Diagnosis and treatment of common infectious diseases in severely ill sub-Saharan African patients
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Chapter 6

Pharmacokinetics and pharmacodynamic target attainment of benzylpenicillin in an adult severely ill sub-Saharan African patient population

Bos JC, van Hest RM, Mistício MC, Nunguiane G, Lang CN, Beirão JC, Mathôt RA, Prins JM

Under review
Abstract

Background In sub-Saharan Africa (SSA), benzylpenicillin (PEN) is frequently used for the treatment of severely ill patients. Systemic drug exposure of β-lactam antibiotics can be altered in critically ill intensive care (ICU) patients, but information about sources of pharmacokinetic (PK) variability and pharmacodynamic (PD) target attainment in severely ill non-ICU SSA populations is lacking.

Methods We performed a prospective observational population pharmacokinetic study in an adult hospital population in Mozambique, treated with PEN for presumptive pneumococcal infection from October 2014 until November 2015. One trough, one peak and two random blood samples were collected per patient for the measurement of total PEN (PENₜ) and unbound PEN (PENᵤ) concentrations. We developed a PPK model through non-linear mixed effect analysis and performed Monte Carlo simulations for different patient variable and dosing regimen scenarios.

Results One hundred twelve participants yielded 387 PENₜ and 53 PENᵤ concentrations. The median age was 35 years, body mass index 18.3, albumin 29 (range 12-44) g/L. A one-compartment model best described PENₜ PK and creatinine clearance was positively correlated with PENₜ CL. PENₜ and PENᵤ concentrations correlated well (r=0.98) and the PEN fraction bound to albumin was 55%. For infections with a microorganism with an MIC of 1 mg/L, simulations demonstrated that with a 3 million international units (IU) six hourly dosing regimen, only 24.8% of patients would have a PENᵤ concentration above MIC throughout the dosing interval (100% fₜ>MIC), while this was 74.1% when applying the 50% fₜ>MIC target. For infections with a pathogen with an MIC of 0.06 mg/L, these percentages were 72.3 and 98.2%, respectively.

Conclusions Severely ill adult non-ICU SSA adult patients are at high risk for underexposure to PENᵤ during routine bolus dosing, especially when their renal function is intact and when infected with pathogens with intermediate susceptibility.
Background

Pneumococcal infection is an important cause of morbidity and mortality around the world, and a compromised immune status, including HIV infection, predisposes for disease, and worsens disease outcome. In sub-Saharan African (SSA), invasive pneumococcal disease (IPD) is a common diagnosis, with an incidence of pneumococcal bacteraemia as high as 115 per 100,000 person years among populations aged 30-35 years, and up to 36% of patients may die in the hospital during the course of disease. The β-lactam benzylpenicillin (PEN) has remained one of the most frequently used antibiotics for in-hospital treatment of community-acquired pneumonia and presumptive IPD, including sepsis, as it is considered cheap and safe.

Pathophysiological changes associated with severe infection and sepsis, such as hypoalbuminaemia, oedema and organ dysfunction, are known to lead to alterations of volume of distribution (V) and clearance (CL). An increasing amount of evidence shows that such disease-induced pharmacokinetic (PK) changes may give rise to sub-optimal antibiotic plasma concentrations, especially among critically ill intensive care (ICU) patients. β-lactam antibiotics are particularly vulnerable in this respect, as they are hydrophilic drugs with predominant renal clearance. PEN is only moderately bound to albumin at therapeutic concentrations (50-60%), and the unbound plasma concentration is therefore relatively mildly affected by critical illness-induced hypoalbuminaemia. Nevertheless, critical illness-related PK alterations may give rise to systemic underexposure. An increased volume of distribution and augmented renal clearance, as can be seen in sepsis patients, may therefore lead to an inability to attain pharmacodynamic (PD) targets, and ultimately, to adverse clinical outcome.

