

## Amoxicillin Pharmacokinetics in Preterm Infants with Gestational Ages of Less than 32 Weeks

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The multiple-dose pharmacokinetics of amoxicillin (AM [administered twice daily in a 25-mg/kg of body weight intravenous dose]) in 17 preterm infants (11 males; gestational age,  $29 \pm 1.9$  weeks; birth weight,  $1,175 \pm 278$  g) were evaluated on day 3 of life. Blood samples were collected from an arterial catheter at 0, 0.5, 1, 2, 4, 8, and 12 h after the intravenous dose. A high-performance liquid chromatography method was used to determine AM concentrations in serum. AM pharmacokinetics followed a one-compartment open model. The glomerular filtration rates of all patients were simultaneously studied by means of the 24-h continuous inulin infusion technique. The elimination half-life, apparent volume of distribution, and total body clearance of AM (mean  $\pm$  standard deviation) were  $6.7 \pm 1.7$  h,  $584 \pm 173$  ml, and  $62.4 \pm 23.3$  ml/h, respectively. The mean ( $\pm$  standard deviation) AM peak and trough levels were  $53.6 \pm 9.1$  and  $16.0 \pm 4.9$  mg/liter, respectively. All infants had a serum trough level above 5 mg/liter. The total body clearance and apparent volume of distribution of AM and the clearance of inulin increased significantly with increasing gestational age. The total body clearance of AM ( $1.0 \pm 0.4$  ml/min) and the clearance of inulin ( $1.0 \pm 0.3$  ml/min) were similar. The total body clearance of AM increased significantly with increasing clearance of inulin. We conclude that an AM dose of 25 mg/kg every 12 h given to preterm infants in the first week of life with gestational ages of less than 32 weeks results in serum levels well above the MIC for major microorganisms involved in neonatal infections.

Amoxicillin (AM), a penicillin derivative, is an antibiotic frequently used for the treatment of infectious diseases in newborn infants. It is active against common gram-positive neonatal pathogens such as *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Streptococcus faecalis* and against gram-negative bacilli, including non- $\beta$ -lactamase-producing *Haemophilus influenzae* and *Escherichia coli*. The combination of AM with a broad-spectrum cephalosporin or an aminoglycoside is an effective empiric treatment of suspected neonatal septicemia (21). Despite the widespread use of AM in neonatal intensive care units, the pharmacokinetics of AM in preterm infants have not been studied.

The currently recommended dosage for ampicillin in preterm infants of less than 4 weeks of life with a birth weight below 1,200 g is 25 to 50 mg/kg of body weight every 12 h (12, 14, 15). Dosage recommendations for AM are extrapolated from studies of ampicillin which did not stratify patients according to gestational age (GA) or postnatal age (PA) (1, 3-5, 6, 11). However, the glomerular filtration rate (GFR) of preterm infants increases significantly with advancing GA and PA (8, 19). This has a major effect on the pharmacokinetics of antibiotics which are mainly excreted by glomerular filtration, as has been demonstrated for the pharmacokinetics of ceftazidime (7). AM has a low level of serum protein binding ( $\pm 17\%$ ) and is excreted primarily by glomerular filtration (18). We hypothesized that the pharmacokinetic behavior of AM in preterm infants in the first week of life would be influenced by GA-dependent differences in GFR. We therefore investigated the pharmacokinetics of AM (25 mg/kg/12 h) during the first

week of life in 17 infants with a GA below 32 weeks and studied the effect of GA-dependent differences in GFR on the kinetic parameters.

### MATERIALS AND METHODS

**Patients and study design.** Seventeen preterm infants with GAs of less than 32 weeks, admitted to the neonatal intensive care unit with suspected or documented septicemia or invasive infection, were eligible for study. Subjects were recruited by informed parental consent. The inclusion criteria were postnatal age of 3 days, stability of hemodynamic function (diuresis,  $>1$  ml/kg of body weight per h; systolic and diastolic blood pressure above the third percentile adjusted for GA), normal liver function, and no concurrent administration of nephrotoxic or inotropic drugs. All patients had an indwelling arterial catheter. The partial pressure of oxygen in arterial blood was kept at greater than 50 mm Hg (6,666 Pa), and the oxygen saturation was above 92%. Neonates assigned to receive therapy were given AM (25 mg/kg intravenously [i.v.]) every 12 h and ceftazidime (25 mg/kg i.v.) every 12 h. Patients with documented invasive bacterial infections received at least 10 days of i.v. therapy. Patients with sterile cultures and without a focus of infection received a total of 72 h of therapy. From each patient, the following laboratory parameters were obtained: complete blood count with differential and platelet count, blood and urine cultures, and arterial blood-gas analysis.

