Population pharmacokinetics of antibiotics to prevent group B streptococcal disease: from mother to neonate

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Chapter 12

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Summary, conclusions and perspectives.

Introduction

In the peripartum period infections due to group B streptococci (GBS) play an important role in infectious morbidity and mortality. In the 1970s GBS emerged as leading cause of neonatal morbidity and mortality[1]. Neonates suffering from GBS disease usually present with pneumonia, sepsis and / or meningitis. Less appreciated is the fact that GBS can also cause a variety of maternal clinical infections in the peripartum period. Apart from cervicovaginal colonisation, which is usually asymptomatic, GBS can cause urinary tract infections, vulvovaginitis, intra-amniotic infection, mastitis, bacteremia, sepsis, meningitis, endometritis and wound infections. These infections often have an impact on both the mother and her child (as reviewed in **Chapter 3**).

During the last decades strategies to prevent neonatal GBS disease were implemented in several countries[2]. The cornerstone of these preventive measures is the administration of antibiotics shortly before and/or during labor. To recommend the use of these antibiotics one has to weigh the benefits and the unintended consequences. Overall, most incidence figures of culture-proven GBS disease in the first 7 days of life have decreased, suggesting that antibiotics to be effective[2]. However, these incidence figures are influenced by multiple factors[3]. Furthermore, neonatal GBS disease has also been reported in neonates whose mothers did receive antibiotic prophylaxis. Therefore, concerns are raised on the antibiotic prophylaxis itself. A specific question is whether current guidelines recommend the optimal antibiotic dose and dosing interval. Moreover, although unintended consequences for the mother, such as an anaphylactic shock, are rare, nowadays there is growing concern for potential, unintended consequences for the neonate. Antibiotic use during labor might influence the initial bacterial flora in neonates. A changed initial flora might have impact on the development of the immune system of the neonate[4,5]. Meanwhile there is moderate evidence on the efficacy of the antibiotics and little attention for the unintended consequences of antibiotic use during labor, guidelines recommend this prophylaxis to up to 35% of all pregnant women[2] (as reviewed in **Chapter 2**).

To study the efficacy of antibiotics and to search for the optimal dosing regimen used in the prevention of GBS disease, knowledge on the pharmacokinetics is needed. Pharmacokinetics is the discipline that applies mathematical models to explain and predict the time-course of drug concentrations in the body. Important determinants of the time-course of the drug concentrations are the extent and rate of absorption, distribution, metabolism and excretion.

Pharmacokinetic studies in pregnancy: general approach

Antibiotics in the prophylaxis of GBS disease are administered to the mother, while the antibiotics are also meant to protect the fetus. Since fetal antibiotic concentrations are reached from the maternal circulation, adequate concentration-time profiles in the mother are essential and therefore a first issue in pharmacokinetic studies during pregnancy. In pregnant women there are changes in the absorption, distribution, metabolisation and excretion of drugs. For example, in pregnancy the plasma volume increases, while albumin protein binding decreases and the blood flow to the liver as well as the glomerular filtration rate rise as well[6]. Because all these adaptations occur simultaneously and change with advancing gestational age, extrapolation of pharmacokinetic data from non-pregnant individuals is difficult.

When the administered antibiotics reach an adequate concentration-time profile in the pregnant woman, the second issue is the transfer of the antibiotic over the placental barrier. In this way the antibiotic is able to reach the fetus. The rate of transfer over the placental barrier is related to the maternal-fetal concentration gradient and is inversely proportional to the permeability of the placental membrane[7]. The thickness of the placenta increases with gestational age and in various disease states, like diabetes and hypertensive disorders complicating pregnancy[8]. Antibiotic concentrations determined in venous umbilical cord serum samples can provide information on the transfer over the placental barrier. Unfortunately, postpartum blood samples from the umbilical cord can be obtained only once per patient and also at a time point beyond the control of the investigator.

As a third step, the pharmacokinetics in the child need to be studied. This can partly be achieved using blood samples of the arterial umbilical cord after birth and those of the neonate. It is important to realize that there will be differences in pharmacokinetics in the child before and after birth. In the fetus, blood flow towards the mother via the arterial umbilical cord to the placenta will be the main route of excretion of drug from the neonate. After birth, the only routes of excretion of drugs are through the excreting organs of the neonate. Furthermore, the blood flow will redistribute after birth, what might influence the pharmacokinetics.

