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Fatima Mir Date

High dose oral amoxicillin attains pharmacokinetic efficacy endpoints in young infants (0-59 days) with suspected sepsis - a population pharmacokinetic pilot study

By

Fatima Mir

Master of Science

Clinical Research

Harry L. Keyserling, M.D.	John R. Boring, M.D., Ph.D.
Advisor	Committee Member
John E. McGowan, M.D.	Mitch Klein, Ph.D.
Committee Member	Committee Member

Accepted:

Lisa A Tedesco, Ph.D.

Dean of the James T. Laney School of Graduate Studies

Date

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Fatima Mir

FCPS, College of Physicians and Surgeons, Pakistan 2006

MBBS, Khyber Medical College, Peshawar University, Pakistan 1997

Advisor (Emory): Harry Keyserling, M.D.

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ABSTRACT

High dose oral amoxicillin attains pharmacokinetic efficacy endpoints in young infants (0-59 days) with suspected sepsis - a population pharmacokinetic pilot study

By Fatima Mir

Background: WHO recommends hospital admission and parenteral antibiotics for young infants (aged 0-59 days) with serious bacterial illness in developing countries. Oral antibiotics however are an important, though understudied alternative in settings where these recommendations are poorly accepted or implemented. This pharmacokinetic (PK) pilot study assesses efficacy of oral amoxicillin in young infants in Karachi receiving high dose oral amoxicillin and intramuscular gentamicin for sepsis, heretofore unknown in this population.

Objective: To observe serum levels of amoxicillin and assess PK efficacy targets (Time above MIC) in 0-2 month infants with suspected sepsis receiving 75-100mg/kg/dose of oral amoxicillin

Methods: A pilot study using population pharmacokinetic approach with a minimum of three blood samples per subject was considered optimal to determine serum levels of amoxicillin in sick young infants in Karachi. Timepoints for sparse sampling were 0 (before index dose), 2-3 and 6-8 hours (after index dose). Samples were shipped to Department of Clinical Pharmacology, Childrens Mercy Hospital, Kansas, MO for amoxicillin concentrations by HPLC with mass spectrometry.

Results: Amoxicillin levels were determined in 129 sera samples from 60 young infants. Forty-four infants contributing blood at ≥2 of 3 specified timepoints were included in analysis. Mean amoxicillin levels at 2-3 hours (11.6±9.5 mg/L, n=44) and 6-8 hours (16.4±9.3 mg/L, n=20) following the index dose exceeded the susceptibility breakpoint for amoxicillin (2.0mg/L) against resistant *S.pneumoniae* strains. Of 20 infants with 3 serum levels, 7 showed a classic dose-exposure profile, 13 showed delayed excretion. Two of 7 infants with classic time-exposure curves had atypically prolonged half life unexplained by gestation and weight. In remaining children, patient-specific pharmacokinetics could not be determined. Two of 24 had clinical treatment failure. Six of 44 infants had a positive blood culture with predominance of gram positive organisms.

Conclusion: This pilot study generated observational data showing that oral amoxicillin concentrations in newborns following oral administration exceed the susceptibility breakpoint for >50% of a 12-hour dosing interval. A powered study to look into reasons for delayed excretion of oral amoxicillin in our young infants should follow.

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INTRODUCTION

Over the past decade, Pakistan has inched towards the achievement of various Millenium Development Goals (MDG), with a notable success being the decrease in under 5 mortality from 100.5 per 1000 live births in 2000 to 86.5 per 1000 live births in 2010 (1). When it comes to neonates, however, mortality rates remain largely unchanged (45 per 1000 in 2000 to 41 in 2010) (1).

Sepsis, at 26%, still remains one of three major causes of neonatal mortality across the globe (2). Prominent reasons behind poor outcomes of neonates and young infants with sepsis in community settings in Pakistan are numerous. Inadequate training of health care personnel leads to delays in the identification of serious illnesses. In addition, economic and logistic difficulties often impair health care seeking behaviours among the poor. Consequently, completing a 10-14 day long parenteral antibiotic regimen, as recommended by the World Health Organization (WHO), is typically not feasible in primary health care (PHC) clinic settings (3). In addition, difficulties in accessing secondary and tertiary health care facilities among the most underserved populations leads to hospital referral refusal rates as high as 74% (4).

With this understanding, choosing the right drug at the right dose becomes important in ensuring compliance to therapy and clinical success. Incorporating knowledge of local etiological organisms and their susceptibility to easily available, cost-effective, narrow spectrum antibiotics in empiric antibiotic regimen decisions is critical for minimizing inappropriate use of antibiotics, reducing infectious disease morbidity and limiting the emergence of antimicrobial-resistant pathogens in the community. Successful application of these data to clinical practice requires a solid understanding of dose-exposure-response relationships in populations where physical and metabolic peculiarities can affect drug bioavailability and efficacy. Pharmacokinetic profiling therefore, in the population of interest, for the therapeutic agent of interest is extremely important.

In contrast to hospital cohorts from developing countries which show a high prevalence of penicillin-resistance among gram positive organisms, and, penicillin- and aminoglycoside-resistance among gram-negative pathogens (5-14), community-acquired organisms appear relatively susceptible to simple antibiotics. In isolates from umbilical pus in a community omphalitis cohort, 92-100% gram negative organisms and 100% streptococci were susceptible to aminoglycosides and amoxicillin/penicillin, respectively (15). Other community studies from developing countries show similarly low antimicrobial resistance among pathogens (16, 17). Knowledge of pharmacokinetics of common antibiotics used in young infants and its incorporation in empiric regimens for common bacterial infections is therefore essential in keeping these low resistance trends in the community in firm check by minimizing inappropriate or sub-therapeutic treatment regimens.

The examination of antibiotic pharmacokinetics in healthy newborns following oral administration dates back to the mid-1900s. However, the use of oral formulations for serious infections like sepsis, pneumonia or meningitis is a controversial and hotly debated. The general perception is one of inferiority where oral antibiotics are considered to be less bioavailable and, by extension, less effective than those given by parenteral route. However, well designed comparative effectiveness studies are lacking and the effect of serious illness on oral drug bioavailability has not been adequately studied in this age group.

Case management of pneumonia with amoxicillin in developing country community settings like Pakistan (18, 19) has shown that the oral antibiotic strategy can result in substantial reduction in neonatal mortality. Other antibiotics evaluated include co-trimoxazole for neonatal sepsis in India (16) and Pakistan (20) and cephalexin in India (21). Oral antibiotics, when used appropriately, are increasingly regarded as superior to no antibiotics at all (22) and are a research priority as a “back-up plan” for sick young infants in the community who do not have access to parenteral therapy.

Amoxicillin’s safety profile, cost, availability, ease of administration and palatability make it an antibiotic of choice for serious neonatal infections when given concurrently with once-

daily intramuscular gentamicin. Criticism pointing out the lack of adequate anti-staphylococcal coverage with this regimen are answered, in part, by Edmond et al's report detailing the preponderance of *S. pyogenes* and *S.pneumoniae* among gram positive pathogens causing sepsis in young infants (23). Consequently, amoxicillin remains drug of choice for community acquired pneumonia in children above 2 months of age. However, there have been very few assessments of its role in sick newborns and young infants (<2 months old).