In SSA hospitalized patients, the PK of antibiotics can also be influenced by chronic conditions such as cachexia and liver and kidney dysfunction, resulting from highly prevalent chronic diseases such as tuberculosis (TB), hepatitis B and hypertension. How coinciding acute and chronic conditions influence the PK of β-lactam antibiotics, including PEN, in severely ill non-ICU SSA patients has been poorly investigated. What is clear is that underexposure to the active, unbound antibiotic drug may not only lead to treatment failure, but may also contribute to the emergence of antimicrobial resistance, which is already highly prevalent in South Africa as well as in other SSA countries.

In the present study, we performed a population pharmacokinetic (PPK) analysis of PEN in a Mozambican, severely ill, adult hospital medicine ward population. The specific aims of the study were to describe the population PK of total PEN (PENt) in
order to identify sources of PK parameter variability. Additionally, we aimed to assess the probability of PK/PD target attainment (PTA) of unbound PEN (PENu) with commonly used dosing regimens for the treatment of *Streptococcus pneumoniae*.

**Methods**

**Setting**
The Beira Central Hospital (HCB) in Mozambique is a 733-bed governmental referral health facility with 260 internal medicine beds, admitting up to 1500 patients monthly. The proportion of patients infected with HIV on its medicine ward may be as high as 74%.\(^{14}\) Pneumococcal childhood vaccination with the 10-valent conjugate vaccine (PCV-10) was included in the Mozambican national immunization program in April 2013.

**Study design**
The current study was a prospective, observational, population pharmacokinetic (PPK) study of PEN, as part of a PPK study of antibiotics in adult patients admitted to the HCB medicine ward. In this study, PK data were collected from October 2014 until November 2015 from patients who were treated with intravenously administered ceftriaxone, ampicillin, gentamicin or PEN. The PPK study was reviewed and approved by the Mozambican National Committee for Bio-ethics in Health (CNBS: study registration nr. 118/CNBS/2013). Additionally, a letter of approval was obtained from the general director of the HCB. Participants gave written informed consent. Those unable to read, write and/or understand Portuguese gave a thumbprint and an impartial, literate witness observed the entire informed consent process and subsequently co-signed the informed consent form.

**Recruitment and data collection**
Patients were eligible for study entry if they were hospitalized on the medicine ward of the HCB and were being treated with one or more of the study antibiotics, as documented in a patient’s medication record. Inclusion criteria were age ≥18 years and being willing and able to give informed consent. Exclusion criteria were the use of drugs known to significantly affect PK of the different study antibiotics (probenecid, phenylbutazon, acetylsalicylic acid, indomethacin), a haemoglobin level ≤6 g/dL as measured by the HCB’s laboratory, any condition necessitating a blood transfusion irrespective of haemoglobin level, and an altered level of consciousness. PPK study participants with an intravenous PEN prescription (Benzylpenicillin, Reyoung Pharmaceutical Co. Ltd., Yi Yuan County, Shandong, China) were selected for the present study.
Two trained research nurses captured baseline characteristics and PEN dosing information and measured body weight and length of all study participants. Units of 10 million IU benzylpenicillin powder for infection were dissolved in 10 ml of sterile water for injection, and the appropriate dose was measured and subsequently injected intravenously via a venous catheter in half a minute, according to the responsible physician’s prescription. During a minimum of two days, a maximum of four blood samples were collected for the measurement of PEN concentrations. Sample times were pre-dose (trough level), 30-120 minutes after intravenous administration (peak level) and at 2 random time points during the dosing interval (random levels). PEN administration procedures were observed where possible, and a maximum of 19 ml of blood was collected over a time period of two or more days.

One blood sample was also used for the measurement of albumin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transpeptidase (GGT) and creatinine concentrations. Bilirubin levels were not measured, for local practical reasons in relation to bilirubin’s high photosensitivity. Creatinine clearance (CLCr) was estimated using the Cockroft and Gault (CRGT) formula.15

**Sample handling and drug assay**

EDTA anti-coagulated blood samples were refrigerated at 4-8°C immediately after collection until laboratory processing, which took place within two hours of collection. Samples were centrifuged and plasma was stored at -80°C in the local research laboratory until shipment on dry ice to the Netherlands for biochemical marker and drug concentration analysis.

