. It is important to highlight that after imipenem dose adjustment based on serum monitoring and PK/PD analysis,

the infections caused by gram-negative more aggressive pathogens were eradicated in paediatric burn patients, since 100%PTA was achieved.

On the other hand, considering meropenem susceptible strains, dose higher than 13.23+/-2.82 mg/kg tid were required to treat the septic shock caused *by Acinetobacter baumanii* and *Pseudomonas aeruginosa* (MIC 2mg/L); while high doses 35.33+/-11.58 mg/kg tid were necessary for severe infections caused by *Enterococcus faecalis* and *Enterococcus faecium* (MIC 4-8 mg/L), Table 9. PTA was attained in 55% of patients against susceptible strains (MIC 0.016-2 mg/L) and in 45% of patients, when MIC >2 mg/L strains occurred.

Finally, meropenem dose adjustment based on serum monitoring and PK/PD analysis contribute to desired outcome of paediatric burn patients with severe infections caused by gram-negative pathogens MIC >2mg/L. Then, PTA was achieved in 90% of patients; consequently meropenem therapy was replaced by imipenem in the association with vancomycin combined therapy.

Therefore, both carbapenem agents were effective against more aggressive gram-negative pathogen susceptibles to imipenem and meropenem for the optimal therapy in paediatric burn patients; therefore, the antimicrobial combined therapy of a carbapenem agent with vancomycin seems to be quite effective in those patients against gram-positive and gram-negative pathogen susceptible.

Table 9 Empiric therapy *versus* adjusted dosing for Imipenem and Meropenem in Paediatric Burn Patients for target attainment.

Blood sampling 35sets N=22 Patients	**Imipenem tid 10-20 mg/kg N=12 Patients**	**Imipenem tid >20 mg/kg**	**Meropenem tid 10-20 mg/kg N=10 Patients**	**Meropenem tid >20 mg/kg**
Mean/SD	14.35+/-4.79	30.15+/-4.22 *	13.23+/-2.82	35.33+/-11.58*
Variability %	33.41%	13.99%	21.32%	32.77%
MIC< 2mg/L	Susceptible strains isolated	MIC ≤2 mg/L	Susceptible strains isolated	MIC≤2mg/L
Carbapenem dosing 10-20mg/kg tid MIC 0.016-2mg/L	*Streptococcus pneumoniae* *Streptococcus pyogenes* *Staphylococcus coagulase negative* *Staphylo.epidermidis /haemolyticus* *Streptococcus agalactiae* *Enterobacter aerogenes/cloacae* *Escherichia coli* *Acinetobacter baumanii* *Klebsiella pneumoniae/oxytoca* *Haemophylus influenza*	*0.016* *0.064* *0.125* *0.125* *0.25* *0.5* *0.5* *1* *2* *2*	*Streptococcus pneumoniae* *Haemophylus influenza Proteus* *mirabilis Klebsiella pneumoniae* *Streptococcus C-G group* *Enterobacter cloacae/ aerogenes* *Escherichia coli* *Acinetobacter baumanii* *Pseudomonas aeruginosa*	*0.016* *0.25* *0.25* *0.5* *0.5* *0.5* *2* *2*
MIC> 2mg/L	Suceptible strains isolated	MIC >2 mg/L	Suceptible strains isolated	MIC>2mg/L
Carbapenem dosing >20mg/kg tid	*Proteus mirabilis; Pseudomonas aeruginosa;* *Proteus vulgaris* *Morganella morganii; Enterococcus faecalis/ faecium*	*4* *4* *4* *4*	*Enterococcus faecalis* *Enterococcus faecium*	*4-8* *4-8*
	PTA %		**PTA %**	
Empiric dose	63%	38%	55%	45%
Susceptible strains	(MIC 0.016-2mg/L)	(MIC >2mg/L)	(MIC 0.016-2mg/L)	(MIC >2mg/L)
Dose adjustments	100%	100%	100%	90%
Susceptible strains	(MIC 1-2mg/L)	(MIC 4mg/L)	(MIC 1-2mg/L)	(MIC 4-8mg/L)

Dosing stratification for carbapenems was done for drug effectiveness/safety in paediatrics by comparison of imipenem and meropenem, Table 10.

