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Original article

Population pharmacokinetics and dosing simulations of temocillin in liver-transplanted paediatric patients: a prospective, open-label, non-randomized study

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ABSTRACT

Objectives: Temocillin is a β-lactam antibiotic used for preventing or treating bacterial infections in liver-transplanted children. We characterized its pharmacokinetics in plasma and ascitic fluid and proposed dosing regimens that maximize the achievement of effective drug exposures in this patient group.
Methods: Patients aged 6–36 months received 25 mg/kg/12 h ($n = 14$) or 25 mg/kg/8 h ($n = 23$). Total and unbound temocillin concentrations were measured in plasma and ascitic fluid. Drug safety was monitored. Non-compartmental and population pharmacokinetic analyses were performed, together with Monte Carlo simulations.
Results: No safety concerns were reported. For 25 mg/kg/12 h, the unbound mean (±standard deviation) C_{max} and C_{min} were 38 ± 16 and 2 ± 1 mg/L, respectively. For the 25 mg/kg/8 h dose, the unbound C_{max} remained similar although the mean C_{min} increased to 5 ± 3 mg/L. Protein binding was saturable. Median penetration in ascitic fluid from plasma was 82% (min–max: 63–95%). A three-compartment model with first-order elimination best described unbound pharmacokinetic profiles in plasma and ascitic fluid, with body weight and estimated glomerular filtration rate (GFR) as significant covariates. Monte Carlo simulations suggested that 90% probability of target attainment was achieved in both fluids with 25 mg/kg/12 h for MICs ≤4 mg/L, estimated GFR ≤180 mL/min/1.73 m^2 or weight ≥6 kg, and with 25 mg/kg/8 h, for MICs ≤8 mg/L, GFR ≤120 mL/min/1.73 m^2 or weight ≥11 kg.
Discussion: Although adequate in many instances, the current dosing regimen is likely inadequate for patients with low body weight, high renal function, or bacteria with high MIC, emphasizing the need for patient-specific factors to be considered in dose selection. These data support the importance of paediatric pharmacokinetic studies to optimize drug dosing regimens. **Perrin Ngougni Pokem, Clin Microbiol Infect 2025;31:408**

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Introduction

Liver transplantation (LT), often performed before the age of 2 years [1], is the reference-standard treatment for children with end-stage liver disease [2]. Despite improved hygiene protocols, up to 50% of these patients can develop bacterial infections which can lead to morbidity, mortality, and/or graft loss [3]. Surgical site and intra-abdominal infections are common [4]. Moreover, ascites after LT are associated with an increased risk of abdominal infection [5]. Multidrug-resistant Gram-negative bacteria are frequently incriminated, notably extended-spectrum β-lactamase-producing Enterobacterales [6]. Prophylactic administration of carbapenems is recommended by some protocols but is associated with a risk of emergence of resistance, diarrhoea, or *Clostridium difficile* colonization [7,8].

In this context, the carbapenem-sparing β-lactam antibiotic temocillin is an attractive alternative [9,10]. It covers Enterobacterales, including many extended-spectrum β-lactamase/AmpC producers [9] but preserves the intestinal microbiota, limiting *C. difficile* emergence [11]. Current indications include urinary tract, skin and soft tissue, lower respiratory tract and bloodstream infections caused by Gram-negative bacilli [12]. Temocillin is also a valuable alternative for acute bacterial cholangitis and biliary tract infections [10].

In our institution, temocillin is used (combined with aminopenicillin to cover enterococci) for prophylaxis or infection in febrile children after LT. However, no data are available on temocillin pharmacokinetics (PK) in this fragile population or in paediatric patients in general, making it not possible to know whether current dosing approaches are adequate. The registered paediatric dose is 25–50 mg/kg/d divided into two administrations, with a maximum of 4 g/d, depending on the infection severity [13]. However, there are no studies supporting this dosing regimen.

β-Lactams are time-dependent antibiotics, meaning that the fraction of the dosing interval during which unbound drug concentrations remain above the MIC against the pathogen (fT > MIC) is the PK/pharmacodynamic (PK/PD) index driving efficacy [14]. Although the target drug exposure is still debated [15], a PK/PD target of fT > MIC between 35% and 41% has been defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), together with a resistance breakpoint (MIC >16 mg/L) for target organisms [16]. This would be considered an appropriate minimum drug exposure to be targeted by dosing regimens. In contrast to most β-lactams, temocillin is highly bound to plasma proteins (~85%). This binding is saturable and variable among populations [17], underlining the importance of characterizing temocillin protein binding in relevant patient populations, including paediatrics.

Our first objective was to characterize the PK of total and unbound temocillin in plasma and ascitic fluid in paediatric LT patients upon dosing via intermittent infusion (25 mg/kg/12 h or 8 h) and to evaluate its safety in this population. A second aim was to develop and validate a PK model and to perform Monte Carlo simulations to describe dosing regimens with a high probability of achieving effective drug exposures for a range of clinically relevant MICs.

Patients and methods

Study design and patients

Patients were recruited from the paediatric intensive care unit or the gastroenterology and hepatology unit of the Cliniques universitaires Saint-Luc. Ethical approval was obtained from the institutional Comité d'Ethique hospitalo-facultaire (2015/03NOV/588). The study was registered at the European Union Drug Regulating Authorities Clinical Trials (EudraCT 2014-004224-22) and ClinicalTrials.gov (NCT02260102). Written consent was obtained from the parents or legal representatives in accordance with Good Clinical Practice and local regulatory requirements before any study-related procedure.

This prospective, open-label, non-randomized study included male/female, febrile/non-febrile LT paediatric patients aged 6–36 months (i.e. most frequent population for LT), requiring temocillin for prophylaxis or treatment, and hospitalized for ≥5 days. Exclusion criteria included acute/chronic renal failure (estimated glomerular filtration rate [eGFR calculated using the European Kidney Function Consortium formula [18] <30 mL/min/1.73 m^2], infection by a temocillin-resistant pathogen, penicillin allergy, or participation in another trial with temocillin in the preceding month. If still febrile after 48 hours of temocillin therapy, patients were switched to meropenem and de facto excluded from further sampling.

Safety

Safety was assessed based on reports from parents/legal representatives or nurses/physicians, with a particular interest for diarrhoea (emission of >3 soft/liquid stools per 24 hours) and convulsions.

Intervention

This study was carried out in two phases. Initially, 25 mg/kg/12 h (currently recommended regimen) was administered intravenously (30 minutes infusion; group #1). Blood samples were drawn 0.5, 1, 4, 8 and 12 hours after the start of the infusion. After the recruitment of 14 patients, an interim analysis suggested that this dosing was suboptimal and did not sufficiently achieve the PK/PD target (40% $fT \geq 16$ mg/L). An amendment was made to increase the dose to 25 mg/kg/8 h and collect blood samples after 0.5, 1, 2, 4, and 8 hours (group #2). For both groups, blood was collected after the fourth and eighth doses (i.e. the day after or 2–3 [depending on the administration scheme] days after transplantation) via a central or peripheral venous catheter and centrifuged in EDTA tubes to isolate plasma. In group #2, ascitic fluid was collected via the drain simultaneously with blood whenever possible. All samples were stored at −80°C until analysis.

Analytical method

Total and unbound concentrations were measured by an HPLC-MS/MS method [19] validated for plasma [20] and ascitic fluid (Methods-Results S1; Fig. S1; Table S1).

Non-compartmental PK analysis

Area under the concentration–time curve (AUC) for a dosing interval was calculated using the trapezoidal rule as part of a non-compartmental PK analysis to describe temocillin protein binding and penetration into ascitic fluid. The penetration (total) and the proportion (unbound) of temocillin in ascitic fluid were calculated as $AUC_{\text{total in ascitic fluid}}/AUC_{\text{total in plasma}}$ and $AUC_{\text{unbound in ascitic fluid}}/AUC_{\text{total in plasma}}$, respectively [21].

Population PK models and probability of target attainment

A population PK model was built to describe temocillin unbound concentrations in plasma and in ascitic fluid simultaneously. One- and two-compartment models with the first-order elimination

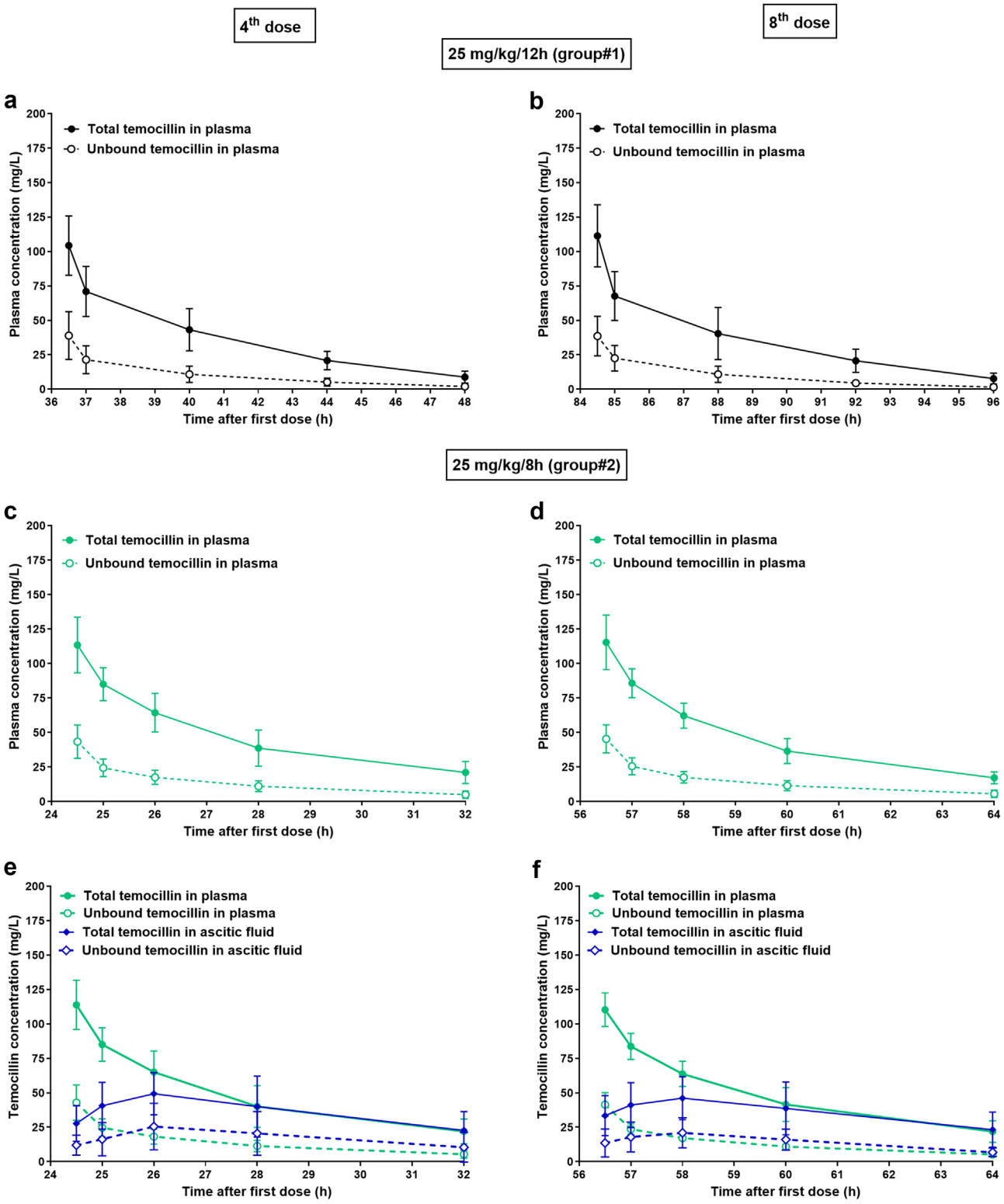

Fig. 1. Concentration-over-time profiles of total and unbound temocillin after multiple intravenous administration (30 minutes infusion). In plasma (group #1: a, fourth dose [$N = 14$]; b, eighth dose [$N = 12$]; group #2: c, fourth dose [$N = 14$]; d, eighth dose [$N = 11$]) and ascitic fluid and plasma from the same patients of group #2 (e, fourth dose [$N = 10$]; f, eighth dose [$N = 6$]). Data are shown as mean ± SD. GFR, glomerular filtration rate; SD, standard deviation.

from the central compartment and inter-compartmental distribution were first tested to fit plasma unbound temocillin concentrations, and an additional compartment was used to fit the ascitic fluid unbound temocillin concentrations, with an additional non-renal elimination from this compartment via the drain (Fig. S2). Further details on model construction selection, and validation as well as on tested covariables are presented in Methods S2. Probability of target attainment (PTA) analysis for different simulated dosing regimens was performed using Monte Carlo simulations ($n = 1000$) of various dosing regimens.

Statistical analysis and curve fittings

GraphPad version 9.3.1 (GraphPad Prism Software, San Diego, CA) was used. Normality of distributions was tested using the Shapiro–Wilk test before applying Student's paired *t*-test, or unpaired *t*-test, two-tailed. Otherwise, Wilcoxon-signed rank test or Mann–Whitney *U* test was used (two-tailed).