In conclusion, to study the efficacy of the GBS prophylaxis pharmacokinetics in both mother and neonate has to be determined. In contrast to maternal blood samples, which can be obtained relatively frequently umbilical cord samples can be obtained once for each patient. This will result in unbalanced study groups. Fortunately, pharmacokinetic data can nowadays be analyzed with a so-called "population approach" in computer programs such as NONMEM (Non-linear Mixed effects Modeling). Using this approach, data of the whole population are simultaneously analyzed while taking into account inter-individual and intra-individual variability. Data of unbalanced groups and sparse data can be analyzed in a meaningful manner by using a population analysis[9,10].

While the availability of pharmacokinetic data is an important prequisite to evaluate the prophylaxis, the relationship between pharmacokinetic properties and clinical effect is also very important. During pregnancy and delivery several antibiotics are used. One of the antibiotics frequently used in Europe in the prevention of neonatal GBS disease is amoxicillin. In some hospitals amoxicillin combined with clavulanic acid is used in the prevention of neonatal infection, including the prevention of GBS disease, because this combination is also active against amoxicillin-resistant *Escherichia coli*. When penicillin allergy is encountered, clindamycin is often used as an alternative. The relationship between pharmacokinetic properties, microorganism susceptibility (as indicated by the minimum inhibitory concentration (MIC)) and clinical effects has been increasingly well understood. The efficacy of the penicillins, like amoxicillin, is primarily correlated to the percentage of time that the serum concentration of the unbound drug remains above the MIC (%fT>MIC)[11-13]. Similar to amoxicillin, clindamycin effect is also dependent on exposure. However, its clinical efficacy is more closely related to the area under the concentration curve over the MIC for 24 hours ($fAUC_{0\text{-}24h}$ /MIC) rather than the time that the unbound serum concentration exceeds MIC[14].

As a final step, Monte Carlo Simulations (MCS) can be performed to evaluate differences in the efficacy of various regimens used to prevent GBS disease. In this context MCS is used to evaluate the probability of target attainment (PTA), using the derived pharmacokinetic parameters, data on the concentration-effect relationship and the inter-individual variability[15-17]. In this way, the pharmacokinetic data of both mother and child, combined with the knowledge on the concentration-effect relationship can be used to optimize the antibiotic prophylaxis in clinical practice.

The aim of the research project presented in this thesis was to describe the pharmacokinetics of the antibiotics frequently used during pregnancy and delivery to prevent GBS infection. Because amoxicillin is the antibiotic most frequently used in the Medical Center Haaglanden, amoxicillin has been used as prototype. Furthermore, limited data on the pharmacokinetics of clindamycin and penicillin G were described.

Pharmacokinetics of antibiotics in pregnancy and delivery: Amoxicillin as a prototype

Pregnant women with preterm premature rupture of the membranes (PPROM) are an important group of patients treated with amoxicillin. In these women, the mechanical barrier of the membranes is disturbed and it is believed that bacteria that are normally present in the lower genital tract might ascend to the uterus and

cause intrauterine infection. The immune system of premature neonates is not fully developed and therefore they are even more at risk for infection.

Estimates of the pharmacokinetic parameters of amoxicillin in women with PPROM did not differ significantly from values reported in the literature for non-pregnant individuals (**Chapter 4**). The only difference between the results of our study and previously reported values for non-pregnant individuals were the peak-concentrations. Peak-concentrations reached in pregnant women were lower compared to non-pregnant individuals. This might be explained by an increase of extracellular fluid or the presence of the fetus, placenta and amniotic fluid. There are previous studies on several drugs that do show differences in the pharmacokinetics between pregnant women and non-pregnant individuals[18,19]. Most of these studies included both patients before the onset of and during labor. Patients with PPROM were all included before the onset of labor. One study on the pharmacokinetics of ampicillin found that the terminal half-life was increased in women during labor when compared to pregnant women before the onset of labor[20]. Since the patients in our study were not in labor this could explain why our study did not show differences in pharmacokinetics between non-pregnant individuals and pregnant women. In other words, not the state of pregnancy, but being in labor might influence the pharmacokinetics. And indeed, for amoxicillin we found a minor influence of labor on the pharmacokinetics (**Chapter 5**). The volume of distribution of the peripheral compartment was decreased, but this did not result in a changed terminal half-life. Thus, although we did observe some slight differences, we do not consider them clinically relevant.