Table 10 %PTA Imipenem versus Meropenem by dosing stratification and MIC data.

Carbapenem regimens (MIC data obtained)	Probability of 40%*f*T>MIC target attainment (%)			
Imipenem	Blood cultures - Strains isolated	PTA %	Dose adjustment	PTA %
10.2-15.5 mg/kg tid (MIC 0.016-0.5mg/L)	*Streptococcus pneumoniae* *Streptococcus pyogenes* *Staphylococcus coagulase negative Staphylo.epidermidis;St.haemolyticus* *Streptococcus agalactiae* *Enterobacter aerogenes; E.cloacae* *Escherichia coli*	100%	NO	100%
18.8-20.0 mg/kg tid (MIC 1-2mg/L)	*Acinetobacter baumanii* *Klebsiella pneumoniae/oxytoca* *Haemophylus influenza*	63%	YES	100%
28.3-32.0 mg/kg tid (MIC 4mg/L)	*Proteus mirabilis; P. vulgaris Pseudomonas aeruginosa* *Morganella morganii* *Enterococcus faecalis; E. faecium*	38%	YES	100%
Meropenem				
12.0-14.5 mg/kg tid (MIC 0.016-0.5mg/L)	*Streptococcus pneumoniae* *Haemophylus influenza* *Proteus mirabilis P vulgaris* *Klebsiella pneumoniae* *Streptococcus C-G group* *Enterobacter cloacae E. aerogenes* *Escherichia coli*	100%	NO	100%
14.7-17.0 mg/kg tid (MIC 1-2mg/L)	*Acinetobacter baumanii* *Pseudomonas aeruginosa*	65%	YES	100%
30.1-40.5 mg/kg tid (MIC 4-8mg/L)	*Enterococcus faecalis* *Enterococcus faecium*	45%	YES	90%

PTA 100% was achieved for imipenem at low dosing 10.2-15.5 mg/kg tid for infections caused by all pathogens isolated (MIC 0.016-0.5 mg/L): *Streptococcus pneumoniae* (MIC 0.016mg/L); *Streptococcus pyogenes* (MIC 0.064 mg/L), *Staphylococcus coagulase negative* (MIC 0.125 mg/L); *Staphylococcus epidermidis, Streptococcus haemolyticus, Streptococcus agalactiae* (MIC 0.25mg/L); *Enterobacter aerogenes, Enterobacter cloacae* and *Escherichia coli* (MIC 0.5 mg/L).

Similarly, PTA 100% was achieved for meropenem at the lowest dosing 11.96-14.50 mg/kg tid for the control of infections caused by MIC 0.016-0.5 mg/L pathogens: *Streptococcus pneumoniae* (MIC 0.016mg/L); *Haemophylus influenza, Proteus mirabilis, Proteus vulgaris and Klebsiella pneumoniae* (MIC 0.25mg/L); *Streptococcus C-G group, Enterobacter cloacae, Enterobacter aerogenes and Escherichia coli* (MIC 0.5 mg/L).

In contrast, increases on imipenem dosing were required (18.8-20.0 mg/kg tid) for 100% PTA achievement against infections caused by *Acinetobacter baumanii, Klebsiella pneumoniae, Klebsiella oxytoca* and *Haemophylus influenza,* MIC 1-2 mg/L susceptible strains. In parallel, increases on meropenem dosing 14.7-17.0 mg/kg tid was required for 100% PTA for infections caused by *Acinetobacter baumanii, Pseudomonas aeruginosa* (MIC 2 mg/L) meropenem susceptible strains.