Results

Population's characteristics

Demographic characteristics were not different among groups #1 and #2 (Table S1). The median eGFR was 139 and 124 mL/min/1.73 m^2 and median paediatric end-stage liver disease score reached 15 and 18.5, respectively. Transaminases, total bilirubin and international normalized ratio were higher in group #2 vs. group #1. Plasma protein and albumin concentrations were comparable in both groups (mean ± standard deviation [SD]: 50.9 ± 7.9 g/L and 54.1 ± 8.8 g/L [total protein]; 33.6 ± 8.1 g/L and 33.7 ± 4.8 g/L [albumin]) but below the normal values. In ascitic fluid, total protein and albumin concentrations were 26.8 ± 3.3 g/L and 18.9 ± 3.3 g/L (mean ± SD). Two patients from group #1 and three from group #2 were switched to meropenem before the administration of the eighth dose because febrile after 48 hours of temocillin treatment. All isolated microorganisms (n = 14) were Enterobacterales with temocillin MICs ≤16 mg/L and associated with cholangitis (n = 6), urinary tract infection (n = 4), bacteraemia (n = 3), or post-operative perforated bile duct cyst (n = 1).

Tolerability

Both dosing regimens were well tolerated and no patients were withdrawn because of adverse events. Phlebitis was observed at the site of injection (2/37 patients), but not directly related to temocillin, given that all drugs (Table S2) were administered via the same catheter. No episodes of diarrhoea or convulsions were observed.

Non-compartmental PK analysis

Plasma concentrations

In group #1 (Fig. 1(a) and (b) and Table S3 for details), mean (±SD) C_{max} and C_{min} were 107.5 ± 21.8 mg/L and 8.3 ± 4.0 mg/L (total temocillin), and 38.7 ± 15.8 mg/L and 1.8 ± 1.1 mg/L (unbound temocillin). The mean unbound AUC_{0-24h} was 27.5% of the total value. The PK parameters were globally similar between patients with negative (prophylaxis) or positive (treatment) microbiological culture, or between the samples taken after the fourth or the eighth dose (Fig. S3 and Tables S4 and S5).

In group #2, C_{max} for total drug and half-life were comparable with those in group #1 (Fig. 1(c) and (d) and Table S3 for details). Importantly, reducing the dosing interval from 12 to 8 hours increased mean (±SD) unbound C_{min} from 1.8 ± 1.1 (group #1) to 5.2 ± 2.6 mg/L (group #2).

Plasma protein binding

The unbound concentrations increased as a function of the total concentrations according to a polynomial function of the second order (Fig. 2(a)), suggesting some degree of saturation (Fig. 2(c)). K_d and B_{max} values were close to 30 and 130 mg/L in both groups (Table S6). The unbound fraction increased linearly over the range of total temocillin concentrations in both groups (Fig. 2(b)).

Plasma and ascitic fluid concentrations

C_{max} was reached in the ascitic fluid after 2 hours (median value) for both the total and the unbound concentrations, with values lower than in the plasma. AUC was lower in ascitic fluid than in plasma for total concentrations. Conversely, C_{min} and AUC values for unbound concentrations were similar in ascitic fluid and plasma (Fig. 1(e) and (f) and Table S7 for details). Median penetration in ascitic fluid reached 82.2% (min–max: 63.4–95.0), corresponding to a median proportion of 36.2% (min–max: 21.8–55.1) for the unbound drug. No difference was seen between samples taken after the fourth or the eighth doses (Table S8).

Population PK model and PTA

The PK model is presented in Results S2, Tables S9 and S10, and Figs. S3–S6. Fig. 3 illustrates the PTA for achieving 40% fT > MIC in plasma and ascitic fluid for various dosing regimens and MICs in a representative patient (median weight and eGFR for our cohort, 9.8 kg and 138 mL/min/1.73 m^2). In both fluids, the 25 mg/kg/12 h dose did not reach a PTA >90% for isolates with MIC >4 mg/L. In contrast, the new therapeutic scheme (25 mg/kg/8 h) resulted in a PTA >90% for isolates with MICs ≤8 mg/L. Dosing simulations suggested that 50 mg/kg/8 h would be needed for MICs of 16 mg/L (Fig. 3 and

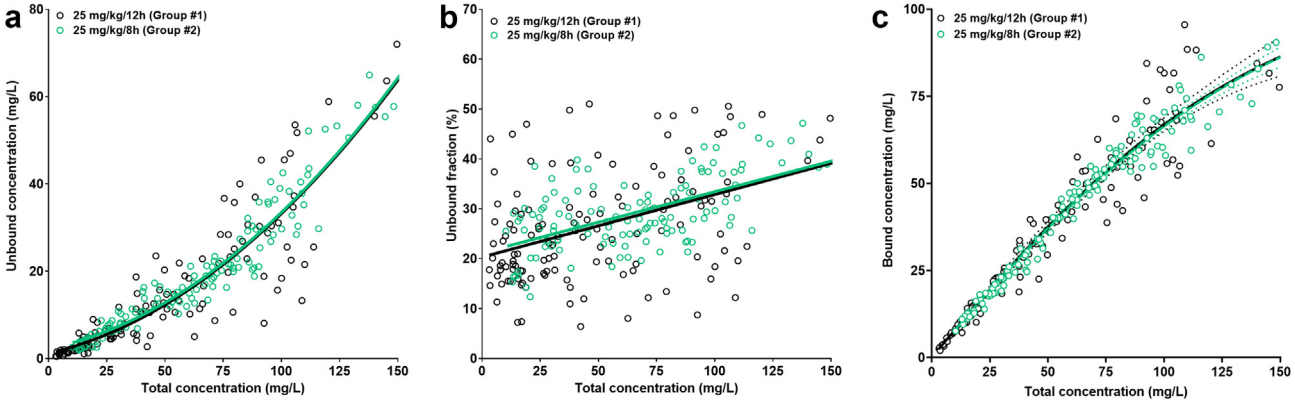

Fig. 2. Binding of temocillin to plasma proteins. Each data point (130 in group #1 and 125 in group #2) corresponds to a unique sample, but samples were obtained from 14 individuals in each group. Abscissa: total concentration; ordinate: (a) measured unbound concentration; (b) calculated unbound fraction; (c) bound concentration (calculated as the difference between total and unbound concentrations). Data were used to fit: (a) a quadratic (second order) polynomial function; (b) a linear function; (c) the Saturation Binding Curve Accounting for Ligand Depletion equation [17]. In (c), curves are shown with 95% CI. Values of the best-fit parameters are provided in Table S6.

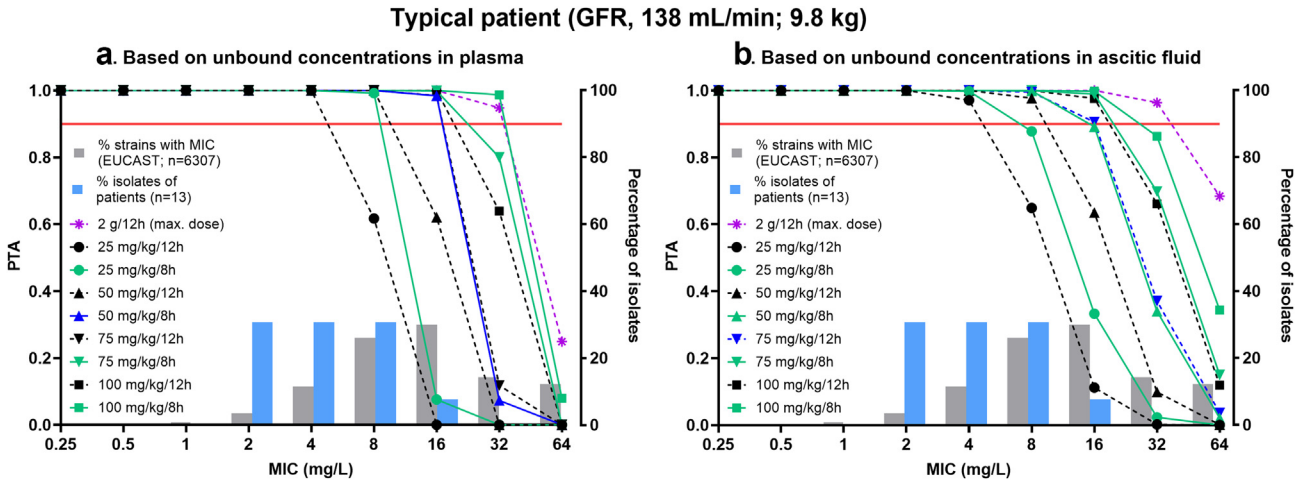

Fig. 3. Monte Carlo simulations and PTA analysis based on unbound concentrations in plasma (a) or in ascitic fluid (b) for doses of 25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg q12 h and q8 h as well as the maximal dose (2 g/12 h) administered to a representative paediatric patient (body weight: 9.8 kg; eGFR: 138 mL/min/1.73 m^2). Red-dotted line: PTA of 90%; blue: lowest dose for which a PTA of 90% is reached for a MIC of 16 mg/L (limit of susceptibility to temocillin); violet: maximal daily dose authorized in paediatrics is 4 g/d according to the leaflet of the product. Blue and grey histograms: MIC distribution of the isolates of this study and of EUCAST for *Escherichia coli* and *Klebsiella pneumoniae*, respectively. EUCAST, The European Committee on Antimicrobial Susceptibility Testing; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; PTA, probability of target attainment.

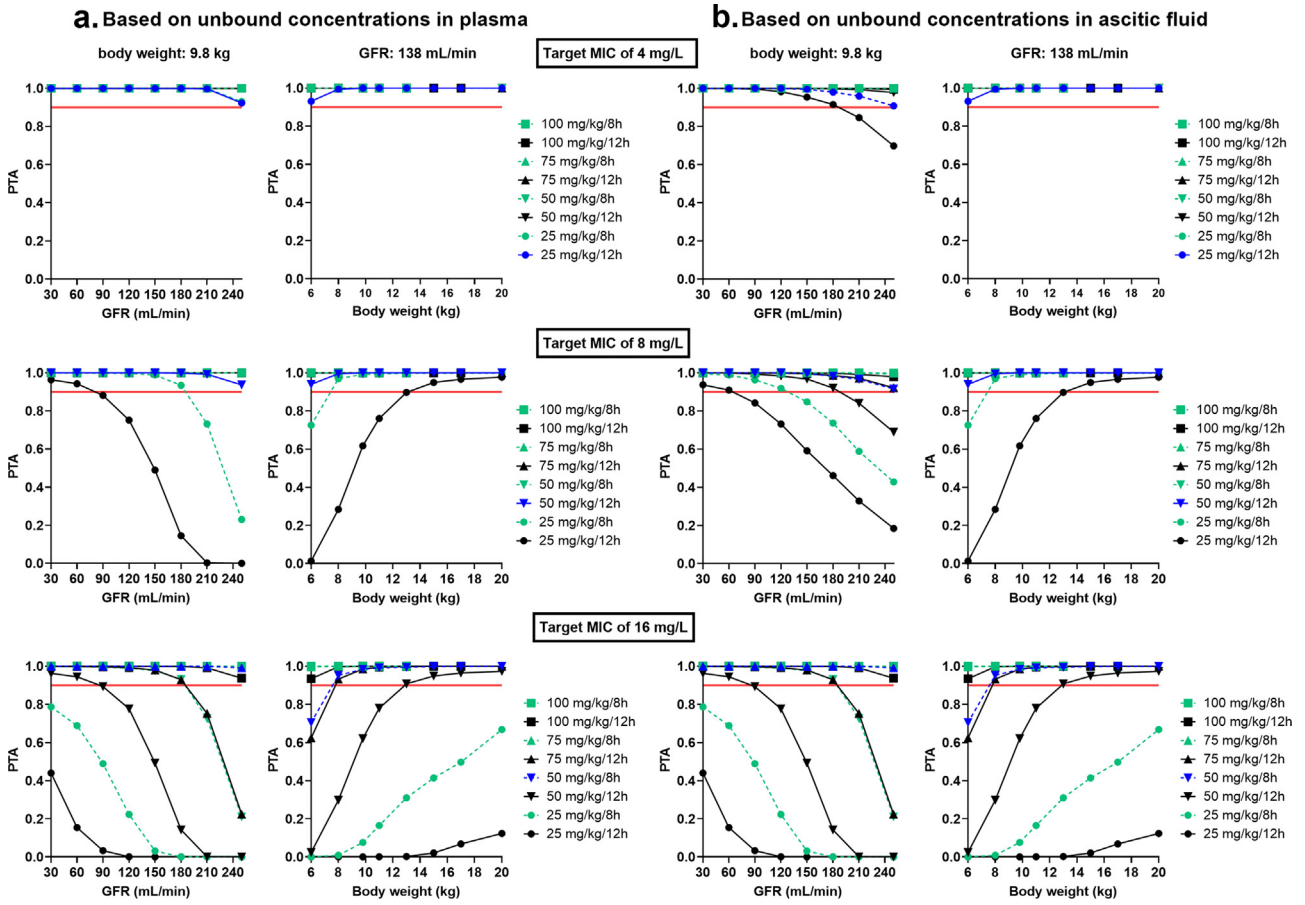

Fig. 4. Monte Carlo simulations and PTA analysis based on concentrations in plasma (a) or in ascitic fluid (b) for 25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg q12 h and q8 h administered to patients with different eGFR and a body weight of 9.8 kg (left) or to patients with different body weights and an eGFR of 138 mL/min (right), against bacteria with MICs of 4, 8 or 16 mg/L. The red-dotted line shows a PTA of 90%. Blue: lowest dose for which a PTA of 90% is reached for the corresponding MIC. The maximal daily dose authorized in paediatrics is 4 g/d according to the leaflet of the product; some simulated doses may be over this limit depending on the weight of the patient. eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; PTA, probability of target attainment.