In order to confirm PEN protein binding’s linearity with PENt concentrations being representative of PENu concentrations, both PENt and PENu were measured in a random selection of 15% of all samples with a measurable PENt concentration. Plasma was ultrafiltrated (centrifugal filters: Millipore Amicon Ultra 0.5 ml/30K, Merck Millipore, Darmstadt, Germany), and the ultrafiltrated plasma was subsequently processed to obtain the PENu concentration. Thus obtained PENu concentrations were used to calculate the sample’s PENu fraction. PENt and PENu concentrations were measured using a validated high-performance liquid chromatography mass spectrometry (LC-MS; LC: LC30 UPLC, Shimadzu, Kyoto, Japan & MS: Qtrap 5500 system, Scieix, Framingham, MA, USA). The lower limit of quantification (LLQ) was 0.5 mg/L and the higher limit of quantification was 40 mg/L. Concentrations higher than 40 mg/L were diluted and re-analyzed. Within- and between-assay variability was smaller than 4.8% and 7.0%, respectively. The accuracy of the assay was between 97 and 102%.
EUCAST dosage specific clinical MIC breakpoints for susceptibility and resistance to PEN of S. pneumoniae, where a pathogen with an MIC ≤0.06 mg/L is considered susceptible and one with an MIC >2 mg/L is considered resistant, and on the range of MICs observed (<0.016-0.75 mg/L) in a pilot study on the susceptibility of S. pneumoniae among adult Mozambican patients with pneumococcal pneumonia.

We screened a total of 762 patients for the larger PPK study and excluded 366 patients (Figure 1). The most common reasons for exclusion were a haemoglobin level ≤6 g/dL, having received or being scheduled to receive a blood transfusion, or having an altered level of consciousness. We included 115 patients in the current study on PEN and remained with 112 participants for analysis, after removing three patients from analysis with PENt concentrations <LLQ only.

**Model development**

The PPK analysis was performed using the non-linear mixed-effect modelling software package NONMEM (7.1.2; Icon Development Solutions, Ellicott City, Maryland, USA). For detailed methodological model building information see the online appendix. In brief, first a structural, compartmental PPK model was developed in which the PK of PENt was described, including its between-patient variability (BPV), and one and two compartment models were tested. Next, patient demographics and pathophysiological factors were tested for their correlation with the identified PK parameters from the structural model in an attempt to explain BPV. During this covariate analysis, which consisted of a univariate analysis followed by a multivariate analysis, the following covariates were tested: age, sex, weight, length, BMI, haemoglobin, albumin, creatinine, creatinine clearance, and gamma-GT, ALAT and ASAT concentrations. Lastly, the model resulting from the multivariate analysis was tested for its robustness in a bootstrap analysis in which the dataset was resampled 1000 times. The model was validated by performing a visual predictive check (VPC) that investigated whether the final model could adequately predict the observed concentration-time course of PENt, including the observed variability. Bootstrap and VPC analyses were performed using Perl-speaks-NONMEM version 3.5.3 software (PsN®, Uppsala, Sweden).

**Monte Carlo simulations**

Using the validated PPK model, PENt concentration-time profiles were simulated based on Monte Carlo simulations. To visualize the effect of covariates identified, concentration time profiles of patients with all median characteristics of the population, but with either the median, 10 or 90-percentile value of a specific covariate, were simulated using the most frequently observed PEN dosing regimen. To generate insight in the PTA, four different dosing regimens were simulated: 1 million international units (IU) q6h (every 6 hours), 3 million IU q6h, 4 million IU q6h and 3 million IU q4h. For the simulations of the PTA, 1000 virtual patients were simulated for each dosing regimen. Based on these data and on the median PENu fraction of 0.45, the PTA, being the percentage of patients with a PENu concentration remaining above a specified MIC during a specified percentage of time of the dosing interval (fT>MIC), was calculated for different fT>MIC targets.