In 2009, a randomized controlled trial in Karachi, Pakistan (24) began evaluating the equivalence of penicillin-gentamicin (x 7 days) to a) penicillin-gentamicin (x 2 days) followed by 5 days of oral amoxicillin alone and b) intramuscular gentamicin (x 2 days) with oral amoxicillin (x 7 days) in 0-59 day old young infants with suspected sepsis. Choice of these treatment arms followed a WHO commissioned review of available literature on etiology of neonatal sepsis, antimicrobial resistance among the commonest neonatal sepsis pathogens (such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *E.coli)* and antibiotic pharmacology in neonates (amoxicillin-gentamicin safest and closest simulator of recommended first line ampicillin-gentamicin combination for serious bacterial infections in facility settings) by experts in the field (25).

A review of the literature for pediatric-specific amoxicillin pharmacokinetic parameters (See Annexure 2) reveals that amoxicillin disposition in sick young infants residing in developing countries had not been described. Priors for pharmacokinetic parameters such as renal clearance, protein binding and Time above MIC (minimal inhibitory concentration) were also incompletely described. However, expected age-dependent changes in the disposition of amoxicillin can be inferred from known data on developmental physiology.

Clinical effectiveness data from the Simplified Antibiotic Therapy Trial (SATT) mentioned above does not allow a true estimate of amoxicillin efficacy in newborns with sepsis because of low blood culture positivity rate (9%) in our sepsis trial enrollees, inadequate information on amoxicillin disposition priors in developing country young infants and the 'polluting' effect of concurrent gentamicin administration in this cohort. Amoxicillin absorption from the

young infant gut and clearance from the renal tract has not been specifically described. Absorption and excretion of β-lactams as a whole however, involves proton-coupled peptide transporters (PEPT 1 and 2) whose expression appears to be influenced by ontogeny, concurrent nutrient intake, infection and malnutrition. All these factors likely influence the absorption, distribution, metabolism and excretion (ADME) of β-lactams in sick newborns living in impoverished conditions in Karachi to a different extent than observed in previously healthy newborns from developed countries from where data on drug disposition exists. We therefore conducted this pilot study to evaluate pharmacokinetic parameters of oral amoxicillin in infants randomized to receive the drug orally. Single dose kinetics were assessed by sampling each infant just before receiving the index dose of oral amoxicillin (0 hours) as per WHO weight bands (See Annexure 1) and 2-3, and 6-8 hours of the index dose. Serum samples were collected after appropriate consent from parents, transported to the laboratory where serum was separated and frozen at -70ºC. At the end of the study period (11 months), serum samples of 60 babies (3 each of 21 infants, 2 each of 27, and baseline of 12) were shipped to the Department of Clinical Pharmacology, Children's Mercy Hospital, Kansas, MO where amoxicillin assays were conducted.

Our pilot study aims to add efficacy metrics to clinical effectiveness data on oral amoxicillin in the parent trial by estimating the time (as percentage of dosing interval) amoxicillin remains above the susceptibility breakpoint for *S.pneumoniae* (2µg/ml) in a subset of young infants with sepsis enrolled in the oral amoxicillin-parenteral gentamicin arm (24). If oral amoxicillin achieves efficacy parameters (plasma levels above 2 µg/ml for at least >50% of dosing interval) in most of the young infant participants, we can further study both amoxicillin and gentamicin using higher breakpoints such 8 µg/ml (*S.agalactiae*), assess effect of weight, gestation, feeding frequency and renal clearance on serum levels of drugs and make a strong case for not only oral-parenteral combinations as a safe, baby friendly alternative in situations where hospital referral is refused but also as alternative/second line combination- or mono-therapy for serious infections. With increasing antimicrobial resistance

and the demand for oral antibiotic alternatives even in the very young, understanding of the disposition profiles for common and uncommon antibiotics and other drugs in our infant and pediatric populations becomes imperative.

BACKGROUND

The reasons behind the need to explore oral antibiotics as treatment options for newborn and young infant infections in resource-restricted settings are numerous. Treating an infection effectively involves understanding the infection process and its impact on the individual, the family and the community as a whole. In Pakistan, where the majority of births and neonatal deaths still take place at home, families are often reluctant to seek care outside the home for neonatal illness. Reasons vary from cultural (e.g., confinement after birth, fatalism) to economic (poverty, high cost of care) and logistic (e.g., lack of transportation, lack of quality referral-level care) (15, 26-31). In these settings, oral antibiotic options for serious bacterial infections, whether primary or step down therapy in parenteral to enteral 'switch regimens,' can effectively simplify therapy for newborns and young infants in the community. Selection of empiric oral antibiotics should be dependent upon target organisms and their susceptibility patterns, spectrum of antibiotic activity, drug resistance, drug distribution, therapeutic index, cost of therapy and convenience in administration (22).

Etiology of neonatal sepsis in developing countries

Very few studies focus on the etiology of early onset sepsis (0-6 days) in home-born young infants in developing countries (11, 32-34). Zaidi et al (35) report an overall gram negative predominance (2:1) in a total of 3209 isolates from newborns in the first week of life accounted principally by Klebsiella spp. (25%), *E. coli* (15%), Acinetobacter and Pseudomonas spp. (total of 11.8%). Among the gram positive organisms, *S. aureus* (18%) predominates in this population.

In the 7-59 day age period, there is parity between gram-negative and gram-positive organisms (0.8:1). Pathogens reported in order of importance immediately following the first week of life are *S. aureus*, *S. pneumoniae*, Group B Streptococcus (GBS) and *S. pyogenes* (10-14% each). Gram-negatives of importance are non-typhoidal Salmonella species (13%),

and *E. coli* (9%). Compared to first week of life, the proportion of Salmonella spp., *H. influenzae*, *S. pneumoniae* and *S. pyogenes* is much higher and that of Klebsiella spp is much lower. More recently, Edmond et al highlight preponderance of *S. pyogenes* and *S. pneumoniae* in young infant sepsis (23).

The case for oral antibiotics for neonatal sepsis

In the early 1990s, WHO and UNICEF developed an Integrated Management of Childhood Illness (IMCI) strategy to address the high under-5 mortality in developing countries. The approach focused on improving case management skills of health workers, strengthening health systems and addressing family and community practices. Notably, the original IMCI modules did not include care of the sick newborn during the first week of life (the time when one in three children die) nor did it emphasize home-based newborn care. These modules have now been revised and incorporate a newborn/young infant component (IMNCI). They recommend hospital referral for all babies under 2 months fulfilling criteria for suspected serious bacterial infection (3) largely because of the paucity of pharmacokinetic and clinical effectiveness data for simple antibiotic regimens administered in community settings. Importantly, hospital referral more often than not is rejected by families.

Standard of care for neonatal sepsis

The best initial empiric therapy of a suspected systemic infection in newborns, and young infants, still remains the combination of ampicillin/procaine penicillin and gentamicin in the community or third generation cephalosporins (eg. ceftriaxone or cefotaxime) and aminoglycosides for 10-14 days. These antibiotics are safe and efficacious when administered at extended intervals (twice daily or daily dosing) (3, 20, 26, 36-45). In general ampicillin is preferred to penicillin G due to wider gram-negative activity encompassing *H. influenzae*, *E. coli*, Proteus spp., Salmonella spp., Shigella spp., and, increased activity against Listeria spp.

and enterococci even though Klebsiella spp., a leading cause of early onset sepsis in newborns, is intrinsically resistant to this amino-penicillin.