**Pharmacokinetics study.** The pharmacokinetics of AM were studied on day 3 after birth. Blood samples were taken from indwelling arterial lines before administration of an i.v. bolus injection ( $t = 0$ ) and at 0.5, 1, 2, 4, 8, and 12 h after the i.v. dose. Serum samples obtained after centrifugation in a microcentrifuge (Merck-type Eppendorf 5414;  $3,000 \times g$  for 1 min) were stored at  $-70^\circ\text{C}$ .

**Measurement of GFR.** The GFR was measured by the continuous inulin infusion technique on day 3 after birth (19). A 10% glucose-inulin solution containing 25 g of inulin per liter was infused at a rate of 0.6 ml/kg/h, beginning at time ( $t$ ) zero of the pharmacokinetics study. After 24 h, the inulin clearance ( $\text{CL}_{\text{in}}$ ) was calculated from the infusion rate ( $R$ ), the inulin concentration in the infusate ( $I$ ), and the serum inulin concentration ( $P_{\text{in}}$ ) by the equation  $\text{CL}_{\text{in}} = (I \times R)/P_{\text{in}}$ .

**AM HPLC assay.** Analysis of serum AM concentrations was performed according to the method described by Haginaka and Wakai with minor modifications (9). HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, Scotland). The other chemicals were purchased from Aldrich-Chemie (Steinheim, Germany). All chemicals applied were of the highest grade commercially available. Chromatographic analysis was performed with a glass-prepacked col-

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TABLE 1. Demographic and laboratory parameters of 17 patients studied on day 3 after birth

Parameter	Mean $\pm$ SD
Age (wk)	29 $\pm$ 1.6/7
Sex (M/F) <sup>a</sup>	11/6
Wt (g)	1,175 $\pm$ 278
AGA/SGA <sup>b</sup>	15/2
Culture positive	0
Hematocrit (%)	44 $\pm$ 7
Leukocytes ( $10^3/\text{mm}^3$ )	15.6 $\pm$ 12.6
Platelets ( $10^3/\text{mm}^3$ )	215 $\pm$ 112
Artificial ventilation (+/-)	10/7

<sup>a</sup> M, male; F, female.<sup>b</sup> AGA, appropriate for GA; SGA, small for GA.

umn (100 by 3 mm) containing ODS-2 Chromospher Spherisorb beads (5- $\mu\text{m}$ -diameter particle size; Chrompack, Middelburg, The Netherlands) combined with a guard column. A Bio LC pump (model 410, Perkin-Elmer, Norwalk, Conn.) was used to deliver the eluent: 15% (vol/vol) acetonitrile, 50 mM sodium phosphate buffer, and 10 mM thiosulfate buffer (pH 4.6) at a flow rate of 0.8 ml/min. The separations were carried out at room temperature. The eluate was monitored with a Perkin-Elmer LC-95 UV/visible spectrophotometer detector at a wavelength of 328 nm.

To a 100- $\mu\text{l}$  aliquot of the serum sample, 100  $\mu\text{l}$  of 10 M urea solution was added. Subsequently the mixture was ultrafiltered with an Amicon MPS-1 micropartition system with Amicon YMT membranes (Amicon Corp., Lexington, Mass.) by centrifugation at  $1,500 \times g$  for 30 min at room temperature. To an 80- $\mu\text{l}$  aliquot of the ultrafiltrate, 80  $\mu\text{l}$  of 0.1 M borate buffer (pH 9.0) and 8  $\mu\text{l}$  of 0.2 M acetic anhydride solution were added, and the mixture was allowed to stand at room temperature for 3 min. Subsequently, 160  $\mu\text{l}$  of 2 M triazole reagent (pH 9.0) containing 1 mM mercury(II) chloride was added. The solution was sealed in a screw-top vial and heated at 60°C for 10 min. Subsequently, 20  $\mu\text{l}$  was transferred by an automatic sample injector (Perkin-Elmer) to the column.