The study endpoints for the PTA were fT>MIC=100% as primary target and fT>MIC=50%, fT>4xMIC=100%, and fT>4xMIC=50% as secondary targets. The choice of PD targets was based on the conclusions of a recent review and critical appraisal of data concerning β-lactam administration and β-lactam PD targets in critically ill patients. The choice of the (range of) target MICs was based on the
EUCAST dosage specific clinical MIC breakpoints for susceptibility and resistance to PEN of *S. pneumoniae*, where a pathogen with an MIC ≤0.06 mg/L is considered susceptible and one with an MIC >2 mg/L is considered resistant, and on the range of MICs observed (<0.016-0.75 mg/L) in a pilot study on the susceptibility of *S. pneumoniae* among adult Mozambican patients with pneumococcal pneumonia.

**Results**

**Patients and benzylpenicillin concentrations**

We screened a total of 762 patients for the larger PPK study and excluded 366 patients (Figure 1). The most common reasons for exclusion were a haemoglobin level ≤6 g/dL, having received or being scheduled to receive a blood transfusion, or having an altered level of consciousness. We included 115 patients in the current study on PEN and remained with 112 participants for analysis, after removing three patients from analysis with PEN concentrations <LLQ only.

**Figure 1.** Study profile.
A total of 387 plasma samples yielded 387 PEN\textsubscript{t} concentrations. 27/387 (6.9\%) participants had less than four plasma samples available, with the most common explanations being a participant’s unforeseen discharge or death during the study period.

**Table 1.** Baseline characteristics of study population (n=112).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex (n/%)</td>
<td>51 (45.1)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 (18-80)</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>49 (29-105)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>18.3 (10.5-31.3)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>9.8 (6.1-19.3)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>29 (12-44)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>55 (0-1343)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18 (3-191)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42 (13-489)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>68 (33-1320)</td>
</tr>
<tr>
<td>Creatinine clearance(^{*}) (mL/min)</td>
<td>80 (3-195)</td>
</tr>
<tr>
<td>Benzylpenicillin dose prescribed (n/%)</td>
<td></td>
</tr>
<tr>
<td>3 million IU q6h</td>
<td>99 (88.4)</td>
</tr>
<tr>
<td>2 million IU q6h</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>4 million IU q6h</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>other</td>
<td>9 (8.0)</td>
</tr>
</tbody>
</table>

**Observed total benzylpenicillin concentration (IQR)**

| PEN\textsubscript{t}, ≤3h after dose (mg/L) | 17.6 (8.6-35.3) |
| PEN\textsubscript{t}, >3h after dose (mg/L) | 0.8 (<0.5-3.9)  |

Results expressed as median (range) unless specified otherwise. \(^{*}\)Creatinine clearance estimated using Cockroft-Gault equation.\(^{15}\) ASAT: aspartate aminotransferase; ASAT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase; PEN\textsubscript{t}: plasma total benzylpenicillin concentration; IU: international units (1 million IU \(\approx\) 600 mg benzylpenicillin); q6h: every six hours. IQR: interquartile range.

Patient characteristics are presented in Table 1. A large majority of patients (87.6\%) had a 3 million IU q6h PEN dosing schedule.
There were 45/387 (11·6%) samples with a PEN<sub>t</sub> concentration < LLQ that had been collected within four hours after the last PEN dose. These samples were excluded from further analysis, as these were unlikely PEN<sub>t</sub> concentrations from a PK perspective, and were most likely to have resulted from a missed dose. Consequently, the study remained with a total of 342 samples for final analysis. There were 84/342 (24·5%) PEN<sub>t</sub> samples with a concentration < LLQ that had been collected more than four hours after the last PEN dose, and that were included in the analysis. The median bound fraction, based on 53/342 (15%) studied samples, was 55% (interquartile range (IQR): 43-62). The observed PEN<sub>t</sub> concentrations are shown in Figure 2.

**Figure 2.** Observed total benzylpenicillin concentration-time data and visual predictive checks (VPC) of the final model.

The black dots are the observed concentrations. The black line is the observed median, and the grey lines the 5<sup>th</sup> and 95<sup>th</sup> percentile of the observed data. The dark and light blue lines are the corresponding simulated percentiles with their 95% confidence intervals.