Meningeal penetration for ampicillin is better than penicillin G but higher doses are required. Co-administration with gentamicin not only provides essential gram-negative coverage but several features make gentamicin an ideal choice for community based administration (30, 31). Its pharmacokinetics are comparable when given by intramuscular (IM) or intravenous (IV) routes, there is significant antibacterial efficacy when given once-daily administration owing to a protracted post-antibiotic effect and a linear concentration-dependent bactericidal effect. Building empiric regimens on the knowledge of indigenous etiology and resistance data is imperative in order to retain the low resistance-rates reported among pathogens from community cohorts.

Clinical Studies where oral antibiotics were used for Neonatal Sepsis

Bhandari et al (21) reported an overall 3.3% case fatality in sick young infants with sepsis and pneumonia treated in outpatient settings in Delhi, India with oral cephalexin. Though hospital referral (standard of care) was refused in 76% of sick young infants (n=273), the low case fatality allows us to infer the clinical effectiveness of cephalexin as oral therapy for sick infants (0-2 months) with serious bacterial infections.

Bang et al (16) reported a 20% reduction in perinatal mortality by using a home-based neonatal care bundle in 39 intervention villages in Gadchiruli district in India (vs. 47 control villages). Though the bundle included sepsis management (oral cotrimoxazole and intramuscular gentamicin daily for 7 days), other components like advice on temperature regulation and breastfeeding ‘contaminated’ clinical effectiveness data of the sepsis regimen and in the absence of pharmacokinetic profiling, cannot be used as adequate evidence supporting use of a potentially dangerous drug (risk of kernicterus due to protein binding) like cotrimoxazole in this specific young population. PK or Population-PK estimates in a real-life scenario such as this would allow better understanding of the pharmacodynamics of an oral

drug like cotrimoxazole, especially in an under-studied population like young infants where drug disposition cannot be extrapolated from adult data; it would also at least partly explain variability in clinical effectiveness of the drug in other regions or populations as seen in a later study in Pakistan by Zaidi et al where significantly higher treatment failure (RR 2.03, 95%CI: 1.09-3.79) and mortality (RR 5.58, 95% CI:1.26-24.72) was reported with daily oral cotrimoxazole and intramuscular gentamicin in sick young infants with sepsis compared to the penicillin-gentamicin combination or ceftriaxone by intention to treat analysis (20). Clinical effectiveness data serves an important purpose in resource-constrained settings by allowing a gross inference of efficacy, however without actual PK parameters defined in specific populations, this is at the cost of external validity (not allowing for population/inter-individual differences in determinants like appropriateness of drug given diagnosis, dosage and dosage and dosing interval, age, gestation, first pass metabolism, renal clearance, etc).

Baqui et al have similarly reported 30-34% reduction in neonatal mortality in village clusters in Sylhet, Bangladesh randomized to receive among other home-based care interventions, procaine penicillin/gentamicin or oral cephalexin/intramuscular amikacin compared to control groups where no antibiotics were given (46). When the absence of culture-proven sepsis is coupled with the sub-optimal negative predictive value of some clinical signs used to diagnose sepsis, treatment success does not necessarily imply drug efficacy. PK data are therefore the best means to optimize drug dose selection and thus efficacy in specific infant populations.

Clinical Studies where oral antibiotics were used for Pneumonia

The fundamental objective of the World Health Organization (WHO)'s Acute Respiratory Infection (ARI) Program in children 2 months to 5 years is to reduce severity and mortality from ARI, especially pneumonia in this age group. Oral antibiotics like co-trimoxazole have been a part of case management of pneumonia in children above 2 months since the 1980s. Success in younger age groups was first reported by Bang et al who showed a reduction in neonatal pneumonia mortality by 44% and all-cause mortality by 20% with the introduction

of cotrimoxazole as home-based therapy (47). In Pakistan, in-vivo effectiveness at 91-92% (48) favored continued use of cotrimoxazole for childhood pneumonia in the community despite reports of increased in-vitro resistance of *H. influenzae* and *S. pneumoniae* (49, 50). More recently, the effectiveness of standard dose amoxicillin has been reported to be comparable to parenteral penicillin (51) resulting in fewer treatment failures than cotrimoxazole in severe pneumonia (52, 53). For non-severe pneumonia however, it shows comparable clinical outcomes to high dose amoxicillin (54), cotrimoxazole (53) and placebo (55).

The importance of supporting clinical effectiveness data with PK studies is highlighted by Fonseca et al who compared amoxicillin pharmacokinetics twice daily (25 mg/kg/dose) with thrice daily (15 mg/kg/dose). These authors found that both dosages were comparable and led to less than 50% of dosing interval below MICs (18).

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Clinical Studies where oral antibiotics were used for Meningitis

B-lactams are generally characterized by a high level of activity against susceptible pathogens, a relatively low toxicity but poor CSF penetration in the absence of meningeal inflammation. Bakken et al classified meningeal inflammation as mild, moderate and severe based on CSF leukocyte and CSF/plasma glucose ratio and demonstrated higher CSF levels of amoxicillin and clavulanate given intravenously in patients with moderate and severe meningeal inflammation in contrast to those with mild inflammation (56). Though no trials evaluate clinical outcomes in neonatal meningitis with oral antibiotics, select pharmacokinetic data in neonates, children and adults supports adequate penetration of oral antibiotics like amoxicillin (57-60) and ofloxacin (61) into the sub-arachnoid space. High dose oral amoxicillin in newborns with suspected sepsis in combination with intramuscular gentamicin may therefore satisfy the PK efficacy surrogates for *H. influenzae* and *S. pneumonia* but should account for changing level of tissue inflammation as treatment progresses.

Tazi-Lakhassi et al (60) described pharmacokinetics of oral amoxicillin in the CSF following intravenous and oral administration at a 150 mg/kg per day or 250 mg/kg per day dosage in the treatment of purulent meningitis. Despite the early introduction of oral therapy and the reduction in dosage following meningococcal and pneumococcal meningitis, no treatment failures could be attributed to this therapeutic regime. This treatment success must however be interpreted with caution and correlated with degree of meningeal inflammation which improves drug penetration but changes with progress of treatment. Though McCracken et al (62) reviewed pharmacokinetic and bacteriologic correlations between an experimental meningitis rabbit model and humans and found the rabbit model useful in predicting penetration of antibiotics in CSF of infants and children with meningitis, he did not address Minimal Bactericidal Concentrations (MBC) (1:8) or MICs in latter phases of treatment when there is minimal meningeal inflammation.

For a summary of clinical studies evaluating parenteral or oral antibiotic therapy for serious young infant bacterial illnesses, see Annex 5.

Population PK versus Classic PK approach

In contrast to traditional pharmacokinetic evaluation, the population PK approach encompasses collection of pharmacokinetic samples from patients representative of target population. By obtaining fewer samples from a larger cohort of patients one should ideally be able to identify factors that explain the inter-individual variability in drug disposition (e.g. demographic, pathophysiological, environmental, etc) (63). With recognition of the importance of developing optimum dosing strategies, (especially important in designing syndromic childhood illness management protocols), use of the population pharmacokinetic approach is increasingly popular even in case of older drugs like amoxicillin where it creates greater understanding of inter-patient variability and therefore appropriate dosing schedules.