Considering the most aggressive gram-positive and gram-negative pathogens, the highest dosing were required for both carbapenems and described as follows: 28.3-32.0 mg/kg tid imipenem was needed for against the most aggressive pathogens like *Proteus mirabilis; Proteus vulgaris, Pseudomonas aeruginosa, Morganella morganii, Enterococcus faecalis* and *Enterococcus faecium* (MIC 4mg/L); while, doses between 30.1-40.5 mg/kg tid meropenem against *Enterococcus faecalis* and *Enterococcus faecium* were necessary to combat them.

3.8 Final considerations about PK and PK/PD analysis in critically ill patients

Pharmacokinetics (PK) is an important tool available to antimicrobial effectiveness by predicting drug exposure, and can be associated to pharmacodynamic (PD) goals, since the pathogen susceptibility must always be considered to ensure desired clinical outcome by drug effectiveness against strain documentation. Therefore, the variability on PK and on PD parameters must be careful and individually considered, since the relationship given by the *in vivo* data and the *in vitro* data concerned to the pathogen susceptibility could express an important index to predict the antimicrobial effectiveness.

PK data are related to the *in vivo* parameter, while PD data are related to the bactericide minimum inhibitory concentration (MIC 90) obtained by the antimicrobial susceptibility testing done for each pathogen documented. Therefore, antimicrobial indices for vancomycin effectiveness will depend on drug plasma concentration over time; while the index of drug effectiveness will be time dependent for β-lactam agents. Thus, the predictive index of vancomycin effectiveness is expressed by $AUC^{ss}_{0\text{-}24}$/MIC ratio > 400; once ratio values above 400 were recommended for target attainment [6-9, 22, 23, 28-30, 35-36]. While, drug effectiveness time dependent occurs for carbapenem (meropenem or imipenem), and its effectiveness index will be estimated on the basis of the obtained through, the elimination rate constant (kel), the time dosing and MIC data strain documented. Thus, drug effectiveness time

dependent for target attainment will be estimated by the PK/PD analysis and defined by the fraction of time dose interval that imipenem or meropenem free plasma concentration is maintained above the MIC, 40%*f*T>MIC strain [10-16, 21,38,40,44].

Today, it must be considered also that the main problem of the physician during the antimicrobial therapy for control of severe infections in critically ill ICU´patients is related to strain documentation and MIC data available for the clinical team in a short period after blood sampling for cultures. On the other hand, it well known that a week almost is needed to gram-positive strains documentation/susceptibility testing MIC data done in the hospital´ laboratory; while, takes a period longer than 10 days, minimum, if a nosocomial gram-negative strain was considered. Then, in that case MIC values can be obtained within the typical clinical setting or from surveillance databases by searching the EUCAST site from the European Society of Clinical Microbiology and Infectious Diseases - The European Committee on Antimicrobial Susceptibility Testing-Eucast/ MIC data/clinical breakpoints [45].

Concerning PK-analysis, it was found in the literature different approaches that can be used. Noncompartmental data analysis, software was applied to individual patients PK investigation, and, in general is related to a small number of patients [7-16,21-23, 41, 42, 44]. In contrast, NONMEM™ software, is a methodology in population PK analysis based on the nonlinear mixed-effect approach, planned to be applied to a large number of patients population with predictive target attainment [6,12,14,21]. On the other hand, population PK-analysis software could present some limitation related to a large number of data-population of patients included; extreme data values based on lower/upper limits for estimated parameters obviously occur, but cannot be extrapolated to all ICU´critical patients, since changes on PK happen as a consequence of many factors discussed in the chapter related/non-related to each patient, including also the main causes of intrapatient variability (haemodynamic status, fluid administration, surgery and inflammatory processes) may influence PK changes during a patient's stay in the ICU, making it difficult to reach and maintain the desired target; while, interpatients PK-variability also occurs.

In the chapter it was discussed changes on vancomycin and carbapenems PK in critically ill patients focusing paediatric burns and differences that occur in children compared to adults critically ill patients and healthy. Therefore, it is important to highlight that PK can be altered in a different manner those patients and changes will depend of many additional factors including the severity of infection, multiresistant strain, immunosuppressed status and co-morbidities related to each individual patient.

