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ORIGINAL ARTICLE

Saliva as a sampling matrix for therapeutic drug monitoring of gentamicin in neonates: A prospective population pharmacokinetic and simulation study

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Aims: Therapeutic drug monitoring (TDM) of gentamicin in neonates is recommended for safe and effective dosing and is currently performed by plasma sampling, which is an invasive and painful procedure. In this study, feasibility of a non-invasive gentamicin TDM strategy using saliva was investigated.

Methods: This was a multicentre, prospective, observational cohort study including 54 neonates. Any neonate treated with intravenous gentamicin was eligible for the study. Up to eight saliva samples were collected per patient at different time-points. Gentamicin levels in saliva were determined with liquid chromatography tandem mass-spectrometry (LC–MS/MS). A population pharmacokinetic (PK) model was developed using nonlinear mixed-effects modelling (NONMEM) to describe the relation between gentamicin concentrations in saliva and plasma. Monte Carlo simulations with a representative virtual cohort ($n = 3000$) were performed to evaluate the probability of target attainment with saliva versus plasma TDM.

Results: Plasma PK was adequately described with an earlier published model. An additional saliva compartment describing the salivary gentamicin concentrations was appended to the model with first-order input (k_{13} 0.023 h^{-1}) and first-order elimination (k_{30} 0.169 h^{-1}). Inter-individual variability of k_{30} was 38%. Postmenstrual age

Amadou Samb and Matthijs Kruizinga contributed equally to this study.

The authors confirm that the Principal Investigator for this paper is Timo R. de Haan and that he had direct clinical responsibility for patients.

(PMA) correlated negatively with both k_{13} and k_{30}. Simulations demonstrated that TDM with four saliva samples was accurate in 81% of the simulated cases versus 94% when performed with two plasma samples and 87% when performed with one plasma sample.

Conclusion: TDM of gentamicin using saliva is feasible and the difference in precision between saliva and plasma TDM may not be clinically relevant, especially for premature neonates.

KEYWORDS

gentamicin, neonates, non-invasive, population pharmacokinetics, saliva, simulation, therapeutic drug monitoring

1 | INTRODUCTION

Neonates admitted to the neonatal intensive care unit (NICU) have a high risk for bacteraemia or sepsis due to premature birth, low birth weight and indwelling central venous lines.[1] Intravenous treatment with the aminoglycoside **gentamicin** provides gram-negative coverage and is part of the first line antibiotic treatment protocols for early and late onset sepsis in premature and term neonates.

Gentamicin has a narrow therapeutic index, with oto- and nephrotoxicity as its possible concentration-dependent adverse drug events (ADE), for which neonates are especially vulnerable.[2] Furthermore, dosing is complicated by high variability in body composition, kidney function and organ maturation of neonates.[3] Gentamicin concentrations can therefore be unpredictable and therapeutic drug monitoring (TDM) is necessary to ensure adequate dosing regimens. TDM requires repeated plasma sampling from central venous lines and via heel lance, which is invasive, painful and may contribute to clinical anaemia or infection.[4] As a result, TDM by plasma sampling is complicated in neonates,[5] possibly leading to suboptimal individual gentamicin doses and thereby causing a decrease in therapeutic efficacy and an increased risk of ADE.

Non-invasive TDM methods in neonates would allow for a decreased burden of plasma collection, an increased sampling frequency and safer and more efficacious dosing. Moreover, saliva collection is easy and cheap, saliva is readily available and, other than oromucosal inflammations and lesions, there are no contraindications for saliva collection.[6] Previous studies have shown that the use of saliva as a matrix for TDM is feasible for several anti-epileptic drugs and caffeine.[6,7] Analyses of salivary gentamicin concentrations and other aminoglycosides during intravenous treatment of children and adults have been published with varying results. Some studies reported a good correlation between gentamicin saliva and plasma concentrations, while others reported undetectable aminoglycoside concentrations in saliva.[8–11] To date, no such studies have been performed in a neonatal population.

The aim of this study was to determine the feasibility of a non-invasive gentamicin TDM strategy by measuring salivary gentamicin concentrations and relating these to gentamicin concentrations in routinely drawn plasma samples in neonates.

What is already known about this subject

- Little is known regarding gentamicin concentrations in the saliva of neonates.
- There is no literature on the feasibility of saliva sampling for the purpose of gentamicin TDM in a neonatal population.

What this study adds

- A detailed description of gentamicin pharmacokinetics in saliva and plasma of premature and term neonates, which is strongly influenced by PMA.
- We found that TDM with four saliva samples results in 81% correct dose regimens based on simulations.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a multicentre, prospective, observational pharmacokinetic (PK) study conducted in the Emma children's hospital (Amsterdam UMC, Amsterdam, the Netherlands) and the Juliana children's hospital (Haga Hospital, The Hague, the Netherlands). Gentamicin concentrations were measured in saliva and compared with plasma concentrations obtained as part of routine TDM. The local ethics committee of the Amsterdam UMC approved this study (number 2018_193). Local feasibility was tested and approved for the Haga hospital. The study was registered in the Dutch Trial Registry (NTR, NL7211).

2.2 | Subjects

Inclusion of subjects took place between 8 October 2018 and 4 March 2020. Any neonate that was treated with gentamicin according to local clinical guidelines was eligible for the study. Patients were included in this study after signed informed consent of both parents was given. For the analysis, three distinct subgroups based on gestational age (GA) were pre-specified and treated with 0.5 h intravenous gentamicin infusion according to local dosing protocols: (1) neonates with GA < 32 weeks (5 mg/kg/48 h); (2) neonates with GA ≥ 32–37 weeks (5 mg/kg/36 h); and (3) neonates with GA ≥ 37 weeks (4 mg/kg/24 h at Emma Children's hospital and 5 mg/kg/36 h at Juliana Children's Hospital). Clinical data were obtained from the digital medical files of the patients (sex, GA, postnatal age [PNA], postmenstrual age [PMA], birth weight [BW], current body weight [WT], perinatal asphyxia, therapeutic hypothermia and concomitant medication).