**Population pharmacokinetic analysis**

*Model development*

For detailed modelling information, including the PK parameter values belonging to the different modelling steps, see the appendix. In brief, model building was
performed using observed PEN\textsubscript{t} concentrations only, as there was a strong correlation between PEN\textsubscript{t} and PEN\textsubscript{u} concentrations in the selection of samples for which both PEN\textsubscript{t} and PEN\textsubscript{u} concentrations were measured (correlation coefficient $r$: 0.98; Figure 3). An additional reason not to use PEN\textsubscript{u} concentrations for model building purposes was that a large number of PEN\textsubscript{u} concentrations was projected to be below the LLQ (at least 105; 31%) based on the total number of PEN\textsubscript{t} concentrations <1mg/L and a median albumin binding of 55% (see appendix)). The PEN\textsubscript{t} data were fitted to several compartmental models and a one-compartmental model best fitted the data. The BPV in clearance, expressed as coefficient of variation, was estimated to be 76%. The covariate analysis was based on 100% availability of covariate results, except for two patients for whom the haemoglobin level was not captured. These were imputed with median haemoglobin level of the population. The covariate analysis yielded a model with significant associations between PEN\textsubscript{t} CL and $CL_{CR}$, explaining 33% of the BPV. The final model had an adequate fit and VPCs are shown in Figure 2. The residual variability for the PEN\textsubscript{t} concentration, an estimate of unexplained variability relating to measurement error, errors in data collection, intra-patient variability and model misspecification, was 57%.

**Figure 3.** Correlation between total and unbound benzylpenicillin concentration.

Unbound benzylpenicillin concentrations plotted against total benzylpenicillin concentrations (dots) from a random sample of 53. There was a strong correlation (line; $r$=0.98) between total and unbound concentrations.
Monte Carlo dosing simulations

The association between PEN CL and CLCR is also illustrated in the benzylpenicillin concentration-time profiles for the most frequently applied dosing regimen of 3 million IU q6h and it shows that patients with a higher CLCR had lower PENt concentrations (Figure 4).

Figure 4. Simulations of benzylpenicillin concentration-time profiles for different creatinine clearances.

Simulations of total benzylpenicillin (PENt) concentration-time profiles for a 3 million IU q6h (every six hours) PEN dosing regimen, for patients with all median characteristics of the population, but with three different creatinine clearances (CLCR: the median, 10th percentile and 90th percentile).

For microorganisms with an MIC of 1 mg/L, the PTA of a 3 million IU q6h regimen for patients with the median CLCR of 80 mL/min was 24.8% for the primary PD target of 100% fT>MIC (Table 2, Figure 5). The PTA of the secondary PD target of 50% fT>MIC, a target used for non-severely ill patients, was 74.1%. The different PTAs of the same PD targets for the PEN regimen frequently advised for the treatment of pneumonia, namely 1 million IU q6h, never exceeded 50%. Augmenting the PEN dose or decreasing the PEN dosing interval did improve the PTA of the 100% fT>MIC PD target, although it remained low.
Table 2. Probability of target attainment with four benzylpenicillin dosing regimens.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>100% fT&gt;MIC</th>
<th>50% fT&gt;MIC</th>
<th>100% fT&gt;4xMIC</th>
<th>50% fT&gt;4xMIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 million IU q6h</td>
<td>6.5</td>
<td>40.1</td>
<td>0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>3 million IU q6h</td>
<td>24.8</td>
<td>74.1</td>
<td>3.8</td>
<td>30.0</td>
</tr>
<tr>
<td>4 million IU q6h</td>
<td>30.0</td>
<td>79.0</td>
<td>6.5</td>
<td>40.1</td>
</tr>
<tr>
<td>3 million IU q4h</td>
<td>54.2</td>
<td>93.3</td>
<td>16.4</td>
<td>60.9</td>
</tr>
</tbody>
</table>

Probability of target attainment, i.e., the percentage of 1000 simulated patients predicted to achieve four different pharmacodynamics targets with four different benzylpenicillin dosing regimens, for patients with a median creatinine clearance of 80 mL/min, while assuming a pathogen’s benzylpenicillin (PEN) minimal inhibitory concentration (MIC) of 1 mg/L. fT>MIC: time of the unbound PEN concentration above the MIC; IU: international units; q6h: every six hours; q4h: every four hours.