Pre-requisite for a well done population PK study is prior knowledge of certain preliminary pharmacokinetic information, the drug's major elimination pathways in humans and the basic pharmacokinetic model of the drug. This is because the sparse data collected during population PK studies may not provide adequate information for discriminating among pharmacokinetic models. In addition, a sensitive and specific assay capable of measuring the parent drug and all metabolites of clinical relevance should be available before a population PK study is undertaken. When properly performed, population PK studies, combined with suitable mathematical/statistical analysis, can be a valid alternative to classical PK studies which require extensive sampling. All of the amoxicillin studies done in neonates so far have been hospital-based and have used the classic PK approach (64-70).

Public Health Implications

Single dose versus multiple dose pharmacokinetics

Single dose pharmacokinetics of amoxicillin in newborns can give a reliable measure of drug efficacy in real-life community settings. PK parameters like maximal concentration (C_{max}), half life ($t_{1/2}$), area under curve (AUC), etc have been found to be similar for most β-lactams after single and multiple doses (71-76). Burkhardt et al in comparing PK of oral linezolid to co-amoxiclav showed excellent agreement between amoxicillin (and clavulanate) on day 1 and 7 serum concentration-time curves (77). Notably, steady state accumulation of drugs can be predicted by their single dose PK profiles.

Pharmacodynamic Surrogate

The singular putative pharmacodynamic surrogate associated with efficacy in β-lactam antibiotics is based upon the duration of time (i.e. fraction of the dosing interval) that circulating plasma concentrations exceed the minimum inhibitory concentration (MIC) of the susceptible pathogen (time above MIC or T>MIC)(78). Adequate T>MIC values for

amoxicillin depend, in part, on target organisms, the age of subject, their underlying immune status, the site of infection, and the extent of protein binding. For our study, we chose the cut-off of >50% T above MIC (79).

Various studies report a range for optimal T>MIC for β-lactams. Barbour et al describe optimal T>MIC for penicillins (gram-negative bacteriacidal effect) as 50–70% of dosing interval (80-82). For gram-positive bacteria, the T>MIC considered adequate is 35–50%. De Hoog et al claim 40-50% T>MIC as adequate for neonatal infections while Van den anker actively argues that since efficacy of amoxicillin is dependent on time the free, non-protein bound drug remains above MIC, the MIC cut-off should be taken as 'several times' higher to correct for protein binding and take in account the relative immaturity of the immune system of neonates (79, 83). His suggestion of 70% T>MIC as appropriate in this age group accounts for both protein bound drug and poor immune status of very young subjects. Pullen et al however, differ in opinion and endorse a lower 40-50%T>MIC as reflective of efficacy due to low protein binding in neonates (10%) (84). Low protein binding has also been reported for ampicillin by Ehrnebo et al (85).

T>MIC cut-offs for β-lactams have been most extensively studied in otitis media determined by eradication of infection from middle ear fluid through tympanocentesis. In general, a T>MIC of 40% achieves an 85-100% bacteriologic cure rate (82, 86, 87). This same cut-off has also been reported as adequate for pneumonia (82, 88). More recent results from a post hoc PD analysis of data from pediatric studies suggested that maintaining a T>MIC in excess of 60% is a better predictor of successful bacteriological eradication with oral amoxicillin (specifically *S. pyogenes* tonsillopharyngitis) (89, 90).

Weinstein et al found that all patients with osteomyelitis who failed therapy had undetectable trough bactericidal titers of β-lactams (91). Slow-growing bacteria in deep infection sites like endocardium and bones may therefore require a much longer T>MIC (85-100%) than the simpler infections discussed above. Though Gras-le guen has reported clinical success with parenteral to oral switch (ampicillin to amoxicillin) in newborns with Group B Streptococcal

(GBS) sepsis, no study has assessed pharmacodynamic surrogates like T>MIC in newborns with sepsis in community settings (67).

Studies of β-lactams in animal infection models have shown mortality was close to 100% if T>MIC was ≤ 20% of the dosing interval but 90-100% survival was reached if T>MIC was ≥ 40-50% of this interval (82). Animal data from a neutropenic murine thigh infection model shows that a reduction of 1 $\log_{10}$ or greater in CFU/thigh at 24 hours is consistently observed when amoxicillin levels exceed the MIC for 25-30% of the dosing intervals (92). Toutain et al reviewed animal model data (92-94) and recommended that T>MIC should be at least 50% and preferably ≥ 80% of the dosing interval to achieve an optimal bactericidal effect (95).

Mechanism of Action

Cell walls of bacteria are essential for their normal growth and development. Peptidoglycan is a heteropolymeric component of the cell wall that provides rigid mechanical stability by virtue of its highly cross-linked latticework structure. The biosynthesis of peptidoglycans in the bacterial cell walls involves approximately 30 enzymes and grossly 3 stages. The first stage ends in dipeptide formation in the cytoplasm. The second stage involves polymer building. The third stage involves cross-linking between peptide polymers. This is accomplished by peptidoglycan glycosyltransferases outside the cell membranes of gram-positive and within the the periplasmic space of gram-negative bacteria. Beta-lactam antibiotics inhibit this last step in peptidoglycation. Further targets for β-lactams in addition to the transpeptidase involved in stage 3 of peptidoglycation, are proteins termed collectively as penicillin-binding proteins (PBPs). β-lactams' disruption of PBP-mediated peptidoglycan assembly results in autolysis (96, 97)

Pharmacokinetics of Amoxicillin

The four most important parameters governing drug disposition (collective term for the processes of absorption, distribution, metabolism and elimination) are bioavailability (the fraction of the drug absorbed as such into the systemic circulation), volume of distribution (a measure of the apparent space in the body available to contain the drug based on how much is given versus what is found in the systemic circulation), clearance (a measure of the body s efficiency in eliminating drug from the systemic circulation) and elimination (a measure of the rate of removal of drug from the systemic circulation). For a tabular review of pharmacokinetic priors from studies on amoxicillin, see Annex 4.

Systemic bioavailability becomes an important consideration when amoxicillin is administered via extra-vascular routes. Where plasma concentrations can be measured

directly, it is determined by comparing the area under the plasma concentration versus time curve (AUC) after administration of an oral dose with the AUC after administration of an intravenous dose ($F=AUC_{PO} * dose_{IV} / AUC_{IV} * dose_{PO}$) (98). It can also be determined indirectly by comparing AUCs of active metabolites via intravascular and extravascular routes, and measurement of drug excreted in urine or other body fluids (99). Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation e.g. whether a drug is taken with or without food, other drugs taken concurrently, intestinal motility and disease states which affect liver metabolism or gastrointestinal function. Most studies have found the effect of food on amoxicillin absorption to be insignificant (100, 101). Amoxicillin bioavailability has been reported as dose-dependent (plasma concentration does not increase or decrease proportionally with dose) due to non-linearity or limited capacity at the absorption level of kinetics (A of ADME) (102, 103). Bioavailability (F) of oral amoxicillin reported in literature ranges from 1 (as good as with intravenous route) in older infants and children (104) to 0.51±0.05- 0.75 in adults (102, 105) (Annexure 4)

Absorption after oral administration of amoxicillin increases and improves from initial week to end of neonatal period to older infancy; during these first 2 months, gastric pH drops from >5 to 2-4, gastric emptying time increases, intestinal surface area and motility increases, biliary function, splanchnic blood supply and microbial colonization in the gut increase to near adult levels (106). Amoxicillin suspension is acid labile. Carrier-mediated active uptake plays an important role in intestinal absorption of β-lactams (107), particularly oligopeptide transporter PepT1 (108, 109) expressed on the brush border membrane of the enterocytes. However, its involvement in amoxicillin absorption is inadequately described: saturable absorption kinetics of amoxicillin (102) support carrier mediated transport, insignificant effect of feeding on amoxicillin plasma levels (100, 101) despite milk-derived peptides being the 'natural' substrates for intestinal PEPT1 suggest alternate transporter mechanism.