Table 1
Recommended dosing regimens against bacteria with MIC of 4, 8 or 16 mg/L for patients with different body weights and eGFR, to reach a probability of target attainment (PTA)[a] >90% in plasma

Recommended dose based on PTA>90% for MIC=4 mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
60	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
90	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
120	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
138	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
150	50mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
180	50mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
210	50mg/kg q12h	50mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
250	75mg/kg q12h	50mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h

Recommended dose based on PTA>90% for MIC=8 mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
60	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
90	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
120	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
138	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
150	75mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
180	75mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h
210	50mg/kg q8h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h
250	75mg/kg q8h	50mg/kg q8h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h

Recommended dose based on PTA>90% for MIC=16 mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h
60	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h
90	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h
120	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h
138	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h
150	75mg/kg q8h	50mg/kg q8h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h
180	75mg/kg q8h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h
210	100mg/kg q8h	75mg/kg q8h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h
250	-	100mg/kg q8h	100mg/kg q12h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h	75mg/kg q12h

Table 2
Recommended dosing regimens against bacteria with MIC of 4, 8 or 16 mg/L for patients with different body weights and eGFR, to reach a probability of target attainment (PTA)[a] >90% in ascitic fluid

Recommended dose based on PTA>90% for MIC=4mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
60	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
90	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
120	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
138	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
150	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
180	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
210	75mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
250	50mg/kg q8h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h

Recommended dose based on PTA>90% for MIC=8mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
60	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
90	75mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
120	75mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
138	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
150	100mg/kg q12h	75mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h	25mg/kg q12h
180	100mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h
210	100mg/kg q8h	100mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h
250	-	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h

Recommended dose based on PTA>90% for MIC=16mg/L

eGFR	Body weight							
	6	8	9.8	11	13	15	17	20
30	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h	25mg/kg q12h
60	100mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q12h
90	75mg/kg q8h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h	25mg/kg q8h
120	100mg/kg q8h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h	25mg/kg q8h
138	-	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h	25mg/kg q8h
150	-	75mg/kg q8h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h	50mg/kg q12h
180	-	100mg/kg q8h	100mg/kg q12h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h	50mg/kg q12h
210	-	-	100mg/kg q8h	100mg/kg q12h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h	50mg/kg q12h
250	-	-	-	100mg/kg q8h	100mg/kg q12h	100mg/kg q12h	75mg/kg q12h	75mg/kg q12h

(-): not applicable (would exceed the maximum paediatric daily dose (4g/day))

Table S11). PTAs were lower for patients with higher eGFR or lower body weight than this representative patient (Fig. 4 and Table S11). Thus, if wishing to cover bacteria with MICs up to 16 mg/L (EUCAST susceptibility breakpoint [16]), a dose of at least 75 mg/kg/8 h would be required for the vast majority of the population studied (i.e. patients with an eGFR of 60–240 mL/min/1.73 m^2 and a body weight of 8–20 kg). Tables 1 and 2 show the minimal dose required to reach PTA >90% in plasma or ascitic fluid for patients with different weights and eGFR.

Discussion

This study reports the multi-dose safety, plasma and ascitic fluid PK and PK/PD of temocillin in LT paediatric patients. On the basis of these results, we propose new, rationally designed dosing strategies for this population.

Temocillin is an effective and well-tolerated drug in children. It has been studied in various children receiving intravenous doses of 25 mg/kg twice a day or 10 mg/kg by continuous infusion [22,23]. In the present study, temocillin peak and trough plasma total and unbound concentrations for the standard (25 mg/kg/12 h) and higher (25 mg/kg/8 h) doses were lower than those observed after a 2 g administration in critically ill adults (i.e. 28.6 mg/kg for 70 kg) [20,24,25], demonstrating that the adult dose cannot be scaled proportionally to body weight for children. We observed that the distribution volume and clearance of unbound temocillin standardized to the average weight of the population studied were also higher here than in critically ill adults (0.363 L/kg and 0.116 L/h/kg [calculated from data in Table S10]) vs. (0.214 L/kg and 0.037 L/h/kg) [21], respectively), possibly because of marked differences in renal function between these two populations, and likely resulting in a shorter plasma elimination half-life (2.15 hours vs. 4.04 hours) [21,24,25].

We also observed that hepatic dysfunction did not directly and significantly affect temocillin clearance. However, an indirect effect is likely via a decrease in albumin synthesis, and, consequently, in temocillin albumin binding [26]. As in adults, temocillin plasma protein binding is saturable in children and similar to that measured in neurotrauma intensive care unit patients with ventriculitis who showed plasma albumin concentrations comparable with those measured here [17]. Interestingly, the K_d and B_{max} binding parameters are however lower than in neurotraumatic adults. This difference may be because of the lack of data at high total temocillin concentrations which may have prevented full saturation of protein binding.

This study observed that temocillin penetration in ascitic fluid was variable among individuals, as previously described [27], probably because of variability in temocillin plasma concentration and protein concentrations. Penetration was also higher than that recently described by our group in adults (median [min–max values]: 82.2% [63.4–95.0%]) vs. 46.0% [30.0–61.6%]), with comparable unbound temocillin proportion in both populations (median [min–max values]: 36.2% [21.8–55.1%] vs. 23.0% [14.4–39.0%]) [21]. This level of penetration of temocillin into ascitic fluid supports a clinical role in the treatment of intra-abdominal infections.

From a dosing perspective, the PK/PD analyses provided considered three key variables: weight, renal function and MIC. Weight, used to calculate the drug dose (mg/kg), is highly variable in children aged 6–36 months. Body weight has also been shown to influence the clearance and distribution volume of temocillin in haemodialysis patients [28] or of the highly protein-bound ceftriaxone in critically ill children [29]. Renal function significantly impacts temocillin PK, as 80% of the administered dose is eliminated unchanged in the urine [30]. Importantly, the estimation of renal function is complex in small children. The revised Schwartz formula [31] has been specifically developed for paediatric populations, but limited to those with chronic renal disease. We therefore rather opted for the European Kidney Function Consortium formula [18]), validated from the age of 2 years, but considering also children with high filtration rates as observed here. Renal maturation, based on post-gestational age, could also be a better variable than renal function to include in the modelling, but these data were not available to us. Lastly, although most temocillin MICs for isolates collected here were ≤8 mg/L, EUCAST recommends using MICs ≤16 mg/L in PK/PD analyses to cover the entire wild-type bacterial population [16]. Monte Carlo simulations considering the 40% fT > MIC target recommended by EUCAST for temocillin [16] suggest that the licensed dose (25 mg/kg/12h) covers >90% of the isolates with MIC <4 mg/L for children with an eGFR ≤210 mL/min/1.73 m^2 or a weight ≥6 kg, and that the higher tested daily dose (25 mg/kg/8 h) covers >90% of the isolates with a MIC ≤8 mg/L for children with an eGFR ≤120 mL/min/1.73 m^2 or a weight ≥11 kg in both plasma and ascitic fluid. Much higher doses (up to 75 mg/kg/8 h) would be required for children with higher clearance rates (eGFR >150 mL/min/1.73 m^2), lower weight ≤8 kg, or infected by less susceptible organisms (MIC, 16 mg/L). These doses were not tested here, because we believed that there is insufficient safety data for the higher peak concentrations that would result from those much higher doses. More stringent targets (e.g. 100% fT > 1-4xMIC) could be required in critically ill children, but were not included in our simulations, as unreachable with registered dosing regimens. Continuous infusion could be a valuable alternative to intermittent dosing to maximize fT > MIC but has not been extensively explored to date in paediatrics.

Although a pioneer in the field, this study was not powered as an efficacy study. Additional trials in larger cohorts should evaluate the safety and pharmacological target attainment, but also the efficacy of the proposed drug regimens using a randomized design. Nevertheless, our work shows that temocillin has favourable PK properties for the prevention and treatment of intra-abdominal infections after LT in children and provides clinical guidelines on how to best adapt the dose based on the weight, eGFR and MIC of the individual patient.

To conclude, this study highlights the importance of a personalized approach to optimize dosing and suggests the importance of monitoring the unbound concentration when PK are altered because of increased renal clearance and/or low body weight. In a broader context, our work shows the risks associated with developing paediatric dosing regimens only based on allometric scaling from adult dosing regimens and supports the development of evidence-based dosing regimens for children.

Transparency declarations

PNP and LE are (or were) employees of the Université catholique de Louvain. XS, DVdL, M-LG, OC, LH, AH, and ES are (or were) employees of the Cliniques universitaires Saint-Luc. G-JW was a recipient of a Beware fellowship from the Region Wallonne. FVB is Research Director of the Fonds de la Recherche Scientifique (FRS-FNRS). The laboratories and/or clinical units of FVB, XS, DVdL, and ES have received research-supporting grants and/or honoraria from various industries for research work and/or presentations unrelated to the topic of the present paper. PNP received financial support from Eumedica to attend ECCMID 2022 (Lisbon, Portugal). Other authors have no conflicts of interest to declare. This study was funded by the programme Beware TMOPHARMACO 1510467 of the Region Wallonne, Belgium. JAR was founded by Australian

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Author contributions

FVB is a study promoter. ES and XS are principal and associated investigators. XS, DVdL, M-LG, OC, LH, AH, and ES were involved in patient's recruitment. PNP, ES, and FVB helped in protocol writing. PNP and G-JW did sample analysis. PNP, XL, SLP, G-JW, JAR, and LE did data analysis and PK/PD modelling. LE and FVB provided supervision. PNP and FVB with the contribution of all coauthors were involved in the writing of the paper.

Data availability

Data will be available upon request to the corresponding author. Part of this work has been presented as a poster (P1957) at 29th ECCMID, Amsterdam, The Netherlands, in 2019.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2024.12.015.

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Title:

Population pharmacokinetics and dosing simulation of the β-lactam temocillin in liver transplanted paediatric patients: a prospective, open-label, non-randomized study

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Supplementary Material

Method S1: Analytical method

Temocillin total and unbound concentrations in plasma and ascitic fluid were measured by an HPLC-MS/MS method using ticarcillin as internal standard. If ascitic fluid samples were hemic, they were first centrifuged (as blood samples) and the supernate was further process as a plasma sample. Total temocillin was measured after methanol precipitation of the proteins. Unbound temocillin was collected by ultrafiltration (Amicon® Ultra-15 devices fitted with centrifugal ultrafiltration filters with nominal molecular weight limit (NMWL) of 30 kDa (95% retention; Merck Millipore Ltd.). The HPLC-MS/MS method has been previously fully validated for assay in serum, and thereafter in plasma [[1]]. It has been validated here in ascitic fluid. Full linearity, accuracy, precision, trueness, recovery and matrix effect were evaluated for total and unbound temocillin quantification according the procedure of the guidelines of European Medicines Agency (EMA) [[2]].

Standard calibration (SC) and Quality control (QC): Temocillin was dissolved in water to prepare a stock solution at a final concentration of 10 mg/mL. For total temocillin concentrations, a working solution was prepared by 1:10 (v/v) dilution of this stock solution with drug-free ascitic fluid. For unbound temocillin concentrations, a pool of ultrafiltrates of ascitic fluid was used to dilute 1:10 (v/v) the stock solution. Aliquots of 1000 µL were stored in micro-centrifuge tubes at -80°C until use. Eight SC levels (1-150 mg/L and 1-100 mg/L for the total and unbound temocillin concentrations, respectively) were freshly prepared by dilution of the working solution with drug-free ascitic fluid or ultra-filtrates. Four different QC samples (1, 3, 60, 120 mg/L and 1, 3, 25, and 90 mg/L for total and unbound temocillin concentrations, respectively) were prepared by a method similar to that described for the preparation of the standard calibration.

Sample preparation: SC and QC samples were thawed on the day of use and discarded at the end of the day. A 100 µL aliquot of ascitic fluid, or its ultrafiltrate containing temocillin was pipetted into a micro-centrifuge tube and mixed with 10 µL of ticarcillin (100 mg/L) as Internal Standard (IS). Then 300 µL of methanol were added to the mixture. After a (3 x 5 seconds) vortex step, samples were centrifuged at 18,000 *g* at 4°C for 10 minutes. Ten microliters of the supernatant were injected into the HPLC/MS-MS.