A calibration curve was made by dissolving 4, 12, 25, 50, and 100  $\mu\text{g}$  of AM per ml in serum. These spiked standard samples were processed according to the procedure mentioned above. A standard line of peak areas versus spiked AM concentrations was determined. A linear calibration curve was obtained over a range of 4 to 100  $\mu\text{g}$  of AM per ml. Because the spiked samples of the calibration curve underwent the same processing procedure as the clinical samples, the clinical samples were directly converted by linear regression from the calibration curve to actual AM concentrations per milliliter of serum. The lower limit of detection of AM in serum was 1  $\mu\text{g}/\text{ml}$ . The coefficients of interassay variation determined at concentrations of 8.6 and 86  $\mu\text{g}/\text{ml}$  were 7.9 and 3.4%, respectively. The intraassay values were 2.1 and 4.7%, respectively. Recovery of 95% of the derivatized AM, which had been incubated for 24 h at room temperature, was established.

**Pharmacokinetics analysis.** Kinetics studies were performed on the third day after birth. Visual inspection of individual model fits gave no indication that a more complex (e.g., two-compartment) pharmacokinetics model was required. Pharmacokinetics parameters were calculated with the multiple-dose formulae described by Rowland and Tozer (16). The basic formula used was  $C_t = \text{dose} / [V \times (1 - r^n)] / [(1 - r) \times e^{-kt}]$ . In this formula,  $C_t$  is the plasma concentration of AM at given times  $t$ ,  $V$  is the apparent volume of distribution,  $n$  is the dose number, and  $r = e^{-k\tau}$ , in which  $k$  is the elimination rate constant and  $\tau$  is the dosing interval. Because doses were given twice daily, the AM concentration-versus-time curve was assumed to be attributable to the 7th dose (and the trough level at  $t = 0$  was assumed to be attributable to the 6th dose). The data were weighted by  $1/(\text{predicted value})^2$ . All calculations were carried out with the nonlinear regression module of SPSS/PC+ V 4.0.1 (SPSS, Inc., Chicago, Ill.), which uses a Levenberg-Marquardt algorithm.

TABLE 2. Pharmacokinetics parameters of AM and  $\text{CL}_{\text{in}}$  data of 17 infants studied on day 3 after birth

Parameter	Mean $\pm$ SD
$t_{1/2\beta}$ (h)	6.7 $\pm$ 1.7
CL (ml/min)	1.0 $\pm$ 0.4
CL (ml/min/kg of body wt)	1.1 $\pm$ 0.2
$V$ (ml)	584 $\pm$ 173
$V$ (ml/kg)	671 $\pm$ 117
$\text{CL}_{\text{in}}$ (ml/min)	1.0 $\pm$ 0.3
Trough level (mg/liter)	16.0 $\pm$ 4.9

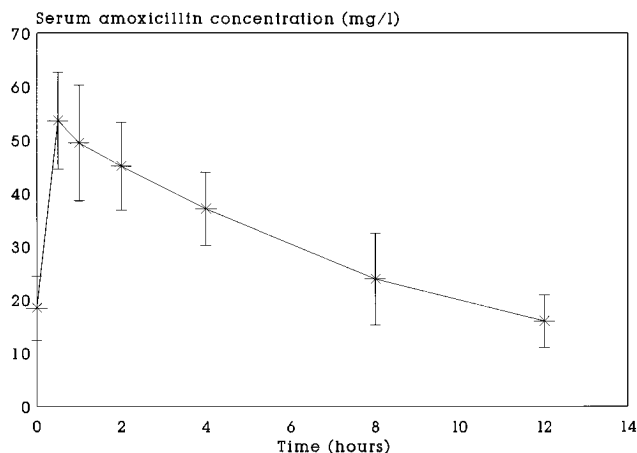


FIG. 1. Serum drug concentration-versus-time profiles of AM (25 mg/kg of body weight) in 17 preterm infants on day 3 after birth. Bars indicate standard deviations.