Excretion of amoxicillin is primarily through the kidneys. Contribution of tubular secretion in its excretory process is suggested by its renal clearance exceeding glomerular filteration rate (GFR), significant reduction in renal clearance of amoxicillin with the use of probenicid (a classic inhibitor of renal organic anion transport system) (110), and demonstration of involvement of human peptide transporter 2 (hPepT2) and to a lesser extent human peptide transporter 1 (hPepT1) (situated in basolateral and apical membranes of proximal renal tubules respectively) in amoxicillin secretion by Li et al (108). Carrier-mediated transport is also supported by a 'genetic component' accounting for variation in renal clearance of amoxicillin (111). At birth, the kidneys are anatomically and functionally immature; though nephrogenesis is complete by 36 weeks of gestation, glomerular function defined as GFR at birth is 2-4 ml/min/1.73 m^2 in term neonates (compared to 130 ml/min/1.73 m^2 in an average adult), it doubles by 1 week of age and reaches adult values by 1 year of age. Tubular function matures later than glomerular function but also reaches adult values by 1 year of age. Drug transporters such as renal organic anion transporters (OATs) known to be involved in β-lactam excretion have been indirectly shown to have low functional capacity at birth which increases over first few weeks of life and then declines to adult values (112-114).

Volume of distribution (VD) represents the apparent hypothetical volume of total body water (TBW) that drug appears to distribute into to produce a drug concentration equal to that in the blood (VD=Dose/ Cp^0 where Cp^0 represents the highest attainable plasma concentration after the administration of a single dose). Volume of Distribution (VD) of oral amoxicillin reported in literature ranges from 0.62±0.23 to 1.4±1.13 in older infants and children (66, 115) to 0.2-0.49 L/kg in adults (103) (See Annexure 2). Total body water when expressed as a percentage of total body weight is influenced by ontogeny; it decreases from 78% at birth to 60% at 1 year of age with a corresponding decrease in extracellular water (ECF) from 45% to 27%; intracellular water (ICF) increases from 34% to 43% in the first 3 months and comprises a higher percentage of the body weight than ECF till 1 year of age when it decreases to 35%. Adult body composition is finally achieved by puberty (116-118).

Clearance (CL) is hypothetically the volume of blood which is cleared of unmetabolized amoxicillin per unit time by any or all pathways possible for drug removal (hepatic, renal, sweat, breast milk etc). CL may be represented as total body clearance, renal clearance (CL_{ren}) or non-renal clearance (CL_{nr}). It can be determined by knowledge of dose and AUC (CL=dose (mg/kg)/AUC (mg/L*hr). Renal clearance requires quantitative 24 hour urine collection to determine amount of drug excreted unchanged. Clearance (CL) reported in literature for newborns and young infants ranges from 0.064 – 0.21L/h (84, 119).

Elimination half life (*t1/2*) of a drug is the time necessary to reduce the drug concentration (in blood serum or plasma) by 50% after absorption is complete and distribution between body compartments has attained equilibrium. Loss of drug from the body ($t_{1/2}$) reflects elimination of the administered parent drug molecule (i.e. not its metabolites) by metabolism, renal excretion, or other pathways capable of elimination of drug. Practically the elimination half-life can be used to determine the period of time required for a drug dosing regimen to produce steady-state plasma concentrations (5* $t_{1/2}$) and the dosing interval required to produce a desired excursion (i.e. peak and trough) in plasma drug concentration. Elimination half life ($t_{1/2}$**)** reported in literature for newborns and young infants ranges from 4.28 ± 2.4 (120) to 6.7±1.7 h (68). This is considerably longer than the 0.94-1.2 hours reported in adults (See Annex 4)

Indications

Oral amoxicillin in children >2 months of age is treatment of choice for community-acquired pneumonia and otitis media. Its use in babies <2 months has not been recommended in the past due to inadequate pharmacokinetic studies in this demographic evaluating ADME of amoxicillin. Evaluation of clinical effectiveness of amoxicillin (among other oral options) in combination with gentamicin for serious bacterial infections (22) in resource-poor settings has led to this amoxicillin pilot PK study in 0-2 month old babies with sepsis.

Resistance

Although all bacteria with cell walls contain PBPs, β-lactam antibiotics cannot kill or even inhibit all bacteria because bacteria can be resistant to these agents by myriad mechanisms.

A microorganism can be intrinsically resistant due to structural differences in the PBPs which are targets of these drugs. Because β-lactams inhibit many different PBPs in a single bacterium, the affinity of many PBPs for the antibiotics in question must decrease in order for the microorganism to be resistant.

Resistance due to altered PBPs with decreased affinity for β-lactams can be acquired by homologous recombination between PBP genes of different bacterial species. Other instances of bacterial resistance are caused by the inability of the agent to penetrate to its site of action.eg *P. aeruginosa* which is intrinsically resistant to a wide variety of antibiotics because it lacks classic high-permeability porins (aqueous protein channels in the outer membrane).

Bacteria can also destroy β-lactams enzymatically. Most bacteria produce only one kind of enzyme. Penicillinases and cephalosorinases are narrow-spectrum. 'Extended spectrum' β-lactamases are less discriminant and can hydrolyze a wide variety of β-lactam antibiotics.

β-lactamases can be divided into 4 classes: Class A includes extended spectrum β-lactamases which degrade penicillins, some cephalosporins and in some instances carbapenems. This class includes the worrisome KPC-carbapenemase which is rapidly emerging in Enterobacteriaciae and confers resistance to carbapenems, penicillins and all extended spectrum cephalosporins. Class B β-lactamases are Zn+-dependent enzymes that destroy all β-lactams except aztreonam, whereas Class C are active against cephalosporins. Class D includes cloxacillin-degrading enzymes (121).

In general gram-positive bacteria produce and secrete a large amount of β-lactamases, most of which are penicillinases. The information for staphylococcal penicillinase is encoded in a plasmid and may be transferred by bacteriophage to other bacteria.

Amoxicillin is effective against gram-positive (enterococci, β-lactamase-negative staphylococci, *S. pneumoniae and* α- and β-hemolytic streptococci) and some gram-negative organisms (β-lactamase-negative *E. coli*, *H. influenzae*, *N. gonorrhoeae*, Proteus spp. and *H. pylori*).

Dosing

Doses of oral formulation have varied from 15 mg/kg/dose three times a day (18) to 100 mg/kg/dose twice a day in newborns and infants (67). Higher doses have been associated with greater T>MIC and clinical success.

Dosage Forms

Amoxicillin is available in suspension, tablet and infusion forms.