No formal sample size calculations were performed. A total of 60 patients (20 patients per group) were scheduled to be enrolled into the study, since 20 patients per subgroup are deemed sufficient for NONMEM analysis.[12]

2.3 | Sample collection

Saliva samples were collected using SalivaBio Infant's Swabs (Salimetrics, Carlsbad, CA, USA). Swabs were placed in the cheek pouch of the patient for approximately 90 seconds, according to the manufacturer's instructions.[13] Nursing staff and researchers received training in sample collection before study initiation. After collection, swabs were centrifuged at 2754 RPM for 5 minutes and extracted saliva was stored at −80 ° C until analysis for a maximum of 3 months. Per patient, a maximum of eight saliva samples were collected up to 48 hours after the last gentamicin dose following a predetermined sampling schedule. However, deviation from the sampling schedule due to clinical practice was allowed. Any adsorption of gentamicin to the swab was assessed through recovery tests prior to analysis. Adsorption of less than 15% was deemed acceptable, as this is a commonly used boundary value for the precision and accuracy of quantitative analytical laboratory techniques. Gentamicin concentrations in plasma were collected from two routine TDM measurements, 1 h after the first dose and 12–48 h after the first dose. Additional plasma levels were determined in residual material, when available.

2.4 | Bio-analytical assay

The major components of gentamicin (C1, C1a and C2) were quantified in saliva samples using a previously published validated LC–MS/MS method.[14] This method has been validated for saliva samples for the purposes of this study. In short, the accuracy and within-run imprecision at the lowest level of quantification (LLOQ) were 118% and 10.2%, respectively. The accuracy and imprecision were 98.4% and 3.3%, respectively, at the middle level of quantification (MLQ). At the upper limit of quantification (ULOQ), accuracy was 98.7% and imprecision was 3.2%. The LLOQ was 0.056 mg/L and minimal sample volume was 10 μL.

2.5 | Pharmacokinetic analysis

Data handling, data visualization and descriptive statistics were performed using R statistics version 4.0.2.[15] A population PK (POP-PK) model was developed using nonlinear mixed-effects modelling, as

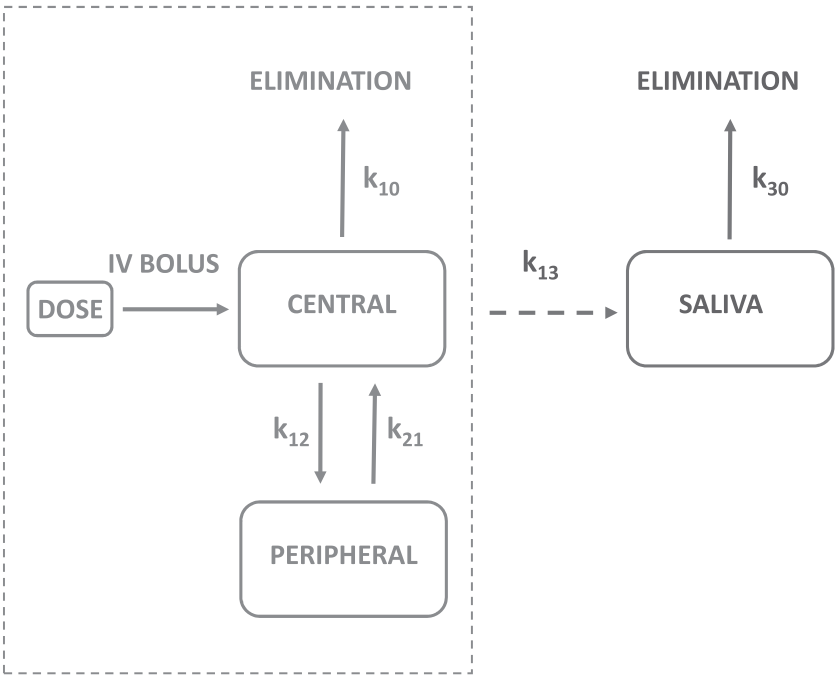

FIGURE 1 Conceptual model for gentamicin PK in plasma and saliva. Within dashed lines: gentamicin in plasma. Dose is administered as a 0.5 h IV infusion to the central compartment. k_{12}: Transport rate from central to peripheral compartment. k_{21}: Transport rate from peripheral compartment to central compartment. k_{10}: Elimination rate from the central compartment. Outside dashed lines: gentamicin PK in saliva. k_{13}: Transport rate from central compartment to saliva compartment. The dashed arrow signifies that gentamicin loss from the central compartment is assumed to be negligible. k_{30}: Elimination rate from saliva

implemented in NONMEM version 7.4.0 (ICON Development Solutions, Dublin, Ireland). Gentamicin concentrations in plasma and saliva were logarithmically transformed.

An integrated model describing gentamicin in plasma and saliva was developed using a stepwise modelling approach. First, plasma PK data was described using a previously published model by Fuchs et al.,[16] fixing the PK parameters. The control stream for this model was provided by the authors. This is a two-compartment model with inter-individual variability (IIV) on clearance (CL) and central volume of distribution (V_c). Further specifications for the plasma model are presented in Table S1 in the Supporting Information. Model performance was evaluated through the assessment of goodness-of-fit (GOF) plots and visual predictive checks (VPCs).