Another factor that might influence the pharmacokinetics of amoxicillin is the simultaneous administration of clavulanic acid. Potential interactions between simultaneously administered drugs might be caused by inhibition of metabolic enzymes, competition for protein binding in plasma or changes in renal transporter-system. When amoxicillin and clavulanic acid are administered simultaneously, the activity spectrum of amoxicillin against both gram-positive and gram-negative bacteria is enhanced. Amoxicillin is inactivated by β-lactamases produced by several microorganisms. When clavulanic acid is administered simultaneously it protects the amoxicillin from enzymatic inactivation[21] by irreversibly binding and inhibiting a wide range of these β-lactamases. The intrinsic antibacterial activity of clavulanic acid alone is negligible[21]. In practice different dose ratios of amoxicillin/clavulanic acid have been used. As there are no reports that the efficacy of the these dose ratios are different, the amount of clavulanic acid may not be that critical[22]. Time-concentration profiles of amoxicillin are therefore even more important. Clavulanic acid and amoxicillin have, in part, similar routes of metabolization and excretion. But, the pharmacokinetics of amoxicillin in maternal serum was found not to be influenced by simultaneous administration of clavulanic acid (**Chapter 6**).

In patients with a intra-amniotic infection (IAI) antibiotic treatment will be continued following delivery. In the immediate postpartum period, physiological changes in the mother start to take place. Such changes may have major influences on the pharmacokinetics as has previously been shown for the antiepileptic drug lamotrigine[23]. After delivery the concentration of lamotrigine rises to toxic levels. Therefore knowledge on the pharmacokinetics after delivery is needed. The peripheral volume of distribution of amoxicillin appeared to be decreased during labor, but decreases further immediately after delivery. A decrease in volume of distribution might result in higher concentrations in maternal serum, as has been shown for lamotrigine. However, for amoxicillin toxic concentrations are not reached and the current dosing regimens do not need to be adapted (**Chapter 5**).

For ampicillin/ amoxicillin, two different dosing regimens are described in the literature to prevent neonatal GBS disease. The Centers for Disease Control and Prevention (CDC) recommend an initial dose of 2 gram, and subsequent doses of 1 gram every 4 hours[2]. In the Cochrane Library a dosing regimen of 1 gram every 6 hours is described as the usual regimen[24]. Although the rate of infusion influences the PTA, infusion rates are not described in the current guidelines. Doses of 2 gram amoxicillin are administered by infusion over 30 minutes, whereas doses of 1 gram amoxicillin may be administered by infusion (i.e. over 15 minutes) or as bolus injection. However, our simulation studies suggest that the 2 gram starting dose does not have additional value over a 1 gram dose. In general, a slower infusion rate will increase the PTA and for that reason infusions are preferred over bolus injections. However, bolus injections are in clinical practice more convenient. In our simulation study, the %*f*T>MIC after a bolus injection was only 4% lower compared to an infusion over 15 minutes. Values for the %*f*T>MIC found for both regimens with the antibiotics administered by bolus injections reached adequate concentrations taking into consideration the mean population and the 99% confidential intervals. Therefore, we conclude that bolus injections can be used in these regimens without a reduction in the efficacy (**Chapter 8**).

To recommend a particular dosing regimen, several aspects should be taken into account. Since the initial dose of 2 gram does not have additional value over a 1 gram dose, the only difference between the two regimens is the dosing interval of 4 hours versus 6 hours. Although larger intervals reduce the %*f*T>MIC, both regimens reach adequate maternal concentration-time profiles. However, to guarantee that the efficacy to prevent neonatal GBS disease is not reduced using a 6-hour dosing interval, time-concentration profiles in the fetus should also be taken into account. To remain on the safe side, we recommend a dosing interval of 4 hours. Another argument to recommend a 4-hour dosing regimen instead of a 6-hour dosing interval is the clinical situation in which the prophylaxis has to be administered. The often urgency of care in delivery rooms can easily result in inaccuracies in the administration. Using a 4-hour dosing interval results in a higher

PTA, also when doses are accidentally skipped (**Chapter 8**).