## RESULTS

Seventeen neonates, including 11 male and 6 female infants, were enrolled in the study. The demographic and laboratory parameters and the  $\text{CL}_{\text{in}}$  data for these patients are shown in Table 1. The pharmacokinetics parameters of AM are indicated in Table 2. Figure 1 demonstrates the serum AM concentration (mean  $\pm$  standard deviation)-versus-time curve. The mean peak and trough levels ( $\pm$  standard deviation) were  $53.6 \pm 9.1$  and  $16.0 \pm 4.9$  mg/liter, respectively. All neonates had serum trough levels above 5 mg/liter.

We examined the relationship between GA and  $\text{CL}_{\text{in}}$  (as a parameter of the GFR) in our patients. We demonstrated that  $\text{CL}_{\text{in}}$  increased significantly with increasing GA ( $r = 0.84$ ,  $P \leq 0.001$ ). With increasing GA, total body clearance (CL) of AM increased significantly ( $r = 0.75$ ,  $P = 0.001$ ) (Fig. 2). A similar increase was detected for  $V$  ( $r = 0.58$ ,  $P = 0.014$ ). AM clearance also increased significantly with weight ( $r = 0.66$ ,  $P < 0.005$ ). A similar increase was seen for  $V$  ( $r = 0.78$ ,  $P < 0.001$ ). After we normalized the parameters  $V$  and CL of AM for weight, the previously significant correlations between GA and  $V$  and between GA and CL disappeared ( $P = 0.735$  and  $P = 0.283$ , respectively). CL of AM ( $1.0 \pm 0.4$  ml/min) and  $\text{CL}_{\text{in}}$  ( $1.0 \pm 0.3$  ml/min) were equal. We determined the presence of

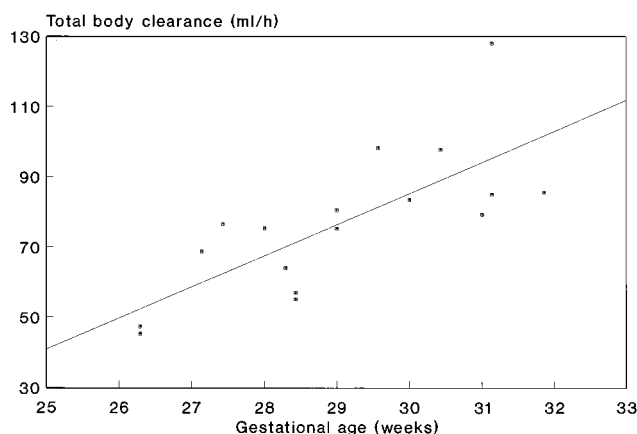
FIG. 2. Linear regression analysis of GA (weeks) versus CL of AM (ml/h) in 17 preterm infants on day 3 after birth ( $r = 0.75$ ,  $P \leq 0.001$ ,  $y = 8.88x - 181.2$ ).

TABLE 3. Correlation coefficients of AM pharmacokinetics parameters with GA and  $CL_{in}$ <sup>a</sup>

Parameter	GA	$CL_{in}$
$t_{1/2\beta}$ (h)	-0.20	-0.09
CL (ml/min)	0.75***	0.72***
V (ml)	0.58*	0.62**
Trough level (mg/liter)	-0.36	-0.21

<sup>a</sup> Statistical significance: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

a regression between  $CL_{in}$  and AM clearance. We found that AM clearance was equal to  $(47.1 \times \text{inulin clearance}) + 28$ . Calculations showed no significant relationships between GA and  $CL_{in}$  versus the elimination half-life ( $t_{1/2\beta}$ ) of AM (Table 3). In addition, the elimination rate constant and  $CL_{in}$  were fitted and no relationship was detected.

## DISCUSSION

During the previous decade, it became apparent that the pharmacokinetics of a large variety of drugs in preterm infants are significantly different from those in full-term children. Clearance rates are usually much slower in the preterm infant, mainly because of immaturity of renal function of hepatic drug metabolism. Dosage recommendations for the use of AM in preterm neonates have been extrapolated from data about ampicillin obtained for infants and adults (10, 17). We questioned the validity of these recommendations and studied the pharmacokinetics of AM in preterm infants with GAs of less than 32 weeks on day 3 of life by using the lowest recommended dose (25 mg/kg/12 h i.v.).