Safety and adverse effects

Safety of intravenous and oral amoxicillin in newborns has been established. Common adverse effects of amoxicillin are the same as those with other penicillins: nausea, vomiting, or diarrhoea. Prolonged or repeated use may result in thrush. Serious but extremely rare side effects include dark urine, persistent nausea or vomiting, stomach/abdominal pain, yellowing eyes or skin, easy bruising or bleeding, persistent sore throat or fever.

Allergic reactions may include rash, itching/swelling (especially of the face/tongue/throat) with difficulty in breathing. Amoxicillin commonly may cause a mild rash, however since viral exanthems are often mistaken for amoxicillin rashes, this may be an overstatement of the frequency of true amoxicillin-related rash.

Pharmacokinetic Studies of Parenteral amoxicillin in newborns and young infants

Pullen et al evaluated plasma amoxicillin levels and T >MIC in 150 neonates after a second dose of intravenous amoxicillin and found toxic plasma concentrations (peak levels >140mg/L earlier associated with neurotoxicity) in many newborns (clinical status not

reported). They proposed a lower dose of 15 mg/kg q8h and 20 mg/kg q8h for neonates with GA ≤ 34 weeks and >34 weeks respectively after simulation with this new dosage regimen showed achievement of satisfactory plasma levels (69). Huisman et al conducted multiple-dose pharmacokinetics of intravenous amoxicillin in preterm neonates <32 weeks of gestation and concluded that a dose of 25 mg/kg q12hourly resulted in serum levels well above MIC for major micro-organisms involved in neonatal infections (68). Adrianzen et al observed a decrease in mean plasma concentration of amoxicillin by 36%, from 73 to 47 mg/mL due to cardio pulmonary bypass surgery (64).

Pacifici et al's (122) review of studies on penicillin pharmacokinetics in neonates pooled data from 6 amoxicillin PK studies. Preterm infants (<32 weeks GA) had a longer half life (6.7 h) than term infants (3.7 h) and neonates. Clearance was reduced and half life prolonged in the neonate compared to the mature infant. Pullen et al looked at the effects of postnatal age and found that the clearance was greater in neonates > 9 days (3ml/min/kg) than had been shown in neonates of a similar gestation (1.6ml/min/kg) (69).

Pharmacokinetic Studies of Oral amoxicillin in newborns and young infants

Gras le Guen (67) demonstrated clinical success (median trough levels 25.8 and 35.15 mg/L well above the target trough level of ≥ 5mg/L) in 222 full-term neonates with definite or possible group B streptococcal infection who received oral amoxicillin after 48 hours of intravenous ampicillin therapy at high doses of 200 or 300mg/kg/day in 4 divided doses.

Alexander et al (115) reviewed pharmacokinetic data on oral amoxicillin in pediatric patients for post-exposure prophylaxis against anthrax and found doses of 45 mg/kg/day given orally to children <40kg in weight achieved 70-100% T > MIC for susceptible *B. anthracis* (≤ 0.5 mg/mL).

Autret et al studied pharmacokinetics of intravenous and oral amoxicillin given at 40 mg/kg/dose q12h. Average levels of plasma amoxicillin with IV and oral routes were not different except at 0.5 hours where they were higher with the IV route (120).

Pharmacokinetic Studies on aminoglycosides co-administered with oral β-lactams in young infants

Testa et al (123) observed a strong inter-individual and intra-individual variability in 68 neonates, recommending therapeutic drug monitoring especially in preterms where aminoglycoside clearance is reduced leading to higher trough levels. Charles et al looked at the effects of gentamicin on amoxicillin in 40 preterm neonates (<32 weeks) and found that there was a significant decrease of 25% in amoxicillin when co-administered with gentamicin. They also found a longer half life of amoxicillin in preterm babies with gentamicin (6.9h) than without (5.2h) (66).

METHODOLOGY

Objectives

- To determine if pharmacokinetic 'surrogate' for efficacy (≥50% T> MIC2.0[†]) of oral amoxicillin can be achieved in young infants (0-59 days) with suspected sepsis when given at a dose of 75-100mg/kg/dose twice daily
- To describe disposition kinetics of oral amoxicillin in young infants suspected of sepsis in primary health-care settings in Karachi, Pakistan

Null Hypothesis:

- Twice daily high dose oral amoxicillin (75-100 mg/kg/dose) does not achieve pharmacokinetic 'surrogate' for efficacy (≥50% time above MIC2.0 for free drug) in young infants (0-59days) with suspected sepsis

Study Design

This was a population-pharmacokinetic study of children enrolled in Group B of the following ongoing, randomized controlled trial (RCT) at Aga Khan University in Karachi, Pakistan:

> ''Simplified Antibiotic Regimens for the Management of Sepsis in Young infants in First level Facilities: Randomized Controlled Trial' (SATT) (24)

SATT was designed to evaluate the equivalence of simpler regimens for neonatal sepsis with the standard of care regimen (parenteral penicillin and gentamicin for 7 days) in community settings. One of the regimens being evaluated (regimen B) is a combination of twice-daily oral amoxicillin for 7 days with once daily intramuscular gentamicin for 2 days. The trial is

[†] MIC2.0 based on breakpoints for resistant *S.pneumoniae*

designed to address the question of whether oral amoxicillin is a feasible replacement for parenteral penicillin/amoxicillin in 'sick' newborns and young infants (<2 months) where parenteral therapy and/or hospital referral is refused. Advantages of the combination in regimen B include a decrease in the total number of daily injections from three to one and easier delivery by primary healthcare centres (PHC).

Study Subjects

Neonates, with 'signs' of sepsis, were identified by active surveillance (Routine peri-partum and postnatal household visits by Community Health Workers in research sites) and self-referral at one of five low-income peri-urban PHC centers run by the Department of Pediatrics and Child Health at Aga Khan University (community sites: Rehri Goth, Bilal Colony, Ibrahim Haidery, Ali Akber Shah Goth and Bhains Colony). Enrollment in the PK study occurred November 1, 2010 to September 30, 2011. A total of 216 infants enrolled in Arm B of the parent SATT study during this time were eligible for recruitment in this pharmacokinetic study. Inclusion and exclusion criteria for this nested study were the same as those of parent study detailed below.

Selection Criteria for oral amoxicillin population-pharmacokinetic study:

Inclusion Criteria

1. One or more 'signs' of sepsis
    a. respiratory rate>60 breaths/minute
    b. temperature <35.5ºC or >38ºC
    c. difficulty feeding
    d. moving only when stimulated
    e. abdominal distension
2. Randomized to Group B of SATT

3. Willing to provide permission for the collection of ≤ three additional blood draws at pre-specified time points.

Exclusion Criteria

1. Serious illness
    a. convulsions
    b. bulging anterior fontanelle
    c. loss of consciousness
2. willingness to accept a hospital referral
3. refusal to participate in SATT

Patients fulfilling eligibility criteria were selected by non-probability purposive sampling. A 1 mL whole blood sample was obtained from each participant prior to amoxicillin administration, 2-3 hours post-dose, and 6-8 hours post-dose. Wherever possible, these blood draws were scheduled to coincide with clinical blood draws (blood culture for parent study). Participants were requested to remain at the PHC, if convenient, for the 2-3 hour sampling. Patients who were unwilling or unable to remain at the center were sent home after the baseline blood draw and retrieved at the time of subsequent sampling by a study vehicle. Families refusing to return for subsequent venepunctures were allowed to withdraw from study. At one PHC clinic (no evening shift), the PI and phlebotomist travelled to the infants' homes for collection of 6-8 hour samples.