Linearity: The linearity was assessed over the 8 SC, in 5 replicates over 3 days. The linearity of the assay was determined by plotting the back calculated concentrations of the validation SC against the introduced concentrations. A linear regression model, based on the least squares method, was fitted and compared to the identity line (y = x). The relative upper and lower 95% β-expectation tolerance limits were calculated to check whether they were inside the acceptance limits (±15% except for the samples at the low limit of quantification (LLoQ) for which the limit is set at ≤20%).

Trueness, precision and accuracy: Trueness, precision and accuracy were assessed on QC samples and LLoQ samples in 5 replicates for 3 days. Trueness was calculated at each concentration level of the QC and expressed as relative bias. Precision was evaluated intra-day (repeatability) and inter-day (intermediate precision) and expressed as coefficient of variation (CV). Accuracy profiles were evaluated as the sum of systematic and random errors of the test values (total error). We then checked that all the trueness and precision CV were ≤15%, except for the LLoQ samples [≤20%], and for accuracy profiles, that the relative upper

and lower 95% β-expectation tolerance were inside the acceptance limits (±15% except for the LLoQ samples [≤20%]).

Recovery and matrix effect: Extraction efficiency (recovery) of temocillin from ascitic fluid was evaluated by comparing concentrations recovered from samples treated as described above for the determination of total concentration with unextracted calibrators (6 replicates for the 4 QC concentrations). For evaluation of matrix effect, SC were prepared in solvent (H_2O) and then in matrix (ascitic fluid) in 5 replicates, and the influence of the matrix was evaluated by comparing the slope and the intercept of the linear regression obtained in water or in matrix.

Results S1: validation of temocillin assay in ascitic fluid

Linearity: Slope values and correlation coefficient (R^2) were (1.00 and 0.999) and (1.00 and 0.997) for total and unbound temocillin concentrations, respectively, and the absolute 95% β-expectation tolerance limits were within the absolute acceptance limits acceptance, attesting the linearity of this method (Figure S1 [upper panel] and Table S2).

Trueness, precision and accuracy: The CV on trueness (relative bias) were <4.310% and 5.537% for total and unbound temocillin concentrations, respectively. The CV were <2.83 and 4.13% on precision (repeatability) and <3.52 and 5.54% on the intermediate precision for total and unbound temocillin concentrations, respectively. Accuracy profiles are shown in Figure S1 [lower panel], illustrating that the relative upper and lower 95% β-expectation tolerance limits are inside the acceptance limits for total and unbound temocillin concentrations. All the accuracy, precision, and trueness results are thus in accordance with EMA guidelines criteria indicating that the method is accurate to measure temocillin in ascitic fluid (Table S2).

Recovery: and matrix effect: Temocillin extraction recovery were between 99.30 and 104.93% for both total and unbound temocillin concentrations. Concerning the matrix effect, no significant difference was observed for the intercept [p=0.100, and p=1.000 for total and unbound temocillin respectively] or for the slopes [p=0.700 and p=0.400 for total and unbound temocillin respectively] (Mann Whitney test, two-tailed, $p > 0.05$).

Method S2: Population pharmacokinetic models and Monte Carlo analyses

Structural model

Population pharmacokinetic analysis was performed using the non-linear mixed-effect modelling program Monolix version 2023R1 (LIXOFT, Antony, France) implementing the stochastic approximation expectation maximization (SAEM) algorithm. Individual estimates for PK parameters were assumed to follow log-normal distribution. The between-subject variability (BSV or ω) was described using an exponential model according to the equation $\theta_j = \theta_p \times \exp(\eta_j)$,where θ_j is the estimate for a PK parameter in the j^{th} patient as predicted by the model, θ_p is the typical population PK parameter value, and η_j is a random variable from a normal distribution with zero mean and variance ω^2, which is estimated.

The PK model was built to describe temocillin unbound concentrations in plasma and in ascitic fluid simultaneously. One- and two-compartment models with first order elimination (for plasma) integrated with the ascitic fluid compartment were compared. Several error models (constant, proportional or combined error model) were tested for describing the residual variability (ε). Model selection was based on accuracy of parameter estimates, visual inspection of goodness-of-fit (GOF) plots and numerical assessment of objective function value (OFV) and the corrected Bayesian information criteria (BICc).

The differential equations used to take into account the elimination form the two-compartments and the ascitic fluid are here below:

$\frac{dA_1}{dt} = \frac{Q}{V_2} \times A_2 - \frac{Q}{V_1} \times A_1 - k_{13} \times A_1 - \frac{CL_1}{V_1} \times A_1$
$\frac{dA_2}{dt} = \frac{Q}{V_1} \times A_1 - \frac{Q}{V_2} \times A_2$
$\frac{dA_3}{dt} = k_{13} \times A_1 - \frac{CL_3}{V_3} \times A_1$

Covariate model

From the base model, the effects of the following covariates on PK parameters of temocillin were evaluated: (i) demographic data (age, weight, BMI), (ii) physiological and biological parameters (plasma total proteins and albumin, GFR, CRP), and (iii) PELD score [[3;4]]. Continuous covariates were modelled using linear or power functions.

The covariate model was built using a stepwise procedure with forward inclusion and backward deletion. The addition of covariates was stopped when no more decrease of OFV was obtained. The statistical significance of covariate was individually evaluated during the stepwise deletion using the likelihood ratio test (LRT). Decrease of OFV at least 3.84 ($P < 0.05$) and increase of OFV over 6.63 ($P<0.01$) was required for a covariate to be considered significant in the forward inclusion and the backward deletion step, respectively.

Model evaluation

Evaluation of the model was based on GOF plots, including observations versus individual and populations predictions, plots of normalized prediction distribution error (NPDE) versus population predictions and time. The visual predictive check (VPC) was performed using 500 simulations with the final model. This plot shows the time course of the 5th, 50th, and 95th percentiles of the simulated profiles and compared with observed data.

The accuracy of the final model was also examined using a bootstrap method. A 1000-run bootstrap resampling procedure was performed in Monolix using the Rsmlx (R Speaks 'Monolix', version 4.0.2) package in R software (version 4.1.3). The median, 2.5% and 97.5% values obtained from the 1000 bootstrap runs for each parameter were calculated and compared to the estimates from the original data.

Probability of target attainment (PTA)

To define the appropriate dose for each paediatric patient based on renal function status and body weight, we performed a PTA for each GFR and body weight with eight different simulated temocillin dosing regimens. Monte-Carlo simulations were performed by Simulx version 2023R1 (LIXOFT, Antony, France) based on the final PK model to generate 1000 PK profiles for each GFR-weight-dosing scheme combination. The PTA were then calculated to evaluate the chance of reaching the define therapeutic goals for each simulated set of profiles. The tested intravenous dose regimens of temocillin were 25mg/kg (q12, and 8h), 50mg/kg (q12 and 8h), 75mg/kg (q12 and 8h) and 100mg/kg (q12 and 8h). The extra dose of 150mg/kg (q12 and q8h) was tested for patients with low body weight equal to 6 kg. Eight different levels of renal function (GFR 30, 60, 90, 120, 150, 180, 210, and 250 mL/min), and seven levels of body weight (6, 8, 11, 13, 15, 17, and 20 kg) were also tested. The PTA were also calculated for a representative paediatric patient (GFR = 138 mL/min and body weight = 9.8 kg; median values in our population). For temocillin, clinical breakpoints were established to maintain unbound concentrations above the MIC during 35-41% of the dosing interval (target %*f*T>MIC of 35 to 41%), and the target MIC were 4, 8 and 16 mg/L [[5-8]]. Therefore, the PTA for achieving 40% fT > target MIC of temocillin in plasma was calculated given that this population is critically-ill [[9;10]].

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Results S2: Population pharmacokinetic models and diagnostics

Unbound temocillin concentration-time profiles in both plasma and ascitic fluid were best described by a three-compartment model (two plasma compartments integrated with one ascitic fluid compartment) with a first order elimination from both central plasma and ascitic fluid compartment. Figure S3 shows the model structure diagram. Residual variability was best described by a combined additive plus proportional error model for plasma concentrations and by a proportional error model for concentrations in ascitic fluid.

According to covariate selection criteria (Table S9), the effect of body weight on volume of distribution of central compartment (V_1) and GFR on plasma clearance (CL_1) was remained in the final model with relationship as shown in equation below:

$$V_1 = V_{1,pop} \times \left(\frac{BW}{BW_{median}}\right)^{effect\ of\ BW} = 3.56 \times \left(\frac{BW}{9.8}\right)^{0.63}$$

$$CL_1 = CL_{1,pop} \times \left(\frac{eGFR}{eGFR_{median}}\right)^{effect\ of\ eGFR} = 1.14 \times \left(\frac{eGFR}{138}\right)^{1.87}$$

As evidenced by the goodness of fit plots (Figure S4 and S5) and VPC (Figure S6), the final model adequately described the PK profiles of unbound temocillin in both plasma and in ascitic fluid.

The pharmacokinetic parameter estimates form the final model were presented in Table S10. The value of all estimated parameters was close the median value from 1000 bootstrap results indicating the final model was stable.

Table S1 – validation of the HPLC method for the quantification of total (A) and unbound (B) temocillin in ascitic fluid

(A) Total temocillin concentration		
Validation criterion		
Trueness (n=4, J=3, k=3)	**Relative bias (%)**	**Recovery (%)**
1mg/L	1.58	101.58
3mg/L	4.31	104.31
60mg/L	4.93	104.93
120mg/L	1.72	101.72
Precision (n=4, j=3, k=3)	**Repeatability (RSD %)**	**Intermediate precision (RSD %)**
1mg/L	2.83	3.05
3mg/L	2.58	3.52
60mg/L	2.13	3.08
120mg/L	1.52	1.61
Accuracy (n=4, j=3, k=3)	**Relative β-expectation lower limit (%)**	**Relative β-expectation upper limit (%)**
1mg/L	-8.27	11.44
3mg/L	-2.77	11.39
60mg/L	-1.45	11.33
120mg/L	-3.49	6.94
Linearity (n=8, j=3, k=3)		
Slope	1.00	
Intercept	0.32	
R^2	0.999	
LOQ (mg/L)	1.00	
(B) Unbound temocillin concentration		
Validation criterion		
Trueness (n=4, j=3, k=3)	**Relative bias (%)**	**Recovery (%)**
1mg/L	2.92	102.92
3mg/L	1.35	101.35
25mg/L	-0.69	99.30
90mg/L	2.61	102.61
Precision (n=4, j=3, k=3)	**Repeatability (RSD %)**	**Intermediate precision (RSD %)**
1mg/L	4.13	4.53
3mg/L	3.73	5.54
25mg/L	3.09	3.17
90mg/L	2.29	2.43
Accuracy (n=4, j=3, k=3)	**Relative β-expectation lower limit (%)**	**Relative β-expectation upper limit (%)**
1mg/L	-11.78	17.62
3mg/L	-10.14	12.84
25mg/L	-10.84	9.45
90mg/L	-5.24	10.46
Linearity (n=7, j=3, k=3)		
Slope	1.00	
Intercept	-0161	
R^2	0.997	
LOQ (mg/L)	1.00	

n, number of independent concentrations; j, number of series; k, number of repetitions per series; RSD, relative standard deviation

Table S2: Demographic and biochemical parameters of the patients included in the study

parameters	Groups		
	Group #1 25mg/kg/12h (N= 14)	Group #2 25mg/kg/8h (N= 23)	P value
Age (month)			0.9875[a]
mean ± SD	19.29 ± 11.57	18.78 ± 10.44	
median	17.00	18.00	
range	6.00 - 36.00	6.00 - 36.00	
Male gender, n (%)	6 (42.84)	8 (34.78)	0.7321[c]
Mode of administration (schedule)	30 min infusion (Q 12h)	30 min infusion (Q 8h)	
Pharmacokinetic sampling at dose (number)	4 and 8	4 and 8	
Patients with fever and positive microbiological culture, n (%)	5 (35.7%)	9 (39.1%)	
Treatment duration (days; median [range])	5 [5 – 10]	5 [2 – 10]	
Body weight (kg)			0.0875[a]
mean ± SD	11.37 ± 3.30	9.45 ± 2.72	
median	12.00	8.4	
range	6.60 – 18.00	6.00 – 15.20	
Body mass index (kg/m^2)			0.1991[a]
mean ± SD	11.05 ± 3.64	9.51 ± 3.21	
median	12.00	8.40	
range	6.10 – 18.60	6.00 – 17.00	
Plasma protein level (64-83 g/L)[1]			0.2691[b]
mean ± SD	50.89 ± 7.92	54.13 ± 8.84	
median	52.16	56.00	
range	36.00 – 61.23	36.00 – 66.00	
Plasma albumin level (35-52 g/L)[1]			0.9547[b]
mean ± SD	33.62 ± 8.11	33.74 ± 4.82	
median	33.91	35.00	
range	18.00 – 48.69	24.00 – 43.00	
Ascitic fluid protein level[2]			NA
mean ± SD		26.76 ± 3.27	
Median	No samples collected	27.15	
Range		21.80 – 32.00	
Ascitic fluid albumin level[2]			NA
mean ± SD		18.85 ± 3.33	
median	No samples collected	18.45	
range		14.20 – 23.50	
INR (0.80-1.20)[1]			0.1682[a]
mean ± SD	1.44 ± 0.31	1.69 ± 0.53	
median	1.43	1.53	
range	1.08 – 2.28	1.03 – 2.74	
Alanine transaminase (7-35 U/L)[1]			0.2629[b]
mean ± SD	145.8 ± 102.8	197.0 ± 147.5	
median	141.5	200.0	
range	19.00 – 403.0	9.00 – 572.0	
Aspartate Transaminase (9-36 U/L)[1]			0.4155[a]
mean ± SD	145.1 ± 136.1	189.0 ± 161.6	
median	84.50	209.0	
range	34.00 – 434.0	14.00 – 668.0	
gamma-glutamyl transferase (< 40 U/L)[1]			1.0000[a]
mean ± SD	109.8 ± 119.1	97.60 ± 89.05	
median	75.50	70.00	
range	23.00 – 485.0	27.00 – 451.0	