Our results indicate that administration of this dose results in high serum drug levels during the complete dosing interval. The MIC of AM for AM-susceptible microorganisms, including non- $\beta$ -lactamase-producing *H. influenzae* and *E. coli*, does not exceed 5 mg/liter (20). Taking this level as a reference, serum AM levels are sufficiently high. The prolonged  $t_{1/2\beta}$  of AM ( $6.70 \pm 1.67$  h) and the high serum drug trough levels ( $16.0 \pm 4.9$  mg/liter) suggest that the dosing interval may even be extended to  $\pm 18$  h. A study of 10 infants with a mean age of 10 months showed a substantially lower serum drug half-life (mean  $t_{1/2\beta} = 1.22$  h) (17). A serum drug half-life of 1.1 to 1.3 h after administration of different i.v. doses of AM was also found in seven healthy adults (10). AM is predominantly excreted in the urine in unaltered form.

It has been shown that CL of penicillin in the newborn is not significantly greater than creatinine clearance (13). These findings suggest that glomerular filtration, rather than tubular secretion, is primarily responsible for most of the renal elimination of penicillin in neonates. CL increases with age and surpasses creatinine clearance, which probably reflects the development of renal function (both filtration and secretion) throughout the neonatal period. Our results showed that the GFR ( $1.0 \pm 0.3$  ml/min) and the CL ( $1.0 \pm 0.4$  ml/min) of AM were equal. We therefore assume that AM, like penicillin, is almost completely cleared by glomerular filtration in preterm infants with GAs of less than 32 weeks during the first days of life. This is also in agreement with previous studies of penicillins in older neonates and infants (14, 15).

The GFR increased in our patients with advancing GA. These data are in agreement with those from other studies in which a positive relationship between the GA and the GFR was detected (8, 19). We also found a positive correlation between the GA and the CL of AM ( $r = 0.75$ ,  $P = 0.001$ ). This

was not surprising in view of the relationship between GA and GFR and GFR and AM clearance.

The V of many drugs is altered in infants during the first month of life because of the difference in body composition. Approximately 75% of total body weight in the newborn is water. Hence, drugs that are water soluble and that are distributed in extracellular water (e.g., aminoglycosides or penicillins) have an increased V per kilogram of body weight (2). Our data show that the V of AM, as expected, increased with body weight ( $r = 0.78$ ,  $P < 0.001$ ).

A significant relationship between the  $t_{1/2\beta}$  and the GA could not be demonstrated in our patients. One must take into account that not only the clearance but also V affects the  $t_{1/2\beta}$  of AM. With increasing GA, CL increased, which would be expected to lead to a decrease in  $t_{1/2\beta}$ . The V also increased, which would be expected to result in an increase in  $t_{1/2\beta}$ . The interaction between these two developmental mechanisms may explain the absence of a significant relationship between GA and the  $t_{1/2\beta}$  of AM.

The present data indicate that preterm infants in the first week of life with GAs of less than 32 weeks should receive a maximum AM dosage of 25 mg/kg/12 h. This is only one-half or less of the dosage given to more mature infants but will result in serum drug levels well above the MIC for the major microorganisms involved in neonatal infections. Further studies are necessary to elucidate if higher dosage recommendations are necessary in preterm infants with PAs of between 1 and 4 weeks.