Figure 5. Probability of target attainment with four different benzylpenicillin dosing regimens.

Percentages of 1000 simulated patients with a median creatinine clearance (80 mL/min) achieving an unbound benzylpenicillin (PEN) concentration above the minimal inhibitory concentration throughout the dosing interval (fT>MIC=100%), for four different PEN dosing regimens and a range of MICs. The clinical susceptibility breakpoint for *S. pneumoniae* according to EUCAST is 0.06 mg/L.19

When treating a pathogen with an MIC of 0.06 mg/L, which is the EUCAST susceptibility breakpoint, the PTA of the target 100% fT>MIC was 72.3% for the
most frequently used 3 million IU q6h regimen and 56.1% for the 1 million IU q6h regimen, in patients with the median CL\text{CR} of 80 mL/min (Figure 5). The PTA target of 50% \text{fT}>\text{MIC} for the same dosing regimens were 98.2 and 94.7%, respectively.

**Discussion**

This study provides a PPK model of PEN in a SSA adult hospital population. The study population’s severity of illness was clearly illustrated by its low median BMI and low median haemoglobin and plasma albumin concentrations. The model-based simulations suggest that severely ill non-ICU patients, much like critically ill ICU populations, are at risk for underexposure to PEN\text{u} during intermittent bolus dosing with standard dosing regimens, especially when a patient’s renal function is intact and when they are infected with pathogens with intermediate susceptibility.

There was a high between-patient variability of PEN PK in the study population and a substantial part could be explained by the relationship between the clearance of PEN and renal function, as can be expected with a predominantly renally cleared drug. The implication of this covariate relationship is that the risk for underexposure to PEN\text{u} is particularly high for patients with a preserved renal function, as was clearly demonstrated by the current study’s Monte Carlo simulations. This risk is even higher for patients with augmented renal clearance, a condition occurring in 15-65% of ICU patients that is thought to result from increased cardiac output with glomerular hyperfiltration. Renal function was also the most important factor influencing PK in two European studies with PEN.\textsuperscript{21,22} Our findings confirm outcomes from other studies that investigated the PK of the mildly albumin-bound β-lactam piperacillin in critically ill ICU patients, where renal function significantly influenced PK and the risk of underexposure for patients with a normal renal function and standard empirical dosing was high.\textsuperscript{23,24}

Shortening the dosing interval as well as prolonged or continuous infusion improved the PTA in these studies. Even though these methods of administration have proved to be more advantageous for critically ill sepsis patients in terms of the PTA than standard bolus dosing in a number of studies, they are unlikely to be feasible options for (non-ICU) severely ill SSA patients on the short term.\textsuperscript{17}

In the current study’s case, shortening the PEN dosing interval, however impractical this may be under real life practice circumstances, also improved the PTA, even though the probability of the PEN\text{u} concentration remaining above the MIC throughout the dosing interval remained low for an infection with a pathogen with an MIC of 1 mg/L. The use of the less stringent target of \text{fT}>\text{MIC}=50% with the same regimen made the PTA increase from 54.1% to 93.3%, but it has to be
emphasized that there is a growing body of evidence suggesting that for patients with severe disease, who may be at higher risk for impaired drug distribution and tissue penetration, the more stringent β-lactam PD target of 100% $fT>MIC$ improves clinical outcome, and reduces selective pressure and the emergence of antimicrobial resistance, as compared to the more conservative target of 50% $fT>MIC$.\textsuperscript{8,16,25,26}