Blood samples (0.8-1 mL) were collected through a 22 gauge butterfly cannula into a lavender topped EDTA collection tube (BD, Becton, Dickinson and Company) and transported to Infectious Disease Research Laboratory (IDRL), Aga Khan University, Karachi at 4ºC. Blood was centrifuged, separated, and the plasma frozen at -70ºC in cryovials. Samples were shipped on dry ice to the Department of Clinical Pharmacology at Children's

Mercy Hospital, Kansas City, MO. Annex 1 shows a flow diagram elaborating the venepuncture schedule. **Error! Not a valid bookmark self-reference.** shows study flow.

Variables of Interest

Predictor Variables

Demographic variables included; postnatal age at enrolment (continuous: days, categorical:0-6,7-27 and 28-59), gestational age (continuous: weeks, categorical: <37 weeks and >37 weeks), gender, weight (continuous: grams, categorical: <2.5kg, >2.5kg), length (cm), fronto-occipital circumference (FOC) (cm), body surface area (BSA, m^2) (mostellar formula=([Height(cm) x Weight(kg)]/3600)^{½}, maternal age (years), maternal weight (kg), and maternal and infant drug history (categorical: antibiotics, painkillers, none).

Clinical variables included history of risk factors of early onset sepsis (mode of delivery, place of delivery, level of birth attendant, appearance of liquor, premature rupture of membranes, immediate cry, previous neonatal deaths, gravidity, parity, tetanus toxoid status), presence or absence of signs of sepsis in the neonate/young infant (fever/hypothermia, poor feeding, convulsions, decreased consciousness and chest indrawing), presence or absence of associated symptoms in the young infant (vomiting, diarrhea, cough, cold, lethargy, blood in stool, umbilical discharge, diaper dermatitis etc) (categorical: absent/present) on day 1-7 of treatment, and day 10 and 14 (scheduled follow up as per protocol), blood/umbilical/nasopharyngeal/rectal culture results (if sent), treatment failure (presence/absence) and day of treatment failure and/or mortality.

Pharmacokinetic variables included WHO weight band (categorical: See Annexure 1), dose of amoxicillin per unit weight (mg per kg per patient), time of second and third sampling beyond baseline (minutes), time interval between sampling and the most recent feed (minutes), feed intensity before samplings (frequency of feeds reported by mother between baseline and second and second and third sampling), co-administration of gentamicin with amoxicillin

(categorical: yes, no); amoxicillin blood levels (mcg/mL=mg/L) at different time-points (zero, 2-3 and 6-8 hour time points).

Outcome Variables

The primary outcome variable (>50%T>MIC2.0) was binary (achievers, non-achievers). As we could not estimate this outcome parameter in all patients, alternate outcome variables assessed for associations with historical predictors listed above included delayed excretion, clinical failure (definition as per SATT protocol) and culture proven sepsis.

Sample size

Two age groups which are used to stratify differences in PK parameters as a function of renal, gastrointestinal and hepatic maturation are newborns (0-27 days) and older infants (28-59 days). An arbitrary figure of 20% difference in proportion of babies achieving >50%T>MIC 2.0 was considered as representative of amoxicillin efficacy in the two age groups of interest. We assumed decrease in %T>MIC with increasing age due to maturation of renal function and increases in protein binding of amoxicillin. Sample size of the study was calculated with hypothesis tests for two population proportions (two-sided test) using WHO Sample Size Calculator (Sample Size Determination in Health Studies, Software version by KC Lun and Peter Chiam, University of Singapore). Anticipating 90% proportion of population 1 (0-27 days) and 70% proportion of population 2 (28-59 days) to achieve PK surrogate of efficacy (>50%Time above MIC), with 80% power and 0.05 level of significance, a total sample size of 62 (31 subjects per arm) was calculated.

	0-27 days	28-59 days	Total
Sample size targeted	31	31	62
Sample size achieved	29	15	44

Study Antibiotic

Amoxicillin trihydrate was supplied by Glaxo Smith Kline (Sindh, Pakistan). For dosage per weight band, see Annex 3.

Amoxicillin Assay Method Development and Validation

Reagents

Amoxicillin was purchased from Sigma-Aldrich (St. Louis, MO) and had a potency of ≥900 µg/mg. Optima LC/MS grade acetonitrile and formic acid was purchased from Fisher Scientific (Fairlawn, NJ). Cefotaxime sodium (> 99%) was obtained from Hanmi Pharmaceutical Co, LTD (Seoul, S. Korea). Ultra-pure, deionized water was prepared using a Barnstead E-Pure™ water system (Dubuque, IA). Drug-free pooled human plasma (n=5 donors) was purchased from Bioreclamation (Westbury, NY) and used as blank plasma. Plasma was aliquoted and stored at -80ºC until use. All other reagents were of analytical grade.

QC samples and standard solutions

A standard stock solution of amoxicillin was prepared by dissolving the required amount of authentic standard in ultra-pure water to achieve a 1 mg/mL concentration. Intermediate stock solutions were prepared by dissolving various amounts of the standard stock solution in ultra-pure water, resulting in eight working standard solutions spanning the range from 5 to 1000 µM. Calibration curves were prepared by diluting one part working standard with 9 parts drug-free human plasma (or ultra-pure water in experiments designed to assess extraction efficiency). Quality control solutions were prepared similarly, with working standard concentrations of 0.5, 1.5, 40 and 80 µM. All standard solutions were stored at -80ºC until use. A stock solution of the internal standard, cefotaxime, was prepared by dissolving standard in ultra-pure water to achieve a 1 mg/ml concentration. Prior to analyses, a freshly

prepared working internal standard solution was prepared by adding 1 part cefotaxime stock solution to 99 parts chilled acetonitrile.

Sample Preparation

To 20 µL of plasma, 10 µL of the internal standard and 70 µL of ice-cold acetonitrile were added. The sample was vortex-mixed for 10 seconds (s). After centrifugation at 16,000 *g* for 10 min, 50 µL of the supernatant was transferred to a clean glass test tube. 450 µL of ultra-pure water was added to the tube, vortex-mixed for 10 s, followed by the addition of 500 µL of dichloromethane, and vortex-mixed for an additional 20 s. Following centrifugation at 3200 *g* for 15 min at 4ºC, an aliquot of the aqueous supernatant was transferred to a glass autosampler vial, placed in the autosampler tray and maintained at 4ºC until analysis.

HPLC/MS conditions

The concentration of amoxicillin in plasma samples was determined by a reverse-phase HPLC/MS method, adapted from the UPLC/MS/MS method of Ahsman *et al* (reference). Amoxicillin and cefotaxime (internal standard) were resolved on a reversed-phase Phenomenex (Torrance, CA) Luna^{TM} C-8 (2) column (4.6 x 150 mm, 5 µm particle size) preceded by a Phenomenex C-8 guard column (4 mm x 3mm i.d., 5 µm particle size) using a Hewlett Packard 1100 HPLC system equipped with a HP1100 de-gasser, binary pump, thermostatted auto-sampler, column heater (Hewlett Packard Instruments, Santa Clara, CA) and an Agilent 1946D single quadrupole mass spectral detector (Wilmington, DE, USA). The mobile phase consisted of 0.1% aqueous formic acid (75%) and acetonitrile (25%) and was delivered at a constant flow of 0.5 mL/min. The column temperature was maintained at 30ºC. A 2 µL aliquot of each sample was injected onto the column: plasma samples were analyzed in duplicate. Under these conditions, amoxicillin and the internal standard (cefotaxime) eluted at ~2.8, and 8.8 min, respectively.