So far, we conclude that the recommended dosing regimens reach adequate concentration-time profiles in maternal serum. Being in labor as well as the co-administration of clavulanic acid does not result in clinically relevant changes in the amoxicillin pharmacokinetics. Since the most important question is whether these regimens are also adequate to prevent neonatal GBS disease, we have also studied the transfer of amoxicillin over the placental barrier and the pharmacokinetics in the fetus.

Antibiotics administered to the mother reach the fetus via the umbilical cord. Venous cord blood reaches the fetus after placental exchange, whereas arterial umbilical cord blood originates from the fetal circulation. Drug concentrations in the arterial umbilical cord serum are therefore representative of the concentrations in the fetus. In the investigation described in **chapter 7** these concentrations have been analyzed together with samples from the neonates taken approximately 20 minutes after birth with a heel puncture. Specifically in this investigation data on the maternal pharmacokinetics, transfer over the placental barrier and pharmacokinetics in the fetus were all analyzed simultaneously in a 5-compartment model. This gave us the unique opportunity to study the different concentration-time profiles in the mother and the fetus in relation to the time. The peak-concentration in fetal serum was lower and delayed compared to the maternal peak-concentration. The peak-concentration in venous umbilical cord serum was lower compared to the maternal peak-concentration, but higher in comparison to the fetal peak-concentration. After similar values for venous umbilical cord and fetal serum were reached, the fetal concentrations exceeded the concentrations in umbilical cord. Considering the population estimates for the pharmacokinetics in the venous umbilical cord and the fetus, the initial infusion of 2 gram amoxicillin is adequate to prevent GBS infection in the fetus (**Chapter 7**). However, data on the inter-individual variability were not included and these results are therefore only valid for the average pregnant women with an average fetus. Therefore no final conclusion on the efficacy of amoxicillin in the prevention of neonatal GBS disease can be drawn from these data.

In clinical practice, the period between the start of the first antibiotic dose and birth is often used as measure of the adequacy of the prophylaxis. A period of less than 4 hours is considered as inadequate and antibiotic therapy is often continued in these neonates. In contrast, when neonates are born more than 4 hours after the start of the first dose, prophylaxis is considered adequate and they are usually not treated with antibiotics. This clinical measure of adequacy is based on two premises: first on the relation between the number of positive blood cultures and the time after a single antibiotic dose and secondly on the number of vertical transmissions from the maternal vagina to the neonatal mucocutaneous surfaces. Both the number of positive blood cultures and the number of vertical transmissions will decrease with time, because a minimum amount of time is needed to eradicate microorganisms.

After the peak-concentration in the fetus has been reached (31 minutes after the maternal peak-concentration) the concentration will decrease. Antibiotic present in the fetus will eradicate microorganisms and this eradication will occur independent from birth. Therefore, the current clinical measure of efficacy is not correlated to the true efficacy of the antibiotic prophylaxis (**Chapter 2 and 7**).

Patients treated with amoxicillin in our study groups were all relatively healthy. One patient was diagnosed with PPROM and suffered from severe vomiting during the first part of the study (**Chapter 9**). Her concentration-time profile during the first amoxicillin administration deviated substantially from the normal profile, but after she stopped vomiting the profile became comparable to the profiles described in chapter 4. As after both antibiotic administrations, the entire doses were absorbed in the body, improper infusion could not explain the difference between the two profiles. We hypothesized that several (patho)physiological changes had occurred, that all influenced the peripheral blood flow and thereby changed the distribution of the amoxicillin. As a possible explanation it was suggested that the arm in which the amoxicillin was infused might act as depot for the amoxicillin. After the blood flow slowly normalizes the amoxicillin is steadily released from the depot. The registration of this profile is unique because blood samples were taken frequently in an acute, emotional and very stressful situation. This indicates that although the pharmacokinetics is quite well described in healthy volunteers and several groups of patients, unexpected differences in the pharmacokinetic profile can occur in (critically) ill patients.

To conclude, for amoxicillin the concentration-time profiles in pregnant patients before the onset of labor, during labor and in the immediate postpartum period were such that the currently advised dosing regimens in the prevention of GBS disease may be considered adequate. The results of the MCS showed that even when a dose is accidentally skipped, the percentage of time the concentration exceeded the MIC for GBS still reached the threshold value. The pharmacokinetic profile for the average pregnant women and the predicted concentration-time profile in the average fetus indicated that the dosing regimen is also adequate in preventing neonatal GBS disease, albeit that in this prediction the inter-individual variability could not be taken into account.