Unfortunately, adult SSA pneumococcal susceptibility surveillance data reporting MICs are scarce. In an earlier pilot study performed in this hospital on the susceptibility of \textit{S. pneumoniae} in adult patients with pneumococcal pneumonia, we found 3/16 pneumococcal strains recovered from sputum to have an MIC of 0.75 mg/L.\textsuperscript{20} A study from South Africa suggested that 23-29\% of IPD isolates from children and adults had an MIC $>0.6$ mg/L and a Mozambican study, investigating children <5 years of age, estimated that 14\% of IPD isolates had an MIC $>0.064$ and $<2.0$ mg/L.\textsuperscript{27,28} These studies were performed prior to the introduction of childhood pneumococcal conjugate vaccination programs, and there is a need for SSA post-vaccination pneumococcal MIC data for PEN that can underpin antibiotic drug and dosing regimen choices.\textsuperscript{29,30} Comparable data may also be needed for other pathogens sensitive to PEN, such as leptospira serovars. Leptospirosis appears to be a severely underdiagnosed disease, while being a common cause of febrile illness among SSA adult hospitalized patients.\textsuperscript{31}

There are limitations to our study. Firstly, we did not capture information about a patient’s fluid balance and the presence of excess fluid in the interstitial and transcellular space, and we are therefore not informed about how hydration status, edema and so called ‘third spacing’, may have affected PK BPV. It is our experience that fluid therapy is a relatively neglected part of the treatment in adult hospitalized patients in SSA hospitals, and our observations are supported by repeated reports from Uganda showing that fluid therapy was being severely underprovided for in-patients with presumptive sepsis.\textsuperscript{32,33} The possibility of an inadequate hydration status may imply that PENu concentrations as well as PTAs could turn out to be even lower than what was found in the current study once proper volume therapy is applied.

Secondly, although we did not find our measured liver enzyme concentrations to have a significant relationship with PEN clearance, still, part of the remaining BPV in clearance may contain a relationship between liver function and PEN elimination that was left unaccounted for, as we did not include bilirubin concentration as a covariate in our analysis, for local practical reasons.
Thirdly, there was substantial residual PK variability as expressed by the PEN\(_t\)\(_i\) proportional error. We removed a set of samples from the analysis with PEN, concentrations <LLQ which had been drawn less than four hours after the last PEN dose as we supposed that they had resulted from erroneous drug dispensing. An explanation for the high residual PK variability may therefore lie in drug dispensing and administration procedures. Although we cannot exclude that some of the retained concentrations <LLQ could result from erroneous drug administration, overall, these concentrations fitted the rest of a patient's concentration-time profile, that was more likely to include a sample with a measurable concentration preceding the one with a concentration <LLQ. In addition, our low measured plasma concentrations during the second half of the dosing interval seem to be in accordance with PEN\(_u\), concentrations from a PK study with severely ill endocarditis patients treated with a 5 million IU q6h PEN dosing regimen.\(^{21}\)

Finally, even though studies investigating antibiotic PK performed in non-SSA ICU patient populations do show comparable tendencies towards underexposure,\(^{23,24}\) extrapolations to non-SSA severely ill (ICU) patient populations should be made with caution as our study population is likely to differ from other populations in terms of underlying sufferings as well as in treatment aspects, such as antibiotic infusion time and fluid therapy.

In conclusion, this study's results indicate that intermittent bolus dosing of the β-lactam antibiotic benzylpenicillin in severely ill adult SSA patients is likely to lead to underexposure to PEN\(_u\), especially when their renal function is intact. Although underexposure was projected to occur more frequently when treating pathogens with higher MICs, even infections with pathogens that would normally be considered susceptible could suffer from severe under-treatment. The finding of low PTAs in this population during intermittent bolus dosing whilst applying the increasingly recognized stringent PD targets for severely ill patients raises questions about which patients should be targeted with PEN in a SSA hospital setting where microbiological analyses are not routinely done. The extent of the risk of underexposure underlines the need for PK/PD studies with β-lactams in SSA settings against the background of locally derived MICs, and for exploring the effect of simple clinical testing algorithms that would select patients who could still benefit from treatment with PEN.

**Contributors**

JCB, RM and JP designed the study. JCB performed the literature search. JCB obtained ethical approval. JCB, MM, GN and MD implemented the study and JCB supervised data collection and study progress on a daily basis. JCB and RVH
analysed the data. JCB and RVH drafted the manuscript. JP and RM critically examined the analysis and findings and all authors critically read and commented on draft versions of the report. All authors approved the final version.

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