Total Bilirubin (< 1.2 mg/dL)[1]			0.5519[a]
mean ± SD	4.399 ± 3.684	5.422 ± 4.368	
median	3.050	4.200	
range	0.700 – 13.70	0.200 – 17.90	
Conjugated ("direct") bilirubin (< 0.3 mg/dL)[1]			0.9003[a]
mean ± SD	3.886 ± 3.395	4.409 ± 3.713	
median	2.50	4.800	
range	0.800 – 12.00	0.200 – 15.00	
Urea (15-50 mg/dL)[1]			0.0451[a]
mean ± SD	23.89 ± 26.65	32.83 ± 28.92	
median	17.50	26.00	
range	5.00 – 111.0	8.00 – 150.0	
Serum creatinine (0.60-1.30 mg/dL)[1]			0.0898[a]
mean ± SD	0.212 ± 0.100	0.329 ± 0.410	
median	0.170	0.2	
range	0.060 – 0.430	0.170 – 2.160	
***Estimated glomerular filtration rate (> 60 mL/min/1.73 m^2)[1]**			0.2978[b]
mean ± SD	146.1 ± 64.75	126.0 ± 50.65	
median	139	124.0	
range	45.00 – 253.0	56.30 – 239.0	
C-reactive protein (< 5.0 mg/L)[1]			0.2341[a]
mean ± SD	57.51 ± 44.40	84.48 ± 72.76	
median	38.65	58.10	
range	16.00 – 150.4	7.700 – 292.6	
Paediatric end-stage liver disease score (PELD score)[[11]]			0.4082[b]
mean ± SD	14.71 ± 4.48	16.17 ± 5.499	
median	15.00	18.50	
range	7.00 – 22.00	5.00 – 25.00	
Comedications (number)			
• Alburex 5%: Human albumin solution for infusion for compensation of 1/2 or 2/3 of drain losses	14	20	
• Tacrolimus 0.025 – 0.05 mg/kg/day (CI) q24h, followed by 0.30mg/kg/day (po) q12h (monitoring of trough concentration)	14	18	
• Morphine: 0.025 – 0.1mg/kg/day (IV) every 5 to 10 minutes (depending mainly on the age of the patient) (monitoring of pain)	14	14	
• Paracetamol 15 mg/kg (IV) every 6hours	14	23	
• Ampicillin[$]: 25 - 50 mg/kg/day (IV) q8h	14	0	
• Amoxicillin: 100 - 200 mg/kg/day (IV) q8h	0	23	
• vitamin K (phytomenadione) 1 - 5 mg (IV) (monitoring INR)	14	17	
• Basiliximab 10 mg/12h (IV)	14	19	

[a]Mann Whitney U-test, two-tailed; [b]Student's Unpaired t-test, two-tailed; [c]Fisher's exact test, INR, International Normalized Ratio; [1] normal values in the local laboratory; [2]ascitic fluid samples collected from 10 patients; NA, no applicable; [$]withdrawn from the Belgian market in January 2018; N, number of patients *The estimate of GFR in children is based on the EKFC (European Kidney Function Consortium) formula published by Hans Pottel *et al.* [12] and measured in the 24h period of PK sampling.

Table S3: Pharmacokinetic parameters of temocillin in plasma (pooled data after the administration of the 4th and 8th doses)

Parameter	Plasma total temocillin			Plasma unbound temocillin		
	Group #1: 25mg/kg/12h N=14; n=130[a]	Group #2: 25mg/kg/ 8h N=14; n=125[b]	P value	Group #1: 25mg/kg/12h N=14; n=130[a]	Group #2: 25mg/kg/ 8h N=14; n=125[b]	P value
C_{max} (mg/L)			0.1247[c]			0.0848[d]
Mean±SD	107.5±21.87	114.3±19.63		38.74±15.72	44.23±11.09	
Median	103.3	111.3		34.82	42.08	
Range	75.44–157.2	85.50–151.6		18.42–72.00	28.38–64.93	
C_{min} (mg/L)			<0.0001[d]			<0.0001[d]
Mean±SD	8.274±4.089	21.69±8.196		1.767±1.136	5.232±2.632	
Median	7.236	20.62		1.390	4.661	
Range	3.099–15.26	10.25–41.05		0.501–4.953	1.996–10.83	
$AUC_{\rightarrow t}$ (mg.h/L)			0.2623[d]			0.9474[d]
Mean±SD	394.2±121.5	354.6±80.65		108.5±51.02	100.2±29.53	
Median	364.8	317.8		95.27	95.99	
Range	219.3–698.7	242.8–543.5		48.09–245.3	61.46–165.5	
$AUC_{\rightarrow 24h}$ (mg.h/L)			0.0002[d]			0.0012[d]
Mean±SD	788.3±243.0	1064±241.0		217.1±102.0	300.6±88.58	
Median	729.5	953.4		190.5	288.0	
Range	438.6–1397	723.3–1630		96.18–490.6	184.4–496.6	
$T_{1/2}$ (h)			0.8285[c]			0.5866[c]
Mean±SD	3.353±0.680	3.303±0.690		2.757±0.409	2.685±0.521	
Median	3.151	3.224		2.791	2.690	
Range	2.491–5.105	2.156–4.688		2.094–3.568	1.894–3.752	

[a]70/60 samples after 4th/8th doses; [b]97/55 samples after 4th/8th doses; [c]Student's Unpaired t-test, two-tailed; [d]Mann Whitney U-test, two-tailed

C_{max}, Peak concentration after 30min infusion; C_{min}, trough concentration at 12 or 8 hours; AUC, area under curve (from 0 →t or from 0→24h); $T_{1/2}$, half-life; N, number of patients; n, number of samples

Table S4: Pharmacokinetic parameters of temocillin in plasma: comparison between the prophylaxis and treatment (group #1 or group #2) from patients with fever and negative microbiological culture (**prophylaxis**) vs. patients with fever and positive microbiological culture (**treatment**) after the 4th and 8th administration. Pooled data from the 4th and 8th doses.

Group #1: Administered dose, 25mg/kg/ 12h						
Parameter	**Plasma total temocillin**			**Plasma unbound temocillin**		
	Prophylaxis N=9	**Treatment N=5**	**P value**	**Prophylaxis N=9**	**Treatment N=5**	**P value**
C_{30min} (mg/L)			0.4874			0.0628
mean ±SD	105.6 ± 22.24	11.5 ± 21.98		34.78 ± 15.00	47.66 ± 14.31	
median	101.3	106.3		30.95	52.63	
range	75.44 – 157.2	85.20 – 145.2		18.42 – 72.00	22.84 – 63.60	
C_{12h} (mg/L)			0.2548			0.1735
mean±SD	7.737 ± 4.370	9.583 ± 3.109		1.665 ± 1.281	1.995 ± 0.736	
median	5.900	9.620		1.365	1.881	
range	3.100 – 15.26	4.389 – 13.82		0.500 – 4.953	1.090 – 3.209	
$AUC_{\rightarrow 12h}$ (mg.h/L)			0.1410			0.1566
mean±SD	372.5 ± 124.4	430.9 ± 115.7		104.5 ± 58.50	117.5 ± 29.15	
median	348.7	464.0		82.44	128.6	
range	219.3 – 698.7	246.9 – 566.4		48.09 – 245.3	64.11 – 151.1	
Group #2: Administered dose, 25mg/kg/ 8h						
Parameter	**Plasma total temocillin**			**Plasma unbound temocillin**		
	Prophylaxis N=11	**Treatment N=3**	**P value**	**Prophylaxis N=11**	**Treatment N=3**	**P value**
C_{30min} (mg/L)			0.7302			0.3531
mean ±SD	115.9 ± 19.45	119.2 ± 22.49		44.51 ± 11.46	50.21 ± 12.04	
median	111.3	128.9		42.08	52.52	
range	85.50 – 151.6	128.9 – 140.4		28.38 – 64.93	32.11 – 64.93	
C_{8h} (mg/L)			0.1445			0.6329
mean±SD	21.84 ± 8.946	27.33 ± 9.394		5.556 ± 2.730	6.240 ± 3.582	
median	18.26	23.14		5.496	4.661	
range	10.25 – 41.05	18.94 – 41.05		1.996 – 10.83	2.552 – 10.83	
$AUC_{\rightarrow 8h}$ (mg.h/L)			0.2540			0.5417
mean±SD	358.9 ± 86.33	398.6 ± 101.9		103.0 ± 30.12	113.0 ± 39.60	
median	317.8	383.0		95.99	107.0	
range	242.8 – 543.5	302.4 – 543.5		64.50 – 165.5	61.46 – 165.5	

Mann Whitney U-test, two-tailed; C_{30min}, Peak concentration after 30min infusion; $C_{12h \text{ or } C8h}$, trough concentration; AUC, area under curve; Ke, elimination constant; $T_{1/2}$, half-life; N, Number of patients in whom 5 blood samples were taken at the fourth and eighth doses

Table S5: Pharmacokinetic parameters of temocillin in plasma: comparison between the 4th and 8th doses in group #1 or group #2

Group #1: Administered dose, 25mg/kg/ 12h						
	Plasma total temocillin			Plasma unbound temocillin		
Parameter	4th dose N=14; n=70	8th dose N=12; n=60	P value	4th dose N=14; n=70	8th dose N=11; n=60	P value
C_{30min} (mg/L)			0.2036[e]			1.0303[e]
mean ±SD	104.2 ± 21.55	111.3 ± 22.56		38.92 ± 17.34	38.53 ± 14.36	
median	101.3	105.4		37.00	32.35	
range	75.44 – 149.6	88.70 – 157.2		18.42 – 72.00	23.66 – 70.60	
C_{12h} (mg/L)			0.6853[d]			0.1294[e]
mean±SD	8.789 ± 4.341	7.672 ± 3.873		2.029 ± 1.233	1.461 ± 0.974	
median	8.253	6.511		1.801	1.340	
range	3.099 – 15.26	3.294 – 14.27		0.501 – 4.953	0.579 – 4.218	
$AUC_{\rightarrow 12h}$ (mg.h/L)			0.6775[d]			0.7420[d]
mean±SD	401.7 ± 109.6	385.3 ± 138.5		109.4 ± 54.70	107.5 ± 48.76	
median	389.3	352.0		120.6	107.5	
range	240.2 – 644.4	219.3 – 698.7		48.09 – 242.6	66.20 – 245.3	
$AUC_{\rightarrow \infty}$ (mg.h/L)			0.6347[d]			0.7370[d]
mean±SD	483.3 ± 148.3	445.7 ± 161.2		164.3 ± 76.51	157.6 ± 73.55	
median	508.1	440.8		140.0	131.6	
range	251.3 – 767.5	231.7 – 773.4		90.64 – 349.0	80.56 – 332.9	
ke (h^{-1})			0.8660[d]			0.1031[d]
mean±SD	0.209 ± 0.045	0.219 ± 0.031		0.243 ± 0.036	0.272 ± 0.035	
median	0.210	0.228		0.240	0.273	
range	0.135 – 0.278	0.156 – 0.267		0.194 – 0.307	0.222 – 0.330	
$T_{1/2}$ (h)			0.6347[d]			0.0858[d]
mean±SD	3.465 ± 0.802	3.222 ± 0.507		2.907 ± 0.423	2.582 ± 0.327	
median	3.299	3.032		2.884	2.532	
range	2.491 – 5.105	2.596 – 4.4.35		2.256 – 3.568	2.094 – 3.116	
Group #2: Administered dose, 25mg/kg/ 8h						
	Plasma total temocillin			Plasma unbound temocillin		
Parameter	After 4 doses N=14; n=70	After 8 doses; N=11; n=55	P value	After 4 doses N=14; n=60	After 8 doses; N=11; n=55	P value
C_{30min} (mg/L)			0.7499[d]			
mean ±SD	113.4 ± 20.16	115.3 ± 19.85		43.37 ± 12.05	45.32 ± 10.21	0.8104[d]
median	108.6	111.6		41.50	42.08	
range	85.56 – 151.6	90.41 – 148.2		28.38 – 64.93	29.75 – 58.01	
C_{8h} (mg/L)			0.9658[e]			
mean±SD	21.02 ± 8.019	22.55 ± 8.729		4.986 ± 2.685	5.546 ± 2.656	
median	19.37	21.76		4.231	5.496	0.9661[d]
range	13.43 – 41.05	10.25 – 40.23		2.197 – 10.72	1.996 – 10.83	
$AUC_{\rightarrow 8h}$ (mg.h/L)			0.9658[e]			
mean±SD	346.4 ± 82.82	365.1 ± 80.50		98.50 ± 31.64	102.4 ± 27.95	
median	313.1	383.0		90.91	105.3	0.6403[d]
range	242.8 – 543.5	277.2 – 535.5		61.46 – 165.5	67.72 – 151.5	
$AUC_{\rightarrow \infty}$ (mg.h/L)			0.9658[e]			
mean±SD	497.5 ± 214.7	525.7 ± 217.1		186.2 ± 59.96	135.4 ± 56.04	
median	454.3	476.2		117.1	126.3	0.5815[d]
range	315.1 - 1170	319.3 - 1119		70.00 – 303.5	76.22 – 279.9	
ke (h^{-1})			0.4720[d]			
mean±SD	0.2216 ± 0.04164	0.2148 ± 0.04981		0.2718 ± 0.04818	0.2620 ± 0.05623	
median	0.2258	0.2039		0.2685	0.2577	0.9201[d]
range	0.1479 – 0.2789	0.1547 – 0.3215		0.2092 – 0.3410	0.1847 – 0.3660	
$T_{1/2}$ (h)			0.7170[d]			
mean±SD	3.244 ± 0.6798	3.377 ± 0.7290		2.627 ± 0.4686	2.760 ± 0.5969	0.7730[d]
median	3.071	3.399		2.591	2.690	
range	2.485 - 4.688	2.156 – 4.481		2.033 – 3.313	1.894 – 3.752	