An additional compartment describing the salivary gentamicin concentrations was appended to the model. The conceptual model for gentamicin in plasma and saliva is depicted in Figure 1. The first-order transport rate from the central (plasma) compartment to the saliva compartment was expressed as k_{13}, whilst the first-order rate of gentamicin elimination from the saliva compartment was expressed as k_{30}. No transport from the saliva compartment back to the central and peripheral compartments was modelled, since the oral bioavailability of gentamicin is negligible.[17] Central gentamicin mass decrease due to transport from the central compartment to the saliva compartment was assumed to be negligible, as this was expected to be proportionally diminutive compared to the total amount of gentamicin in the central compartment, similar to a hypothetical effect compartment model.[18] Both fixed and random effects of rate constants k_{13} and k_{30} were estimated using the ADVAN6 subroutine in NONMEM. Model parameters were evaluated by assessing changes in the objective function value (OFV), relative standard error (RSE) assessment and diagnostic plots. A ΔOFV of −3.81 corresponds with $P = .05$, which was the significance level for inclusion of any parameter. Gentamicin concentrations in saliva below LLOQ were accounted for with the M3-method.[19] First, the structural model was estimated, describing the relations between parameters, as well as estimation of IIV on the parameters. Thereafter, the error model was developed, describing the residual error structure in the model. Finally, the covariate model explains part of the variability based on covariates.

GA, PNA, PMA, BW, WT, sex, perinatal asphyxia, therapeutic hypothermia and concomitant drugs were evaluated as covariates on the saliva distribution parameters for this model. Covariate analysis was performed with stepwise forward inclusion ($\alpha = 0.05$) and backwards elimination ($\alpha = 0.01$). Continuous covariates were included in the model as a power equation function:

$$p = \theta_p * \frac{cov}{median}^{\theta_{cov}} \quad (1)$$

Parameter p was calculated from typical parameter θ_p, multiplied by the fractional deviation from the median value of the covariate. The magnitude of the covariate effect was estimated as θ_{cov}. Dichotomous covariates were coded in NONMEM as shown in Equation (2):

$$p = \theta_p + cov * \theta_{cov} \quad (2)$$

Dichotomous covariates could take the value of either 0 or 1. Reference parameter value θ_p was estimated and the parameter difference between covariate parameters was estimated as θ_{cov} to calculate parameter p.

Assessments of diagnostic tools such as GOF plots, RSE, η-shrinkage and ε-shrinkage were used for model evaluation during all steps. Non-parametric bootstrap analyses ($n = 1000$), as well as the simulation-based prediction-corrected VPCs (pcVPC) were employed for assessment of the model robustness and internal validation of the final model.[20]

2.6 | TDM performance simulation

R version 4.02 and the mrgsolve[21] package were used for Monte Carlo simulations. A simulation cohort ($n = 3000$) with a uniform distribution of GA and corresponding WT (Figure S1 in the Supporting Information) [22] was prepared and a single administration of 5 mg/kg/48 h (GA < 32 weeks), 5 mg/kg/36 h (GA ≥ 32–37 weeks) or 4 mg/kg/24 h (GA ≥ 37 weeks) was simulated for each subject in accordance with Dutch dosing guidelines.

For plasma and saliva TDM, different sampling schedules were simulated with measurements at different time-points after the first dose. First, a schedule with a single intermediate (14 h post-dose) sample was simulated and the performance of this schedule in the context of TDM was appraised. Second, a two-sample schedule with a peak (1 h for plasma and 3 h for saliva post-dose samples) and trough (0.5 h before next dose) sample was evaluated. Next, the combination of peak, intermediate and trough samples was evaluated. Finally, schedules were evaluated in which samples were added (at 7 h post-dose; at 7–18 h post-dose; at 1 h pre-dose and 7–18 h post-dose). Bayesian maximum a posteriori (MAP) optimization was used to estimate the empirical Bayes estimates of the individual CL, V_C and k_{30} for each subject based on the simulated concentrations.[23] Based on the estimated CL and V_c, true peak and trough plasma concentrations were estimated for each subject, who then entered a basic decision rule optimizing the dose to reach a targeted peak plasma concentration between 9 and 11 mg/L and trough concentration < 0.8 mg/L after the third dose. Target ranges were deliberately set stricter than clinical guidelines (peak 8–12 mg/L and trough < 1 mg/L) to account for residual error in the estimations. For each subject, two additional dose intervals of gentamicin were simulated after dose adjustment. Finally, the proportions of subjects with true peak and trough concentrations within clinical guideline reference ranges (target attainment) after the third dose were calculated.

Simulations were performed for plasma TDM (1–6 samples), saliva TDM (1–6 samples), model-based dose optimization ('M' samples) and 'no TDM' (standard dosing, 0 samples). Model-based dosing was performed using the typical PK parameter estimates based on the covariates included in the population model published by Fuchs et al.[16] The proportion of subjects with target attainment after each

simulated scenario was calculated and compared in order to appraise the added value of saliva and plasma TDM.

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[34]

3 | RESULTS

3.1 | Demographic characteristics

Table 1 depicts the demographic characteristics of the included patients. In total, 54 of the planned 60 neonates were enrolled in this study. The SARS-CoV-2 pandemic resulted in the early termination of the study. A total of 267 saliva samples were collected during the study, 73 of which (27.3%) could not be analysed, either due to low sample volumes (23.5%) or blood contamination (3.8%).