Clindamycin

Clindamycin is often used as an alternative to amoxicillin in the prevention of GBS infections[2]. The efficacy of the clindamycin dosing regimens in this indication should be studied in a similar manner as performed for amoxicillin. However, to date only a limited number of patients treated with clindamycin could be included

in the study. Therefore no final conclusions can be drawn from this study (**Chapter 10**).

With regard to the pharmacokinetics of clindamycin plasma protein binding is a critical factor as it is an important determinant of efficacy. As clindamycin binds largely to alpha-1-acid glycoprotein wide variations in the degree of protein binding may be expected. Most values on the plasma protein binding of clindamycine reported in the literature range between 80-90%[25-27]. The percentage might be decreased in pregnant women, but in patients with an infection or stress it is shown to be increased. Therefore, the protein binding in our patients at risk is not likely to be less than 80%. For the average pregnant women the currently used dosing regimen leads to adequate concentrations assuming that the protein binding will not exceed 80% of the total concentration. However, to recommend a dosing regimen for the entire population of pregnant women, an adequate concentration-time profile for the average women is not sufficient and the inter-individual variability has to be taken into account. When taking into account the 99% confidence intervals, this regimen was shown not to be adequate when protein binding exceeds the value of 80% (**Chapter 10**).

There were insufficient data of the transfer of clindamycin over the placental barrier to incorporate these data in the pharmacokinetic model. Therefore the individual concentrations in umbilical cord serum are presented which ranged from 0.1 mg/L to 4 mg/L. Considering the protein binding, dosing interval of 8 hours and the MIC value for GBS (0.5 mg/L)[28], these data indicate that with the current dosing regimen the exposure of the fetus to clindamycine may be inadequate for the prevention of neonatal GBS disease.

It has been demonstrated that not all women with a penicillin-allergy in their history are actually allergic to penicillins[29,30]. It may therefore be useful to re-evaluate the hypersensitivity to penicillin. It has previously been shown that allergy testing is safe during pregnancy and that penicillins can be used in women with a negative allergy skin test[31]. Until further studies performed demonstrating adequate pharmacokinetics of clindamycin in pregnancy and especially in the arterial umbilical cord or fetus for the prevention of GBS disease in the mother and the infant, allergy skin testing can be performed to avoid the use of clindamycin.

Treatment with penicillin G in premature neonates

In neonates with suspected GBS infection, the administration of antibiotics to the neonate will be started immediately after birth. For several drugs it has been demonstrated that the pharmacokinetics in neonates differs substantially from that in adults[32-34]. Especially in premature neonates the terminal half-life is often prolonged. Similar to this, we found a prolonged terminal half-life of penicillin G

of 3.9 h in neonates with a gestational age of less than 32 weeks on the third day of life. Taking into account the inter-individual variability, the currently used dosing regimen of 50.000 U/kg every 12 h was shown to be adequate for the treatment of neonatal infections caused by common microorganisms on day 3 of life (**Chapter 11**).

The prolonged half-life in (premature) neonates is particularly beneficial for antibiotics with a time-dependent mechanism of action, since the time the concentration remains above the MIC will be increased. The prolonged half-life is most likely caused by immature renal function. This supports our opinion that the clinically used measure of efficacy (i.e. a period of 4 hour between the start of the antibiotic administration and birth) may need to be reconsidered. After the peak concentration in fetal serum has been reached the concentration will decrease. Since the terminal half-life in the fetus is likely to be lower compared to the neonatal half-life, the highest values for the T>MIC will be reached when birth takes place soon after the peak concentration has been reached.

Future perspectives

The frequency of the use of antibiotics in the peripartum period to prevent GBS disease has increased the last decades. This is the result of the change in prevention strategy to a screening-based strategy and an increase in the prevalence of GBS in pregnant women. In the near future more sensitive detection methods are likely to become available. As a result also women with a low number of GBS in their rectovaginal tract will be identified as GBS carrier. It is doubtful whether these women indeed have an increased risk on giving birth to a neonate with GBS disease. Nevertheless since increasing percentages of pregnant women will be candidates for antibiotic prophylaxis, it is important that the dosing regimen is adequate for the entire population. Suboptimal concentrations pose patients at risk for failure of prophylaxis and might cause selection of resistant bacterial strains.