Table S6: Parameters of temocillin protein binding based on assay of unbound and total temocillin.

Parameters	**Group #1 25mg/kg/12h N=14; n=130**	**Group #2 25mg/kg/8h N=14; n=125**
Unbound concentration (Figure 2 panel A; fitting of a 2^{d} order polynomial function ($Y = B0 + B1 * X + B2 * X^2$))		
R^2 (goodness of fit)	0.815	0.928
B0 (95% CI); mg/L	1.60 (-1.58 to 3.90)	2.26 (-0.24 to 0.47)
B1 (95% CI)	0.12 (0.02 to 0.23)	0.11 (0.03 to 0.18)
B2 (95% CI)	1.94 (1.13 to 2.74) x 10^{-3}	2.02 (0.14 to 2.55) x 10^{-3}
Unbound fraction (Figure 2 panel B; fitting of a 1st order polynomial function ($Y = B0 + B1 * X$))		
no. of data points	130	125
R^2 (goodness of fit)	0.176	0.358
B0 (95% CI); mg/L	20.31 (17.36 to 23.26)	21.18 (19.01 to 23.35)
B1 (95% CI)	0.13 (0.08 to 0.17)	0.12 (0.09 to 0.15)
Binding parameters (Figure 2 panel C; fitting of a "Saturation Binding Curve Accounting for Ligand Depletion" (SBCALD) equation)		
R^2 (goodness of fit)	0.925	0.966
K_d (95% CI); mg/L [a]	29.52 (18.64 to 50.70)	31.77 (24.35 to 42.61)
B_{max} (95% CI); mg/L [b]	126.5 (104.1 to 169.4)	129.1 (114.4 to 150.5)

[a] equilibrium dissociation constant (temocillin concentration needed to achieve a half-maximum binding at equilibrium; evaluation of the binding affinity).

[b] maximum specific binding (specific binding extrapolated to very high concentrations of temocillin; evaluation of the density of binding sites)

Table S7: Key pharmacokinetic parameters of temocillin in plasma/ascitic fluid after administration of 25mg/kg/8h (patients of group #2, pooled data after the administration of the 4th and 8th doses)

Parameter	Total temocillin			Unbound temocillin		
	Plasma N = 10; n=80	Ascitic fluid N = 10; n=80	P value	Plasma N = 10; n=80	Ascitic fluid N = 10; n=80	P value
C_{30min} (mg/L)			< 0.001[a]			0.0021[b]
Mean±SD	112.50±15.16	29.83±12.94		42.35±10.77	12.56±7.95	
Median	110.08	28.23		39.71	10.97	
Range	85.50–144.57	9.40–52.24		28.38–64.92	2.50–31.40	
C_{8h} (mg/L)			0.5999 [a]			0.0654[b]
Mean±SD	21.93±8.04	22.93±12.47		5.35±2.38	9.10±8.45	
Median	21.33	18.80		4.66	7.25	
Range	10.24–41.04	2.20–45.89		2.19–10.72	0.40–39.48	
T_{max} (h)						
Mean±SD	0.5	2.437±1.116		0.5	2.437±1.116	
Median	0.5	2		0.5	2	
Range	-	1-4		-	1-4	
$AUC_{\rightarrow 8h}$ (mg.h/L)			0.0214[b]			0.3484[b]
Mean±SD	354.19±79.30	291.2±115.4		99.82±26.96	131.89±83.83	
Median	317.72	261.1		97.07	115.0	
Range	242.75–543.47	153.9–516.1		64.49–165.5	52.90–399.4	

C_{max}, Peak concentration; C_{min}, trough concentration at 8 hours; T_{max}, maximal time to reach peak concentration in ascitic fluid; AUC, area under curve (from 0 →8h); N, number of patients; n, number of samples
[a]Student's Unpaired t-test, two-tailed; [b]Wilcoxon matched paired signed rank test

Table S8: Pharmacokinetic parameters of temocillin in ascitic fluid from group #2 patients, comparing data obtained after the 4th or 8th administration.

Group #2: Administered dose, 25mg/kg/ 8h					
	Ascitic fluid total temocillin			Ascitic fluid unbound temocillin		
Parameter	After 4 doses N=10**; n=50	After 8 doses; N=6**; n=30	P value	After 4 doses N=10**; n=50	After 8 doses; N=6**; n=30	P value
C_{max} (mg/L)						
mean ±SD	53.33 ± 16.75	49.40 ± 18.10		27.24 ± 16.34	22.83 ± 15.41	
median	48.72	51.91	0.1563	21.20	16.92	0.2188
range	32.59 – 79.32	25.46 – 72.86		12.00 – 61.64	10.40 – 49.30	
T_{max} (h)						
mean ±SD	2.50 ± 1.08	2.25 ± 1.47		2.700± 1.16	2.00 ± 1.095	
median	2.00	2.00	0.7500	2.00	2.00	0.2500
range	1.00 – 4.00	0.50 – 4.00		1.00 – 4.00	1.00 – 4.00	
C_{min} (mg/L)						
mean±SD	23.78 ± 14.67	23.34 ± 12.34		10.49 ± 10.77	6.736 ± 3.142	
median	20.04	18.95	0.1563	7.655	5.874	0.8125
range	2.200 – 44.71	12.69 – 45.90		0.400 – 39.48	4.100 – 12.40	
$AUC_{\rightarrow 8h}$ (mg.h/L)						
mean±SD	290.4 ± 120.3	292.5 ± 128.8		139.0 ± 99.14	91.46 ± 32.42	
median	262.9	236.5	0.5771	115.0	92.15	0.1563
range	153.9 – 516.2	173.0 – 461.3		52.90 – 399.4	52.90 – 129.6	

Wilcoxon signed rank test, Two-tailed; C_{max}, Peak concentration after 30min infusion; T_{max} , time to reach peak concentration; C_{min}, trough concentration at 8 hours; AUC, area under curve; **ascetic fluid sampling only in 10, and 6 patients due to non-production at the time of blood sampling; N, Number of patients in whom 5 blood samples were taken at the fourth and eighth doses; n, number of samples

Table S9: Covariate analysis and the final model selection

	Model	OFV	∆OFV
Forward inclusion	Base	1841.08	
	Add GFR effect on CL_1	1813.69	-27.39
	Add BW effect on V_1	1804.55	-9.14
Backward exclusion	Remove GFR effect on CL_1	1830.87	+26.28
	Remove BW effect on V_1	1813.69	+9.14

Table S10: Population parameter estimates from the final PK model.

Parameter	Estimate (%RSE) [shrinkage %]	Bootstrap median (95%CI)
Fixed effects		
CL_1 (L/h)	1.14 (13.7)	1.29 (0.77-1.76)
V_1 (L)	3.56 (10.9)	3.62 (2.87-4.39)
Q (L/h)	10.7 (18.2)	10.4 (7.98-14.8)
V_2 (L)	6.97 (9.39)	6.84 (5.65-8.26)
k_{13} (h^{-1})	0.42 (9.09)	0.37 (0.24-0.54)
V_3 (L)	3.60 (29.9)	3.09 (1.70-7.13)
CL_3 (L/h)	1.30 (28.3)	1.08 (0.58-1.72)
Effect of weight on V_1	0.63 (28.3)	0.68 (0.26-1.24)
Effect of GFR on CL_1	1.87 (10.2)	1.43 (0.70-2.83)
Random effects		
BSV_CL_1 (%)	9.68 (92.1) [-5.43]	10.4 (3.44-28.6)
BSV _V_1(%)	25.9 (31.0) [-6.34]	30.1 (7.44-58.3)
BSV _Q (%)	92.1 (16.4) [6.24]	80.3 (42.5-123)
BSV _V_2 (%)	53.6 (14.2) [-12.9]	50.5 (31.2-67.9)
BSV _k_{13} (%)	22.6 (31.6) [8.85]	18.7 (6.61-34.5)
BSV _V_3 (%)	50.5 (72.9) [4.73]	42.7 (16.6-99.9)
BSV _CL_3 (%)	43.7 (27.3) [2.43]	37.0 (15.1-74.7)
Residual error		
Additive (mg/L) – plasma concentration	0.27 (47.0)	0.24 (0.14-0.54)
Proportional – plasma concentration	0.078 (18.9)	0.078 (0.052-0.12)
Proportional - ascitic fluid concentration	0.45 (10.6)	0.43 (0.32-0.57)

CL_1, clearance from the central compartment; *V_1*, volume of central compartment; *Q*, intercompartmental clearance between central and peripheral compartment; *V_2*, volume of peripheral compartment; *k_{13}*, the transport rate constant from plasma to ascitic fluid; *V_3*, volume of ascitic fluid compartment; *CL_3*, clearance from the ascitic fluid compartment; RSE, relative standard error; CI, confidence interval; BSV, between subject variability

Table S11: Probability of target attainment (PTA)[a] for various temocillin dosing regimens against bacteria with MICs of 4, 8 or 16 mg/L according to (i) the eGFR, and (ii) the body weight values

A. In plasma

Target MIC (mg/L)	eGFR (mL/min/1.73 m²)	Body weight (kg)	Temocillin doses							
			Studied		Simulated					
			25mg /kg/12h	25mg /kg/8h	50mg /kg/12h	50mg /kg/8h	75mg /kg/12h	75mg /kg/8h	100mg /kg/12h	100mg /kg/8h
4mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	100	100	100	100	100	100	100	100
	60	9.8	100	100	100	100	100	100	100	100
	90	9.8	100	100	100	100	100	100	100	100
	120	9.8	100	100	100	100	100	100	100	100
	138	**9.8**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	150	9.8	100	100	100	100	100	100	100	100
	180	9.8	100	100	100	100	100	100	100	100
	210	9.8	99.7	100	100	100	100	100	100	100
	250	9.8	92.3	93	100	100	100	100	100	100
	(ii) PTA (%) according to the body weight									
	138	6	93.1	100	99.9	100	100	100	100	100
	138	8	99.4	100	100	100	100	100	100	100
	138	**9.8**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	138	11	100	100	100	100	100	100	100	100
	138	13	100	100	100	100	100	100	100	100
	138	15	100	100	100	100	100	100	100	n.a.
	138	17	100	100	100	100	100	100	100	n.a.
	138	20	100	100	100	100	100	n.a.	100	n.a.
8 mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	96.3	99.9	100	100	100	100	100	100
	60	9.8	94.2	99.8	100	100	100	100	100	100
	90	9.8	88.1	99.8	100	100	100	100	100	100
	120	9.8	75.1	99.6	100	100	100	100	100	100
	138	**9.8**	**61.7**	**99.2**	**100**	**100**	**100**	**100**	**100**	**100**
	150	9.8	49	98.8	100	100	100	100	100	100
	180	9.8	14.5	93.4	99.8	100	100	100	100	100
	210	9.8	0.3	73.1	99.2	100	100	100	100	100
	250	9.8	0	23	93.6	100	100	100	100	100
	(ii) PTA (%) according to the body weight									
	138	6	1.3	72.5	93.9	100	99.9	100	99.9	100
	138	8	28.4	96.8	99.5	100	100	100	100	100
	138	**9.8**	**61.7**	**99.2**	**100**	**100**	**100**	**100**	**100**	**100**
	138	11	76	99.3	100	100	100	100	100	100
	138	13	89.7	99.7	100	100	100	100	100	100
	138	15	94.9	99.7	100	100	100	100	100	n.a.
	138	17	96.6	99.7	100	100	100	100	100	n.a.
	138	20	97.7	99.8	100	100	100	n.a.	100	n.a.
16 mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	26.4	78.7	96.2	99.9	99.9	100	100	100
	60	9.8	15.4	68.9	94.4	99.9	99.8	100	100	100
	90	9.8	3.3	48.9	89.3	99.5	99.6	100	100	100
	120	9.8	0.1	22.3	77.7	99	99.2	100	100	100
	138	**9.8**	**0**	**7.6**	**62.1**	**98.4**	**98.5**	**99.9**	**100**	**100**