In summary, antimicrobial target achievement for the control of severe infections caused by nosocomial pathogens, susceptible or resistant to the recommended doses previously established, PK/PD analysis have been performed by applying the traditional noncompartmental PK-data analysis software for PK-data estimation to a small number of patients´ population since parameters were estimated for each individual patient allocated in the study [7-9,11,13,15,16,22,23,41-44].

In addition, Monte Carlo simulation population software implemented is applied to predict the proportion of patients in different population groups who will achieve the desired PK/PD target, when different strains or pathogens are treated. Using this kind of data for large number of patients, Monte Carlo simulations have been used by several investigators to predict target achievement in specific patient population, in spite of PK-data obtained in the most part of studies; have been realized in a small number of subjects included in the protocol. Monte Carlo simulation has been implemented by several investigators as useful technique to predict the target achievement in patient populations [6,12,14,21].

The control of severe infection unfortunately must be done in each critically ill ICU´ patient by the medical team; thus, target achievement must be reached to save patients´ lives soon and independently of MIC data strain available or not to the physician in the Institution. The integrated PK/PD approach permits to consider the variability in PK-data and also on PD goals of the strain, to obtain a desired outcome for a defined population with unpredictable pharmacokinetics like paediatrics, infants, massive burns and also aged patients population of critically ill patients, because variability in the MIC can be obtained from surveillance studies [45].On the other hand, during the clinical follow-up of an individual ICU critically ill patient with severe infection, laboratory data, drug plasma monitoring and PK/PD analysis based on MIC value/eucast database will guide the physician to change or not the dose regimen of an antimicrobial or if an antimicrobial alternative therapy could be chosen, always considering daily ICU´ patient status during the therapy. Finally, PK/PD analysis is a quite specific tool that must be applied carefully in a real time to allow an earlier medical intervention to save lives of ICU´patients critically ill.

4. Conclusion

The current empiric daily dose of approximately 40–60 mg/kg for vancomycin recommended to hospitalized children with normal renal failure is inadequate for achieving target trough levels and desirable AUCs in paediatric burns. Thus, an increase on daily doses 80–100 mg/kg are needed to achieve the desired targets in terms of drug effectiveness/safety and for achieving pharmacodynamic goals MIC 2mg/L, strains. Pharmacokinetics of vancomycin was altered in these patients and optimized dosing given every 6 hours was justified by the reduction on Vd^{ss}, since a biological half-life shorter than expected occurs in critically ill paediatric burns. PK-data is similar by comparison of imipenem with meropenem, in spite of pharmacokinetics altered in a different manner for these agents. Target attainment in paediatric

burns was compared after the empiric carbapenem dose (10-20mg/kg tid); consequently, PTA improvement was registered by dose adjustment (20-40mg/kg tid) proportional to increases on MIC-data strains. Consequently, the pathogens documented were eradicated by the control of severe infections caused mainly by gram-negatives pathogens MIC>2mg/L in paediatric burns, even considering the more aggressive of them.

Finally, it was shown that the stratification of daily dose and dosing regimen tid of these antimicrobials according to MIC-data strains were quite useful for drug effectiveness. Then, optimization of therapy was reached by maximization of drug efficacy and minimization of neurotoxicity of carbapenems or nephrotoxicity of vancomycin in critically ill paediatric burn ICU´patients.

In summary, based on high PK-variability of vancomycin, imipenem and meropenem in pediatric burn patients, routine therapeutic drug plasma monitoring (TDM), PK and PK/PD analysis done in a real time provide relevant data for each individual patient to the physician permitting an earlier intervention and the control of severe infections caused by nosocomial pathogens susceptible or intermediate strains. Therefore, this package must be considered an important tool to guarantee adequate dosing during antimicrobial therapy for ICU´ critically ill paediatric burn patients and contributes significantly to improve the clinical desired outcome and to avoid the development of multiresistant strains.

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