TABLE 1 Demographic characteristics of the study population

Demographic	Value
Enrolled patients, *n*	54
Males, *n* (%)	31 (57.4)
GA in weeks, median (range)	34.8 (24.3–41.7)
<32 weeks, *n* (%)	21 (38.9)
32–37 weeks, *n* (%)	13 (24.1)
≥37 weeks, *n* (%)	20 (37.0)
PMA in days, median (range)	244.2 (170.5–294.2)
PNA in days, median (range)	1.5 (0.3–6.8)
Birth weight in kg, median (range)	2.4 (0.7–4.5)
Actual weight in kg, median (range)	2.4 (0.7–4.3)
Total saliva samples, *n* (%)	267 (100)
Analysed, *n* (%)	194 (72.7)
Failed, *n* (%)	73 (27.3)
Analysed saliva samples per patient, median (range)	3 (1–8)
Blood samples, *n*	99
Peak samples, *n*	43
Trough samples, *n*	56
Blood samples per patient, median (range)	2 (1–4)
Oro-esophageal congenital anomalies, *n*	1
Controlled hypothermia, *n*	3
Perinatal asphyxia, *n*	3

Abbreviations: GA: gestational age; PNA: postnatal age; PMA: postmenstrual age.

3.2 | Swab adsorption

Adsorption of gentamicin to the swab was found to be less than 3.1% at the low concentration level and 8.2% at the high concentration level, and therefore below the predetermined acceptable percentage of 15%.

3.3 | Gentamicin pharmacokinetics in plasma

Model diagnostic figures indicated that the model provided by Fuchs et al. could adequately describe the plasma PK data of the study population, based on 97 plasma TDM concentrations. It seems that the model had a slight bias towards underprediction at the low concentration range, though upon inspection of all diagnostic plots, the performance of the model was deemed acceptable (Figure in the Supporting Information). The model was used to estimate individual plasma PK and served as a basis for the construction of the saliva model.

3.4 | Gentamicin pharmacokinetics in saliva

The salivary PK of gentamicin was described by adding a saliva compartment to the plasma model (Figure 1). For the structural model, a k_{13} of 0.036 h^{-1} and k_{30} of 0.267 h^{-1} were estimated, as well as IIV on k_{30} (63.6%) (Table 2). The estimate of IIV on k_{30} had an acceptable η-shrinkage and ε-shrinkage of 26.2% and 0.1%, respectively. A logarithmic proportional error model was used to describe the residual error (58.4%). Twenty-seven (14%) of all analysed saliva samples were below the LLOQ and these measurements were accounted for with the M3 method.[19] Inclusion of additional transit compartments to account for lag in saliva uptake did not improve the model fit; neither did first-order transport from the peripheral compartment to the salivary compartment. Though it was also possible to successfully fit a model with estimations for both IIV on k_{30} and k_{13}, η-shrinkage on these parameters was 56% and 34%, respectively. These levels of η-shrinkage were unacceptable and therefore that model was rejected.[24]

Stepwise forward inclusion of PMA as a power function covariate on k_{13} led to the largest decrease in OFV ($\Delta OFV = -61.33$). PMA was also included as a covariate on k_{30} as a power function ($\Delta OFV = -17.25$). None of the other tested covariates improved the model; controlled hypothermia/perinatal asphyxia was not tested due to a lack of power ($n = 3$). The parameter estimates of the final model are shown in Table 2. Final estimates for k_{13} and k_{30} were 0.023 h^{-1} and 0.169 h^{-1}, respectively. IIV of k_{30} was 38% in the final model, whereas proportional residual error was 49.7%. The exponents of PMA as a covariate on k_{13} and k_{30} respectively were −8.8 and −5.1. This describes a negative correlation between PMA and both the transport and elimination rate of gentamicin in saliva, indicating that gentamicin is more readily available in the saliva of patients of low PMA, such as premature neonates. Evaluation of the GOF plots of the final model demonstrated a good description of the observed

TABLE 2 Population PK parameters and bootstrap results

	Base model OFV = 877.3		Final model OFV = 738.7		Bootstrap results (n = 1000)		
Parameter	Estimate	RSE (%)	Estimate	RSE (%)	Median	2.5th %	97.5th %
θ_{k13} (h^{-1})	0.036	79	0.023	16	0.023	0.016	0.033
θ_{k30} (h^{-1})	0.267	70	0.169	15	0.171	0.123	0.239
$\theta_{PMA\ K13}$	—	—	−8.8	16	−8.7	−11.7	−5.7
$\theta_{PMA\ K30}$	—	—	−5.1	28	−4.9	−8.1	−2.0
σ_{prop} (%)	58.4	9	49.7	7	49.0	40.8	56.4
IIV_{k30} (%)	63.6	12	38.0	17	37.3	30.5	43.8

θ_{k13}: first-order rate constant from central plasma compartment to saliva compartment; θ_{k30}: first-order elimination rate constant from saliva compartment; $\theta_{PMA\ K13}$: power equation exponent PMA on k_{13}; $\theta_{PMA\ K30}$: power equation exponent PMA on k_{30}; σ_{prop}: proportional error; IIV_{k30}: inter-individual variability of k_{30}.

$$k_{13} = \theta_{k_{13}} * \left(\frac{PMA}{244.2}\right)^{\theta_{PMA\ k_{13}}}$$

$$k_{30} = \theta_{k_{30}} * \left(\frac{PMA}{244.2}\right)^{\theta_{PMA\ k_{30}}}.$$