150	9.8	0	3.1	49.2	97.7	98	99.9	99.9	100
180	9.8	0	0	14.2	93.1	92.9	99.9	99.9	100
210	9.8	0	0	1	72.7	75.3	99.9	99.1	100
250	9.8	0	0	0	21	22.4	99.1	93.7	100
(ii) PTA (%) according to the body weight									
138	6	0	0	2.3	70.5	62.4	99.6	93.4	100
138	8	0	0.9	29.8	95.2	93.2	99.9	99.7	100
138	**9.8**	**0**	**7.6**	**62.1**	**98.4**	**98.5**	**99.9**	**100**	**100**
138	11	0.1	16.5	78	99.1	99.5	100	100	100
138	13	0.2	31	90.7	99.4	99.8	100	100	100
138	15	2.1	41.4	94.8	99.8	100	100	100	n.a.
138	17	6.8	49.7	96.4	99.9	100	100	100	n.a.
138	20	12.3	66.8	97.3	99.9	100	n.a.[b]	100	n.a.

B. in ascitic fluid

Target MIC (mg/L)	eGFR (mL/min/1.73 m²)	Body weight (kg)	Temocillin doses							
			Studied		Simulated					
			25mg /kg/12h	25mg /kg/8h	50mg /kg/12h	50mg /kg/8h	75mg /kg/12h	75mg /kg/8h	100mg /kg/12h	100mg /kg/8h
4mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	100	100	100	100	100	100	100	100
	60	9.8	100	100	100	100	100	100	100	100
	90	9.8	99.6	100	100	100	100	100	100	100
	120	9.8	98.2	100	100	100	100	100	100	100
	138	**9.8**	**97.1**	**99.8**	**100**	**100**	**100**	**100**	**100**	**100**
	150	9.8	95.4	99.5	99.9	100	100	100	100	100
	180	9.8	91.5	98	99.8	100	100	100	100	100
	210	9.8	84.6	95.9	99.3	100	100	100	100	100
	250	9.8	69.8	90.8	97.8	100	99.5	100	100	100
	(ii) PTA (%) according to the body weight									
	138	6	69.8	90.8	97.8	100	99.5	100	100	100
	138	8	90.9	98	99.7	100	100	100	100	100
	138	**9.8**	**97.1**	**99.8**	**100**	**100**	**100**	**100**	**100**	**100**
	138	11	98.8	100	100	100	100	100	100	100
	138	13	99.9	100	100	100	100	100	100	100
	138	15	100	100	100	100	100	100	100	n.a.
	138	17	100	100	100	100	100	100	100	n.a.
	138	20	100	100	100	100	100	n.a.	100	n.a.
8 mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	93.7	99.4	100	100	100	100	100	100
	60	9.8	90.9	98.8	99.9	100	100	100	100	100
	90	9.8	84.2	96.2	99.3	100	99.9	100	100	100
	120	9.8	73.1	91.8	98.3	100	99.8	100	100	100
	138	**9.8**	**64.9**	**87.8**	**97.7**	**99.9**	**99.5**	**100**	**100**	**100**
	150	9.8	59.1	84.7	96.7	99.6	99.4	100	99.9	100
	180	9.8	46.1	73.6	92	98.2	98.6	99.9	99.7	100
	210	9.8	32.8	58.8	84	96.6	97.1	99.9	99.1	100
	250	9.8	18.4	42.8	68.9	91.5	92.1	99.2	97.9	99.8
	(ii) PTA (%) according to the body weight									
	138	6	15.4	39.7	66.3	91.4	91.5	99.3	97.8	99.7
	138	8	42.7	73.2	90.6	98.1	98.4	99.9	99.5	100
	138	**9.8**	**64.9**	**87.8**	**97.7**	**99.9**	**99.5**	**100**	**100**	**100**
	138	11	76.9	92.9	98.5	100	99.9	100	100	100
	138	13	88.5	97.4	99.7	100	100	100	100	100

	138	15	93.5	99.1	99.9	100	100	100	100	n.a.
	138	17	97.1	99.7	100	100	100	100	100	n.a.
	138	20	99	100	100	100	100	n.a.	100	n.a.
16 mg/L	**(i) PTA (%) according to the eGFR**									
	30	9.8	46.6	80.3	94.4	99.6	99.4	100	99.7	100
	60	9.8	37.9	72.1	90.8	98.7	98.7	100	99.6	100
	90	9.8	26.2	56.9	82.3	97.1	97.3	99.9	99.2	100
	120	9.8	15.8	41.8	69.2	93.5	94.4	99.6	98.7	99.9
	138	**9.8**	**11.2**	**33.3**	**63.5**	**89.2**	**90.6**	**98.9**	**97.6**	**99.8**
	150	9.8	9.1	28	57.8	58.9	87.4	98.4	96.3	99.6
	180	9.8	5.1	16.5	41.5	76.1	78.8	95.7	91.5	97.9
	210	9.8	2.1	9.7	29.1	61.7	64.7	88.5	84.6	94.9
	250	9.8	1	4	17	43.1	46.4	77.1	72.7	88.6
	(ii) PTA (%) according to the body weight									
	138	6	0.7	3	14.1	41.5	42.1	76	69.8	88.4
	138	8	4.2	14.1	38.8	74.9	77.2	95.1	90.6	98.1
	138	**9.8**	**11.2**	**33.3**	**63.5**	**89.2**	**90.6**	**98.9**	**97.6**	**99.8**
	138	11	19.2	45.6	73.6	94.4	95.7	99.7	98.9	99.9
	138	13	33.9	64.4	88	97.7	98.3	99.9	99.5	100
	138	15	49.2	78.4	95.1	99	99.1	100	99.8	n.a.
	138	17	61.3	87.2	97.6	99.9	99.5	100	100	n.a.
	138	20	76.9	94.4	98.8	100	99.9	n.a.[b]	100	n.a.

[a] PTA > 90%: green background; PTA < 90%: red background, taking into account MIC, eGFR and body weight. Bold values: data for representative paediatric patient (median values of eGFR and body weight for the studied population).

[b] n.a. not applicable (would exceed the maximum paediatric daily dose (4g/day)).

Figure S1. Validation of HPLC-MS/MS method for determination of temocillin in ascitic fluid.

Upper panel; linearity of the assay; Lower panel; accuracy profile of the HPLC-MS/MS method for total (left) and unbound (right) temocillin concentrations. The plain black line is the identity line (y=x) in the top panel and the relative bias in the lower panel; the long broken blue lines are the 95% β-expectation tolerance limits, and the short broken black lines, the acceptance limits (±20%). The crosses represent the measured concentrations (top) or the relative error on each measurement for the validation standards (bottom).

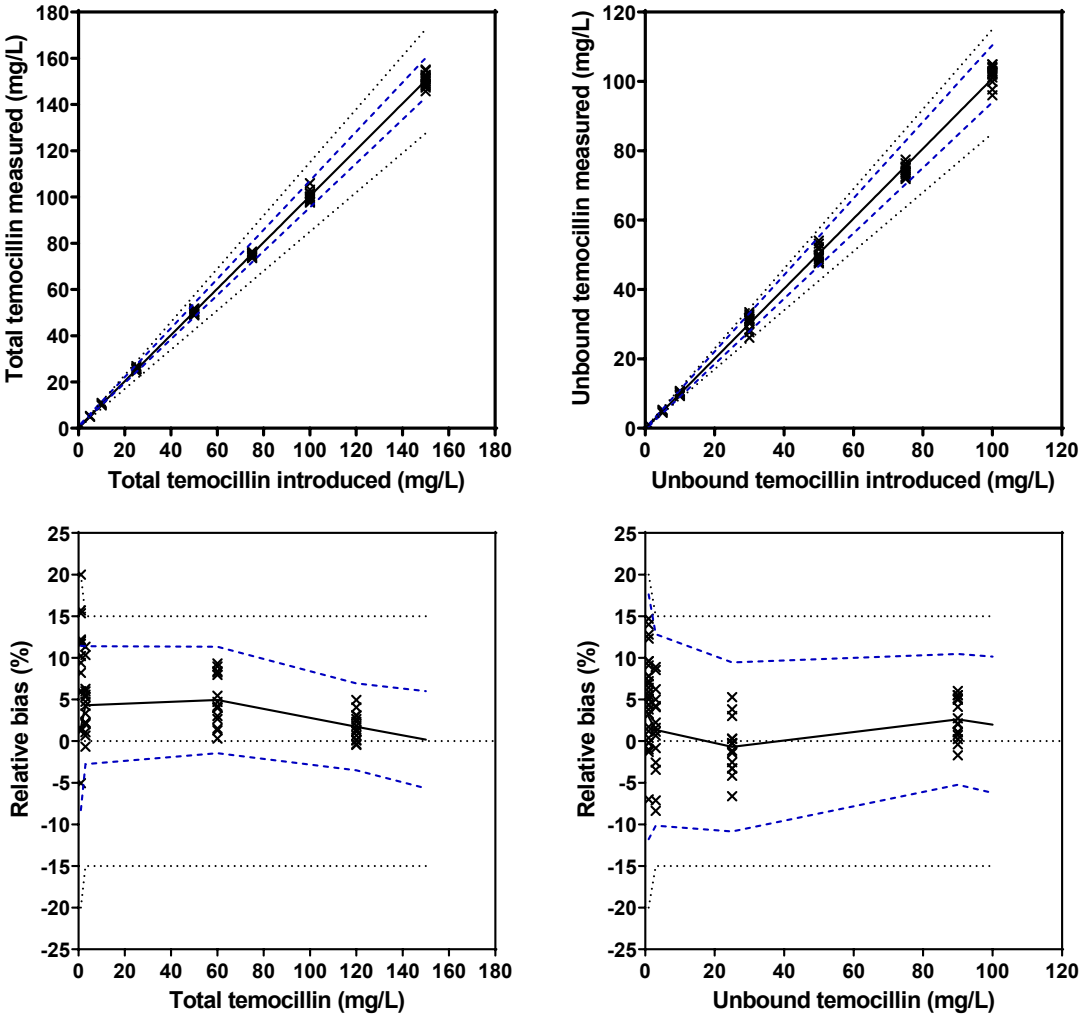


Figure S2. Schematic diagram of the final PK model. C_1, unbound temocillin concentration in central compartment; V_1, volume of central compartment; C_2, unbound temocillin concentration in peripheral compartment; V_2, volume of peripheral compartment; Q, intercompartmental clearance between central and peripheral compartment; C_3, total temocillin concentration in ascitic fluid compartment; V_3, volume of ascitic fluid compartment; CL_1, clearance from the central compartment; k_{13}, the transport rate constant from plasma to ascitic fluid; CL_3, clearance from the ascitic fluid compartment.

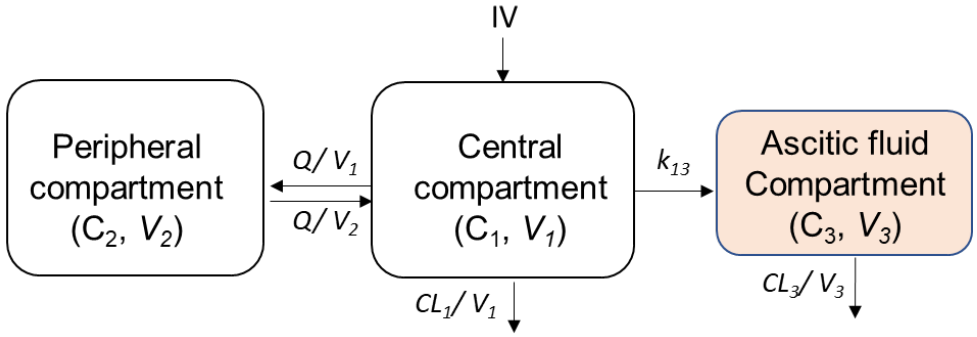


Figure S3: Concentration-time profiles of temocillin: comparison between non-infected and infected patients

The graphs show the total and unbound temocillin concentrations following multiple intravenous administration in plasma from Group #1 (A, 4th dose; B, 8th dose for uninfected patients [N=9]; infected patients [N=5]) and Group #2 (C, 4th dose, D: 8th dose for uninfected patients [N=11]; infected patients [N=3]). Data are shown as mean ± SD.