## REFERENCES

- Axline, S. G., S. J. Yaffe, and H. J. Simon. 1967. Clinical pharmacology of antimicrobials in premature infants. II. Ampicillin, methicillin, oxacillin, neomycin, and colistin. *Pediatrics* **39**:97-107.
- Besunder, J. B., M. D. Reed, and J. L. Blumer. 1988. Principles of drug biotransformation in the neonate. A critical evaluation of the pharmacokinetic-pharmacodynamic interface (part I). *Clin. Pharmacokinet.* **14**:189-216.
- Boe, R. W., C. P. S. Williams, J. V. Bennett, and T. K. Oliver. 1967. Serum levels of methicillin and ampicillin in newborn and premature infants in relation to postnatal age. *Pediatrics* **39**:194-201.
- Colding, H., S. M. Moller, and G. E. Andersen. 1984. Continuous intravenous infusion of ampicillin and gentamicin during parenteral nutrition to 36 newborn infants using a dosage schedule. *Acta Paediatr. Scand.* **3**:203-209.
- Colding, H., S. M. Moller, and M. W. Bentzon. 1983. Kinetics and dose calculations of ampicillin and gentamicin given as continuous intravenous infusion during parenteral nutrition in 88 newborn infants. *Dev. Pharmacol. Ther.* **6**:365-373.
- Dahl, L. B., K. Melby, T. J. Gutteberg, and G. Storvold. 1986. Serum levels of ampicillin and gentamicin in neonates of varying gestational age. *Eur. J. Pediatr.* **145**:218-221.
- De Groot, R., J. N. Van den Anker, A. J. Van der Heijden, and J. Lindemans. 1990. The effect of renal development on the pharmacokinetics of ceftazidime in preterm infants, abstr. 328, p. 141. In Program and abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
- Fawer, C. L., A. Torrado, and J. P. Guignard. 1979. Maturation of renal function in full-term and premature neonates. *Helv. Paediatr. Acta* **34**:11-21.
- Haginaka, J., and J. Wakai. 1985. High-performance liquid chromatographic assay of ampicillin, amoxicillin and ciclacillin in serum and urine using a pre-column reaction with 1,2,4-triazole and mercury(II) chloride. *Analyst* **110**:1277-1281.
- Hill, S. A., K. H. Jones, and L. J. Lees. 1980. Pharmacokinetics of parenterally administered amoxicillin. *J. Infect.* **2**:333-340.
- Kaplan, J. M., G. H. McCracken, L. J. Horton, M. L. Thomas, and N. Davis. 1974. Pharmacologic studies in neonates given large dosages of ampicillin. *Pediatr. Pharmacol. Ther.* **84**:571-577.
- McCracken, G. H., and J. F. Bishara. 1990. Clinical pharmacology of antimicrobial agents, p. 1021-1078. In J. S. Remington and J. O. Klein (ed.), *Infectious diseases of the fetus and newborn infant*, 3rd ed. W. B. Saunders Co., Philadelphia.
- McCracken, G. H., C. Ginsberg, D. F. Chrane, M. L. Thomas, and L. J. Horton. 1973. Clinical pharmacology of penicillin in newborn infants. *J. Pediatr.* **82**:692-698.
- Paap, C. M., and M. C. Nahata. 1990. Clinical pharmacokinetics of antibacterial drugs in neonates. *Clin. Pharmacokinet.* **19**:280-318.
- Prober, C. G., D. K. Stevenson, and W. E. Benitz. 1990. The use of antibiotics

- in neonates weighing less than 1200 grams. *Pediatr. Infect. Dis. J.* **9**:111-121.
16. **Rowland, M., and T. N. Tozer.** 1989. Multiple dose regimens, p. 78-100. *In* M. Rowland and T. N. Tozer (ed.), *Clinical pharmacokinetics: concepts and application*, 2nd ed. Lea and Febiger, Philadelphia.
  17. **Rudoy, R. C., N. Goto, D. Pettit, and H. Uemura.** 1971. Pharmacokinetics of intravenous amoxicillin in pediatric patients. *Antimicrob. Agents Chemother.* **15**:628-629.
  18. **Sutherland, R., E. A. P. Croydon, and G. N. Rolinson.** 1972. Amoxycillin: a new semi-synthetic penicillin. *Br. Med. J.* **3**:13-16.
  19. **Van der Heyden, A. J., W. F. A. Grose, J. J. Ambagtsheer, A. P. Provoost, E. D. Wolff, and P. J. J. Sauer.** 1988. Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. *Eur. J. Pediatr.* **148**:24-28.
  20. **Weingärtner, L., U. Sitka, R. Patsch, and I. Richter.** 1977. Experience with amoxycillin in neonates and premature babies. *Int. J. Clin. Pharmacol.* **15**:184-188.
  21. **Word, B. M., and J. O. Klein.** 1989. Therapy of bacterial sepsis and meningitis in infants and children: 1989 poll of directors of programs in pediatric infectious diseases. *Pediatr. Infect. Dis. J.* **8**:635-637.