From the data described in this thesis, it is likely that for amoxicillin current dosing regimens are adequate for healthy women, also when minor dosing inaccuracies occur. However, to guarantee that the concentrations reached in the fetus are also adequate, Monte Carlo Simulations should be performed using the 5-compartment model for the simultaneous analysis of the concentration-time profiles in the mother and the fetus, taking into the account the inter-individual variability. In this way, an optimal dosing regimen can be chosen for the entire population. Unfortunately, there are no computer programs available to perform MCS with a 5-compartment model.

Patients treated with amoxicillin included in our study were all healthy, with one exception. This patient was included while she suffered from unexplained

severe vomiting. The pharmacokinetic profile in this patient was dramatically different from profiles in relatively healthy patients. This indicates that physiological changes might have major influences on the pharmacokinetics. In our study group it was found that several physiological factors had an influence on the amoxicillin pharmacokinetics. For example, the amount of edema influenced the volume of distribution. Pregnancy and delivery are sometimes complicated by particular disorders. One of these pregnancy related disorders, preeclampsia, is reknowned for an increased amount of edema. This can add up to 10 liter of extracellular fluid. One can therefore not assume that the concentration-time profiles in women with preeclampsia are also adequate. Therefore, pharmacokinetic studies in patients with pregnancy-related disorders should be performed.

For clindamycin limited pharmacokinetic data are available. Considering high plasma protein binding for clindamycin, it appears that the concentration-time profiles may be inadequate for the prophylaxis of GBS infections in both the mother and the fetus. Only the unbound fraction of the drug is active and crosses the placental barrier and therefore changes in protein binding might influence the efficacy of the clindamycin. It is known that protein binding of clindamycin is dependent both on the serum concentration of alpha1-acid glycoprotein, which changes in pregnant women, and on the serum clindamycin concentration. Unfortunately, relatively large blood samples are needed to determine the protein binding in serum. The volume of the blood samples obtained in our study was too small to determine the protein binding. Patients with chronic hart conditions were included in the study. The pharmacokinetics were found to be unaltered in this patient group. Future studies are needed to prove the efficacy of the current dosing regimen and they should include patients with pregnancy-related disorders and information on the protein binding. Finally, a pharmacokinetic model should be developed based on pharmacokinetic parameter estimates of both the mother and fetus, the inter-individual variability as well as data on the clindamycin protein binding to investigate the predicted efficacy of the different dosing regimens using Monte Carlo Simulation.

In the US, *Eschericia coli* is becoming more common as a cause of neonatal infection, especially in premature neonates[35,36]. Many strains of *E. coli* are resistant to amoxicillin and therefore the use of amoxicillin/clavulanic acid might become more commonly used in the future. We showed that the pharmacokinetics of amoxicillin in pregnant women is not influenced by the simultaneous administration of clavulanic acid. However, for inactivation of β-lactamases by clavulanic acid, there has to be a minimal amount of clavulanic acid present. Therefore, future studies should be performed on the pharmacokinetics of clavulanic acid. This harbors a practical problem. Clavulanic acid is a very unstable compound. Therefore, concentrations of clavulanic acid should preferably be determined immediately after the samples are obtained from the patients and a special HPLC method should be developed to

make sure that the clavulanic acid is not degradated during the procedure. Finally, the development of methods for the stabilization in clavulanic acid in plasma should be explored.

In conclusion, the present study indicates that the currently advised dosing regimen for amoxicillin is effective to prevent neonatal GBS disease. But research on the pharmacokinetics of various antibiotics should be continued for optimization of the GBS disease prophylaxis. Especially, the development of pharmacokinetic models describing the maternal and fetal pharmacokinetics simultaneously as well as their relation in time, taking into account the inter-individual variability and degree of protein binding is of importance. Patients with pregnancy-related disorders should be included in these studies. Also for the currently recommended dosing regimen for clindamycin, the results of current study raise some doubt on the efficacy to prevent neonatal GBS disease. The antibiotics of first choice remain the penicillins. When penicillin allergy is encountered, skin testing is advised to ensure that the use of amoxicillin is contraindicated.

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