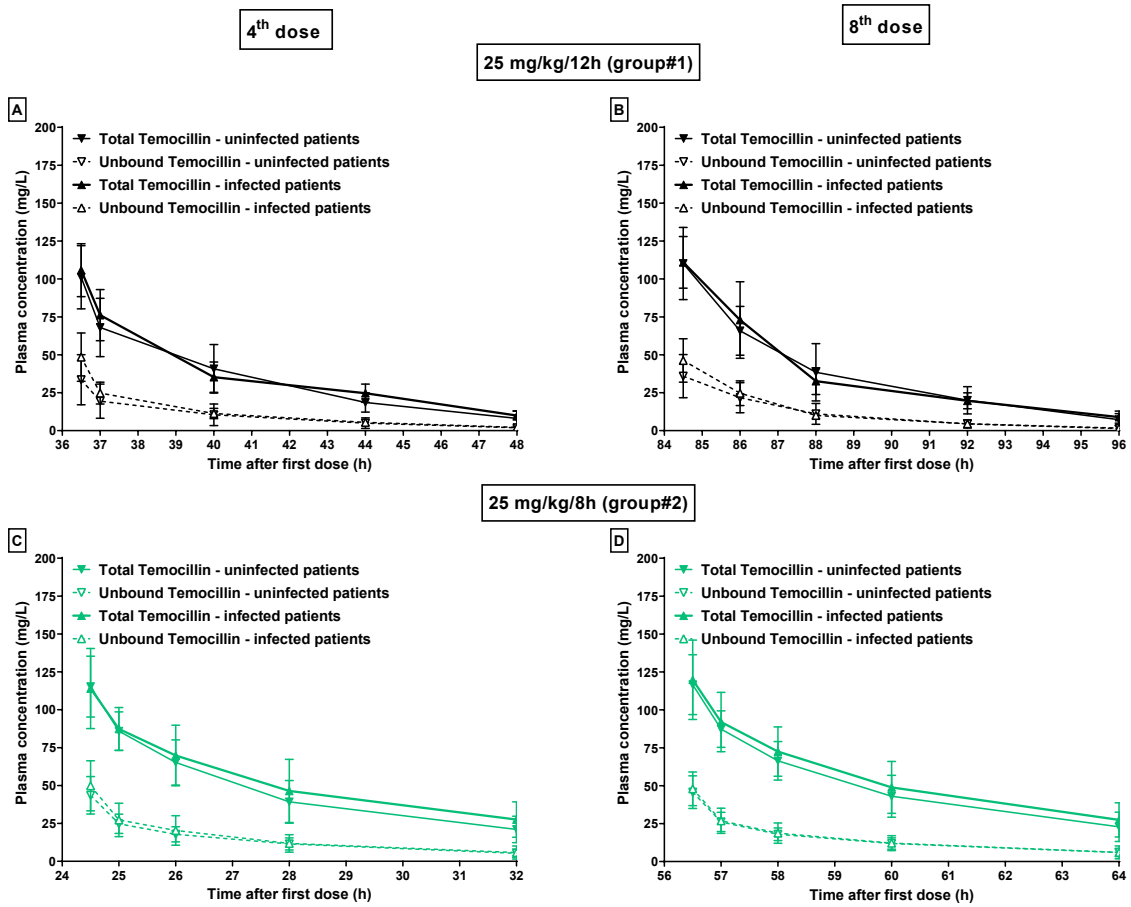


Figure S4. Observed versus population/individual predicted temocillin unbound concentrations in plasma (A) and in ascitic fluid (B). The solid black lines represent the identity line and the solid red lines represent the spline line.

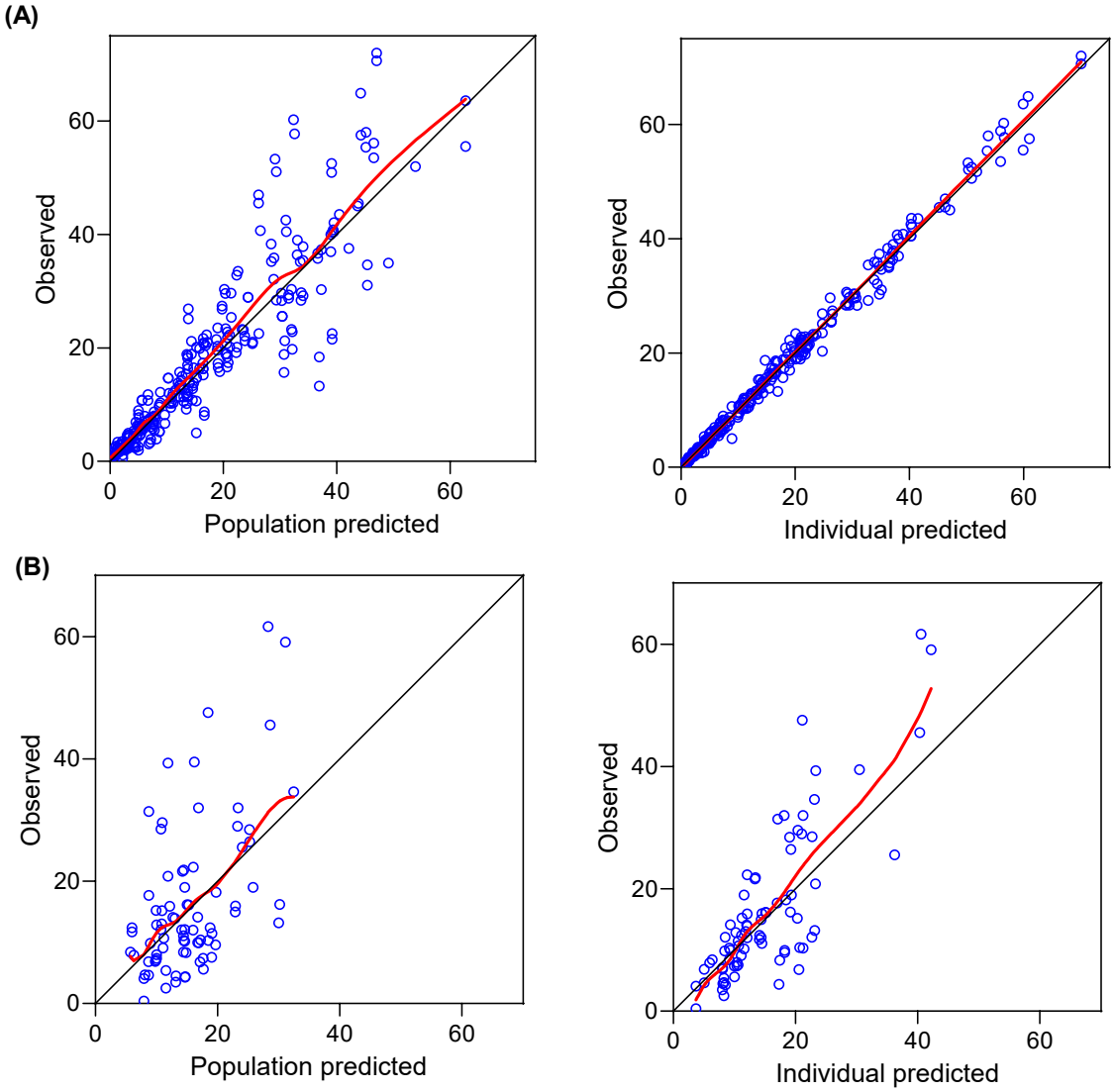


Figure S5. Scatter plot of the residuals. Individual weighted residual (IWRES) versus time (top left) and individual predictions (bottom left) and normalized prediction errors (NPDE) versus time (top right) and population predictions (bottom right) for temocillin unbound concentrations in plasma (A) and in ascitic fluid (B). The solid red lines represent spline line.

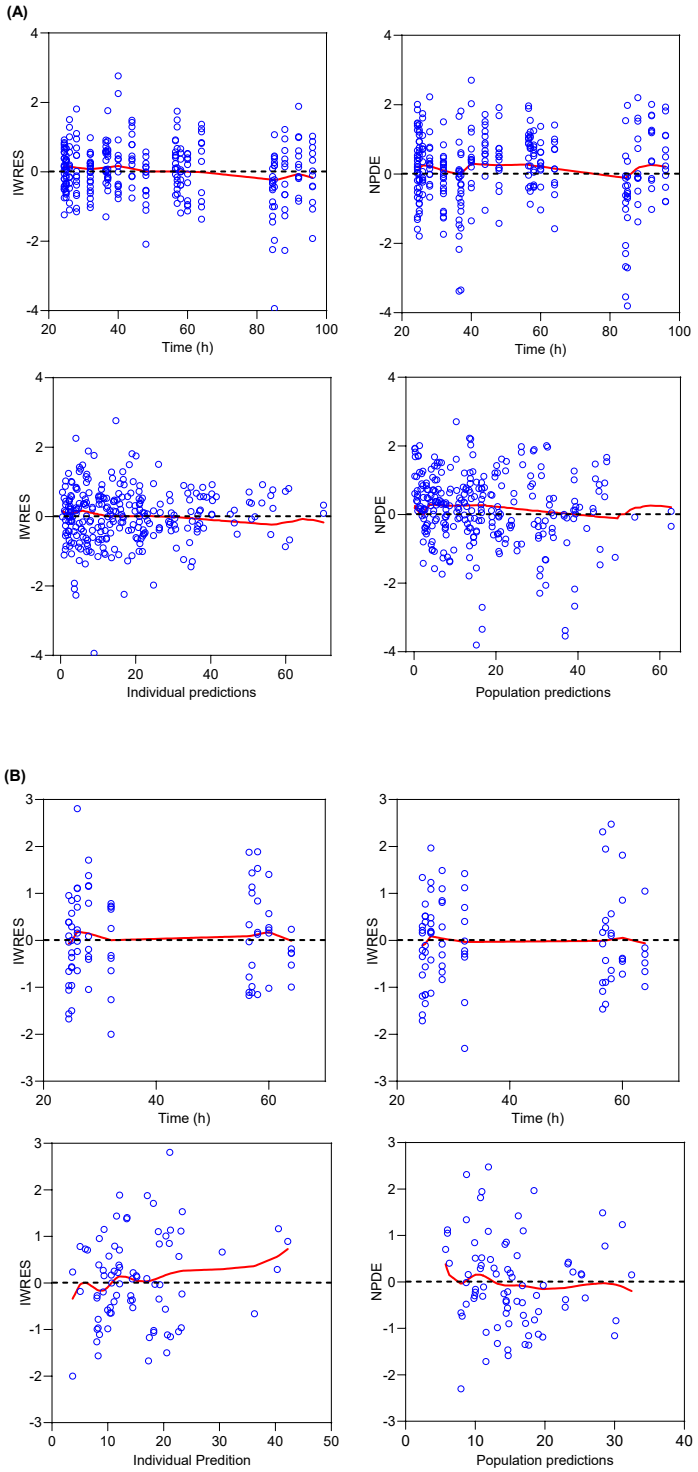
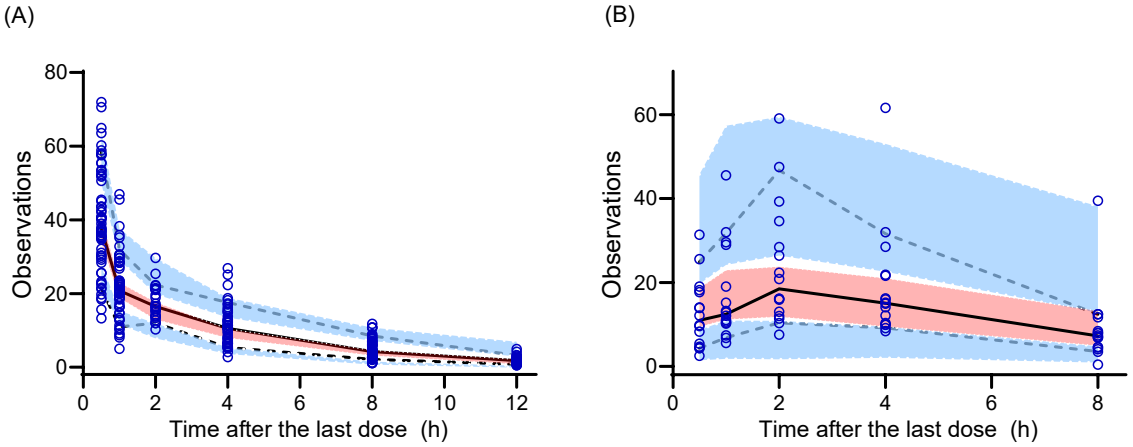


Figure S6. Visual predictive check (VPC) for temocillin unbound concentrations in plasma (A) and in ascitic fluid (B). Dots are observed concentrations, solid lines represent the 10th, 50th and 90th percentile of the observed values, and shaded area represent the spread of 90% prediction intervals calculated from simulations.



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