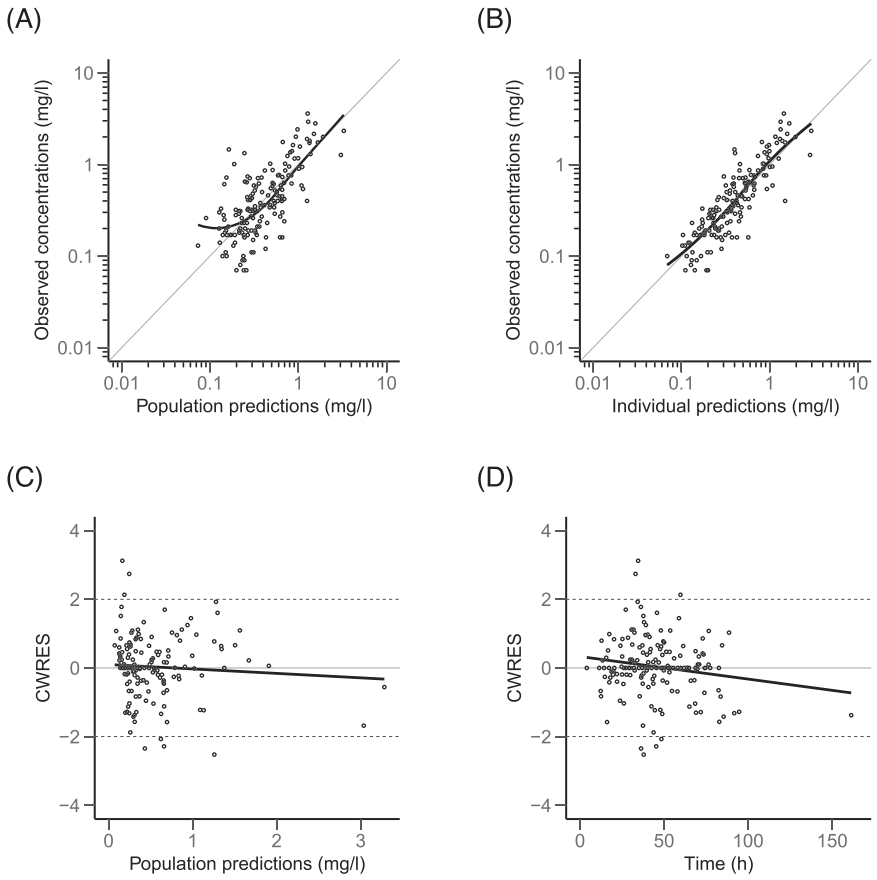

FIGURE 2 Goodness-of-fit plots of the final model. (A) Population predictions *vs* observed concentrations in saliva. (B) Individual predictions *vs* observed concentrations. (C) Population predictions *vs* conditional weighted residuals (CWRES). (D) Time *vs* CWRES

gentamicin concentrations in saliva (Figure 2). For demonstrative purposes, observations and model predictions have been plotted for one representative patient per GA group (Figure 3).

3.5 | Bootstrap and internal model validation

The robustness of the final model was evaluated using a bootstrap procedure (n = 1000) and results are summarized in Table 2. Of the bootstrap runs, 98.3% were successful and the results indicated that the model was robust. For internal validation a pcVPC (n = 1000 samples) of the final model was evaluated (Figure 4). Most of the 10th, 50th and 90th percentiles of the observed values lie within the 95% confidence intervals of the 10th, 50th and 90th percentiles of the simulated values for all bins.

3.6 | Simulations

The simulated proportion of subjects with peak and trough levels within the target range are displayed in Figure 5. Applying TDM using

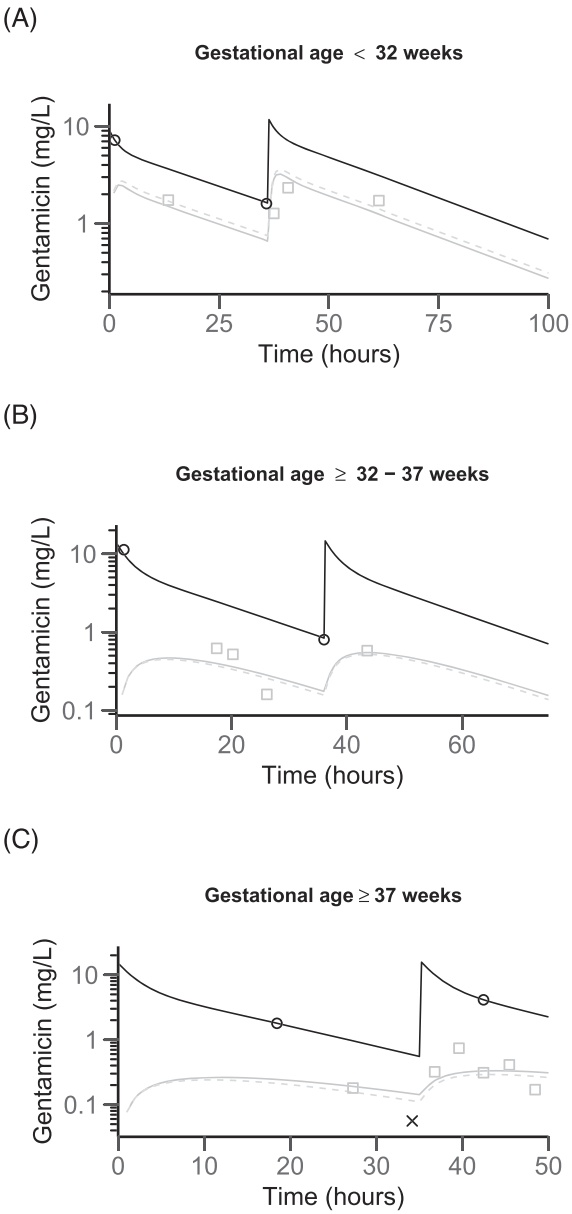

FIGURE 3 Individual pharmacokinetic profiles of gentamicin in plasma and saliva for typical patients of each GA group. (A) Individual patient of GA < 32 weeks; (B) Individual patient of GA ≥ 32–37 weeks; (C) Individual patient of GA ≥ 37 weeks. Black circles: observed plasma concentrations; gray squares: observed saliva concentrations; solid black line: individual predicted plasma concentrations; solid gray line: individual predicted saliva concentrations; dashed gray line: population predicted saliva concentrations; black crosses: observed saliva concentrations < LLOQ

saliva led to a higher percentage of subjects reaching target attainment compared to no TDM (>75% *vs* 48%, respectively). However, saliva TDM led to a lower percentage of target attainment compared to plasma TDM. Obtaining more than four samples for saliva TDM did not result in increased TDM performance. On the contrary, obtaining additional saliva samples at 18 h and 1 h pre-dose led to a slightly decreased performance (−3% and −4%, respectively) compared to the strategy using four samples. Examples of individual TDM simulations are depicted in Figure in the Supporting Information.

4 | DISCUSSION

In this study, we have quantified the PK of salivary gentamicin concentrations in neonates and demonstrated the feasibility of monitoring gentamicin concentrations in saliva. Concentration–time profiles in both plasma and saliva were described with an integrated PK model. The potential use of salivary concentrations in the context of TDM was assessed through Monte Carlo simulations and MAP estimations. Simulations predicted a target attainment of up to 81% for TDM with four saliva samples *vs* 94% when performed with two plasma samples.

In the past, several investigators have assessed the use of saliva for TDM of aminoglycosides with varying results.[6,8–11] Berkovitch et al. reported a good correlation between plasma and saliva concentrations of gentamicin for a once daily dosing regimen in children.[8] Other investigators reported that aminoglycosides did not penetrate into saliva of children with cystic fibrosis or tuberculosis.[10,11] Work regarding saliva TDM in neonates has covered multiple drugs, including caffeine, morphine and antiepileptic drugs.[6] Interestingly, all studies focused on linear correlations. Incorporating saliva concentrations in nonlinear mixed effect models may allow for more flexibility to account for delayed penetration, delayed elimination and variability in saliva/plasma ratio (S/P). To the best of our knowledge, this POP-PK model is the first to apply this principle for gentamicin in saliva and there are only few published models which incorporate this methodology to describe saliva concentrations for other drugs.[25,26]

The model developed during this study was constructed by appending a plasma PK model for gentamicin with a saliva compartment. Initially, a two-compartment model by Bijleveld et al.[27] was used to fit the plasma PK of the study population. This model was chosen because it was developed with data from patients that were admitted to the same NICU as the present study, thereby accurately reflecting the study population. Parameters in this model were allometrically scaled for BW, included IIV on CL and V_c and used PMA as a covariate for CL.[27] Though the model could adequately describe the plasma PK, the full saliva model could not accurately predict V_c through Bayesian MAP estimation using saliva samples during simulation. Therefore, the model by Fuchs et al.[16] (Table S1 in the Supporting Information) was used to describe the plasma PK of the study population (Figure in the Supporting Information). Though this model was highly similar to the model by Bijleveld et al., the added benefit was that a stronger correlation between CL and V_c was included, allowing for more accurate predictions during Bayesian MAP estimations using the full saliva model. Constructing a new plasma PK model with the study data did not result in a better fit.

Gentamicin concentrations in saliva could best be described with drug transport from the central compartment (Figure 1). The final model could accurately describe the PK of gentamicin in plasma and saliva. However, in Figure 4, a slight model misspecification can be

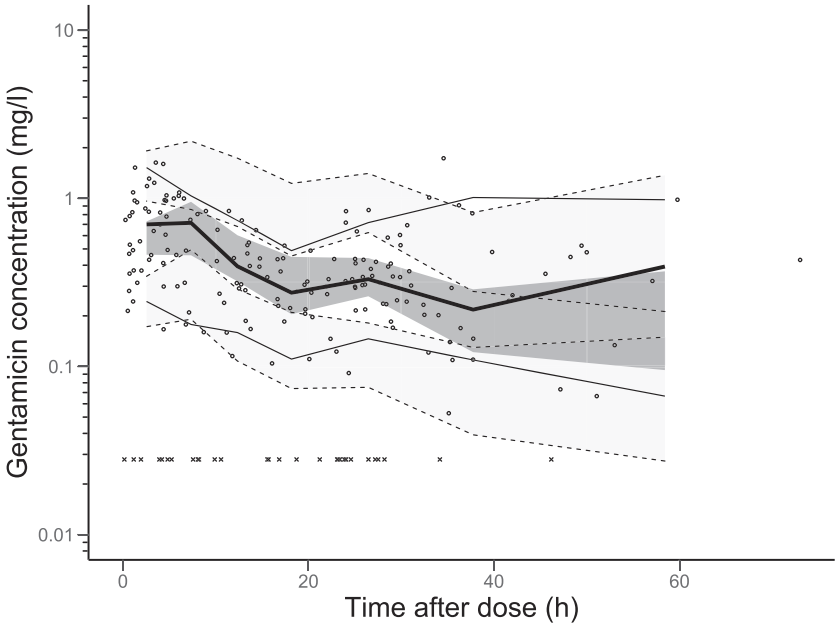

FIGURE 4 Prediction-corrected visual predictive check of the saliva model ($n = 1000$). Black circles: observed gentamicin concentrations; thick black line: median observed concentrations; thin black lines: 80% interval of the observed concentrations; dark gray field: 95% confidence interval of the median prediction; light gray fields with dashed border: 95% confidence intervals of the 10th and 90th percentiles of the predictions; crosses: observations below LLOQ (0.056 mg/L)

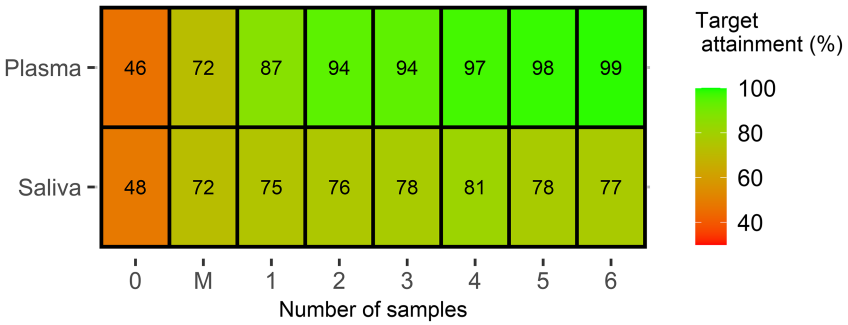

FIGURE 5 Heat map displaying the simulated proportion of subjects who reach target attainment of gentamicin after plasma and saliva TDM using an increasing number of samples. Time-points where samples were simulated: 0: standard dosing according to guidelines without dose optimization; M: a priori tailored dosing without samples; 1: sample (14 h); 2: peak sample (3 h for saliva or 1 h for plasma) and trough sample (0.5 h pre-dose); 3: samples at peak, 14 h and trough; 4: samples at peak, 7 h, 14 h and trough; 5: samples at peak, 7 h, 14 h, 18 h and trough; 6: samples at peak, 7 h, 14 h, 18 h, 1 h pre-dose and trough

seen in later times after the last dose. It should be noted that there were few samples drawn beyond 50 hours post-dose and therefore there was little information regarding this period. Also, in current clinical settings, the dose interval does not exceed 48 hours. Moreover, this misspecification was not present in other diagnostic tools (Figure 2) and therefore this finding was deemed of limited clinical relevance and the model was accepted. Models incorporating drug transport from the peripheral compartment to saliva were evaluated but did not accurately describe the data. Two separate rate constants were estimated for the saliva model. A first-order rate constant k_{13} of 0.023 h^{-1} and an elimination rate k_{30} of 0.169 h^{-1} were estimated. As k_{13} was much lower than k_{30}, transport from the central plasma compartment to the saliva compartment is the rate-limiting step determining the concentration–time profile in saliva.[28] Oromucosal reabsorption of gentamicin was not included in the model, as the contribution of oral absorption cannot be quantified after IV administration of a drug if bioavailability is low, as is the case with gentamicin.[17] Therefore elimination of gentamicin from saliva was best described with a single elimination constant (k_{30}). Moreover, models with zero-order salivary elimination did not result in adequate fits. When predicting gentamicin concentrations in plasma and saliva in typical patients (Figure 3, Figure in the Supporting Information) it seems that the S/P ratio stabilizes hours after the last dose is administered. During this phase, the concentration–time curve of saliva is perpendicular to plasma, indicating that the salivary gentamicin elimination rate is linear to the plasma concentration and therefore is dependent on k_{13}. In a separate population analysis, we could not identify any demographic descriptors to accurately predict individual S/P ratios. Due to the inter-individual variability in the S/P ratio, it is not feasible to use a simple algorithm or guideline to convert saliva concentrations to plasma concentrations.

Considerable IIV was detected. Part of this was accounted for by taking PMA into consideration. It was estimated that IIV on k_{30} was 38% in the final model. PMA had a large influence on the salivary PK

profile of gentamicin. Inclusion of PMA as a covariate on both k_{30} and k_{13} significantly improved the model. The exponents of the power equation functions were −5.5 and −8.8 for k_{30} and k_{13} respectively, demonstrating a strong age dependency of gentamicin disposition in saliva. With increasing PMA, k_{13} and k_{30} decrease by a large margin. Indeed, it was observed that salivary gentamicin levels were generally much lower in term neonates, compared to premature neonates. Furthermore, 75% of samples below the LLOQ were from older neonates (PMA > 260 days). Though the model did not contain a parameter describing the IIV in k_{13}, inclusion of PMA as a covariate on k_{13} significantly improved model fit, decreased RSE on all parameters and decreased residual error. It was quite notable that gentamicin was more freely distributed in saliva of premature neonates. In a development study in rats, it was suggested that tight junctions of the submandibular saliva glands are immature at late gestation.[29] This might result in more permeable saliva glands due to increased paracellular transport of compounds. These findings may be indicative that salivary TDM could be more efficacious and possibly more accurate in premature neonates.

TDM performance was assessed through simulation in a fictional cohort of 3000 neonates with a uniform distribution of GA and corresponding distribution of WT (Figure in the Supporting Information).[22] By applying Bayesian MAP during simulation one can use information obtained from multiple samples to estimate the peak and trough concentrations, which reduces the prediction error in the process. Additionally, the optimization process prevents outlier saliva concentrations being extrapolated to extreme plasma concentrations on which dose adaptations are then falsely made. Results from this simulation may be optimistic, as each virtual subject was subjected to a rigid dose decision rule for dose optimization and inter-occasion variability was not accounted for. In practice, time-dependent factors such as changes in CL are considered during TDM. However, the simulations give an indication of the expected reliability of TDM with saliva samples *vs* plasma samples, as well as the comparative performance of several sampling schedules, and can be used as a proof of concept.

Simulations indicated that a target attainment of 81% is possible with saliva TDM. Obtaining the necessary four saliva samples at 3 h, 7 h, 14 h post-dose and 0.5 h pre-dose is logistically feasible in this scenario. Interestingly, using more than four saliva samples seemed to decrease the accuracy of saliva TDM. However, sampling times during MAP estimation were selected rather arbitrarily and were equal for all dose regimens. A more thorough evaluation of optimal sampling times for MAP estimation was not performed during this study, given its explorative nature. Target attainment following TDM with two plasma samples (94%) was higher than with four saliva samples. This difference in performance for saliva and plasma TDM can be explained by the large difference in residual error between the two matrices. The uncertainty in the Bayesian optimization process introduced by these parameters was too large to address the precision difference in saliva and plasma TDM with additional sampling or different sampling schedules. Moreover, assessed saliva sampling schedules were equal for all dosing regimens, therefore the evaluated additional samples may have had limited value for dosing regimens of 36 or 48 hours. Plasma TDM performs better in settings where collection of two plasma samples is protocol. However, in many clinical settings TDM protocols require a single intermediate concentration sample. In that case, plasma TDM has a predicted target attainment of 87% (Figure 5). This difference with saliva TDM is substantially smaller. Taken together with the uncertainties of the simulations, TDM with four saliva samples may be a suitable alternative to plasma TDM with a single intermediate concentration sample. Moreover, since the same sampling strategies were employed for all dose regimens during simulation, the difference in predicted target attainment may not be clinically relevant for all GA groups. This may be especially true for premature neonates in which gentamicin was more readily available in the saliva. Coincidentally, premature neonates could benefit most from a non-invasive TDM method.

This study has several limitations. First, there was a large proportion of saliva samples with insufficient volumes for analysis. This is unlikely the result of mechanical ventilation or use of anticholinergic drugs, as patients in the study cohort had a nasopharyngeal tube placement not interfering in any way with the oral cavity and anticholinergic drugs were not given. However, the low sample volumes may be due to inadequate sampling technique or insufficient saliva production by subjects, especially with premature neonates. Future studies may employ a different sampling strategy to ensure that an adequate volume of saliva is drawn, such as use of a different swab or cutting the saturated end of the swab.[30,31] Saliva secretion was not stimulated with citric acid as it substantially increases the burden of saliva collection. Currently no standardized method for the collection of saliva from neonates exists. It is important that a standardized saliva collection method is developed in the future, to ensure accurate saliva yields and that saliva collection is comparable between hospitals. Moreover, a small number of samples could not be used due to contamination with blood, therefore did not represent saliva concentrations of gentamicin. However, this occurred rarely (3.8% of all samples were contaminated and all contaminated samples were from two patients). The blood that contaminated the saliva originated from pre-existing lesions as a result of clinical procedures such as intubation or suctioning, rather than being a side-effect of our sampling method. Due to the delicate method of saliva sampling, blood contamination is highly unlikely. In a clinical setting, blood contamination of samples is immediately observed due to the strong red discoloration of the swabs. If this is encountered, subsequent saliva samples are likely to be contaminated as well. For these few patients, saliva sampling is not viable for this purpose and plasma samples should be used for TDM. Nonetheless, a large number of samples was available for model development, thus we do not expect this to have influenced the parameter estimates. Second, due to the low volumes of the collected samples, it was not possible to determine pH of the collected samples. Saliva pH has been proposed to influence salivary distribution of drugs.[32] Though little has been published regarding saliva pH of neonates, we expect that fluctuations in saliva pH have little influence on the protonated fraction of gentamicin since the strongest basic pK_a is 10.18.[33] Third, assumptions made during simulation, such

as the underlying covariate distribution and sampling strategies, have an influence on the proportion of subjects reaching target attainment. However, considering that the goal of the simulation was to compare saliva and plasma TDM, the comparative differences found in these simulation scenarios should be independent of these assumptions. Finally, the final saliva model contained a large proportional residual error of 49.7%. High residual variability in the saliva compartment complicates the predictive power of the model. However, to compensate for this and to obtain reliable predictions, more saliva samples are required. As was found in the simulation study, more samples were required for saliva TDM of gentamicin than for plasma TDM.

Strengths of this study include the use of POP-PK, allowing for the description of nonlinear relations between plasma and saliva gentamicin concentrations with both fixed and random effects, and a relatively large cohort of neonates of different GA receiving varying dosing regimens originating from both a peripheral paediatric ward and NICU, improved the generalizability of the model. Moreover, use of highly sensitive LC–MS/MS allowed for determination of low gentamicin concentrations in small sample volumes with an LLOQ of 0.056 mg/L, which was substantially lower than earlier publications investigating gentamicin in saliva.[9–11] POP-PK modelling allowed for deviation from scheduled sampling times and identification of covariates. Collected saliva samples were evenly distributed, providing information for all time-points of the dose intervals. The TDM simulations of a wide range of sampling strategies give an adequate overview of the expected performance of saliva TDM in different scenarios. Moreover, since a large cohort was simulated ($n = 3000$), it can be assumed that estimations were accurate and standard error was low. Confidence intervals of the target attainments were therefore not calculated, as it was of little added value and repeated calculations would be overly laborious and computationally intensive with a sample size this large.

This study is the first to demonstrate that TDM of gentamicin saliva of an exclusively neonatal population is feasible. A target attainment of 81% was found based on explorative simulations with four saliva samples and performance is close to plasma TDM with one intermediate sample. In the future, the real-life performance of saliva TDM employing an improved sampling technique should be investigated prospectively in premature neonates, as gentamicin appears more readily in the saliva of premature neonates and these most fragile infants may benefit most from non-invasive TDM.

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COMPETING INTERESTS

Nothing to declare.

CONTRIBUTORS

A.S. and M.K. enrolled patients, performed the analysis and wrote the manuscript. Y.T. enrolled patients and reviewed the manuscript. M.v.E. supported data analysis and reviewed the manuscript. Y.B. provided input during analysis and reviewed the manuscript. G.D. and W.v.H. enrolled patients, supervised clinical activities, provided input and reviewed the manuscript. R.S. and A.C. conceptualized the study, provided input during the analysis and reviewed the manuscript. A.v.K. enrolled patients and reviewed the manuscript. T.H. enrolled patients, supervised clinical activities, conceptualized the study and reviewed the manuscript. R.M. conceptualized the study, supervised the analysis and reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy, legal and ethical restrictions.

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SUPPORTING INFORMATION

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