Analytes were detected by electrospray ionization detection with the mass spectrometer operating in a selective positive ion-monitoring mode. Ion detection was optimized for the detection of amoxicillin. The drying gas temperature and flow were maintained at 300ºC and 10 L/min, respectively, the nebulizer pressure was set at 30 psig, the vaporizer temperature was maintained at 400ºC, and the capillary voltage was set at 3 kV. Under these conditions, amoxicillin and cefotaxime yielded $[MH]^+$ ions at *m/z* 366.1 and 456.0, respectively.

Data analysis

Data were collected and integrated with Agilent Chemstation 32 bit V B.03.01 software. Amoxicillin plasma concentrations were quantified by comparison of their peak areas (determined by mass spectral analysis) with nominal concentrations of analytical standards. The resulting concentrations were then normalized using the ratio of the area of the internal standard present in the sample to the area of the internal standard present in blank standards of spiked drug-free human plasma. Standard curves were constructed over the concentration range (0.5 -100 µM) using least-squares regression (Figure 4).

Validation

Validation of the assay was based on the FDA guidelines for bio-analytical method validation. Because the method of Ahsman *et al.,* (which was the basis for the method developed here) was thoroughly validated using procedures based on the same guidelines (CDER), an abbreviated set of procedures (described below) was used to validate the method for the purposes of this study. Additional information regarding amoxicillin stability in aqueous solutions has also been demonstrated by Lugoboni *et al.,* and Yoon *et al.*

i) *Specificity and selectivity* – Chromatograms for aqueous calibration standards (0.2 to 50 µM) were compared to those of six samples of adult drug-free human plasma, pooled adult drug-free human plasma (pooled from the plasma of 5 additional subjects) and nine samples of neonatal drug-free human plasma. Noise levels were

established as the maximum interference (based on peak area) at the retention time for amoxicillin.

ii) *Limits of detection and quantification* – The limit of detection was established as the concentration producing a signal to noise ratio (S/N) = 3 and the limit of detection was established as the concentration producing a signal to noise ratio (S/N) = 5. The limit of detection was determined to be 0.6 pmol (0.3 µM) and the lower limit of quantification for the assay was 1.0 pmol (0.5 µM). The upper limit of quantitation was not determined, hence for this study the effective upper limit of quantification was the highest standard analyzed (100 µM).

iii) *Calibration curves* – The linearity of the method was assessed with calibration curves freshly prepared on each day of analysis. Curves consisted of eight standard concentrations (0.5, 1, 2, 5, 10, 20, 50 and 100 µM) with each point consisting of at least duplicate measurements. A best fit line was determined by linear regression. The analytical method was linear over the standard concentration range used in these experiments, *i.e*., 500 nM to 100 µM ($r^2 > 0.999$).

iv) *Accuracy and precision* – Accuracy was assessed by performing six replicate determinations from the same vial for each of the analytical and QC standard concentrations on three different days. Precision was determined by performing five replicate determinations from individual aliquots of each of the analytical and QC standards. Precision experiments were also conducted on three separate days. Intra-day accuracy and precision deviations ranged from 92-112% and 89-109%, respectively and the CVs for both parameters were <6%. Inter-day accuracy and precision deviations were similar and ranged from 99-105% and 96-102%, respectively with CVs <5% and <10% for accuracy and precision determinations, respectively. All accuracy and precision determinations fell within acceptability criteria (nominal value ± 15%, except for the LOQ which may deviate by 20%).

v) *Extraction efficiency* – The extraction efficiency of the method was evaluated by comparing values for calibration standards (n = 8) dissolved in water with those dissolved in plasma followed by protein precipitation and extraction. Amoxicillin recovery ranged between 87 and 106% among the eight standard concentrations.

vi) *Autosampler stability* – Post-preparative stability was assessed by analyzing extracted calibration curve standards after 12 and 24 hours of storage at 4ºC in the autosampler. The amoxicillin concentrations analyzed in this study were stable for at least 24 hours in the autosampler. Amoxicillin AUCs (area under the curve) ranged from 101 to 114% and 98 to 106% of initial values after 12 and 24 hours, respectively.

Quality Assurance

Mean amoxicillin assay results were double-checked for inaccuracies and correlated with re-running assays in duplicate aliquots shipped to Kansas City from IDRL, Karachi. Four results from one of two study sites were excluded from analysis (5-02, 5-06, 5-07 and 5-09). During quality check visits, we found a protocol deviation in 5-09. Baseline sample was halved into two aliquots, one labeled baseline, and the other 2-3 hour sample. The sample was flagged in our records and sent for analysis. Personnel were counseled and re-trained. Results for three more samples collected at this same center prior to remediation also showed zero levels of amoxicillin at 2-3 hour time point. All four suspect samples were excluded from analysis. Similar results in other center were included due to authentication and error-free quality checks.

Data Analysis

Summary statistics in the form of frequencies for categorical variables and Mean ± S.D for continuous variables were tabulated in Table 2. Twenty of 44 babies gave three blood samples (baseline, 2-3 hour and 6-8 hour of index dose). Primary outcome variable (>50%T>MIC) was assessable for 7 of 20 only. Remaining 13/20 showed delayed excretion with upward

time-concentration curve at 6-8 hour timepoint; 24 with only one datapoint beyond baseline did not allow assumptions about elimination kinetics. Outcome variables were therefore re-grouped based on elimination pattern observed: appropriate elimination kinetics (descending arm of time-concentration curve at 6-8 hours of index dose) (n=7), delayed elimination (n=13) (ascending arm of time-concentration curve at 6-8 hours of index dose) and unknown status (n=24) (time concentration curves showing 2-3 hour datapoint only with no permutation possible for the 6-8 hour timepoint).

To assess association between various predictors and outcome variables based on elimination kinetics status (appropriate, delayed, undetermined), chi square and 95% CI of mean difference was used (Tables 3 and 4). All three outcome groups were comparable.

Individual time versus concentration graphs were plotted for each patient. Three patterns of graphs emerged:

1) Classic time versus concentration curve (with one datapoint each on the ascending (absorption) and descending (elimination) arms, allowing estimation of elimination kinetics) (n=7) (Figure 1)

2) Delayed excretion of amoxicillin (interpreted by still increasing concentrations at 6-8 hours of index doses; no datapoint on elimination curve disallows estimation of elimination kinetics) (n=13) (Figure 2)

Permutation 1 and 2 compare observed time-concentration curve with two hypothetical curves, one showing peak between the 2nd and 3rd timepoint, one showing peak after 3rd timepoint.

3) Undetermined kinetics (no assumption about excretion or elimination kinetics possible due to no information beyond 2-3 hours of index dose) (n=24) (Figure 3)

Permutated Amoxicillin levels 1 and 2 compare observed time-concentration curve with hypothetical curves, one showing downgoing curve after 2nd timepoint, one showing peaking beyond second timepoint and then drop to trough level.

In 7 patients where the time-concentration graph showed a descending curve, we calculated

a) elimination constants: using the formula: $K_{el}= (lnC_1 – lnC_2/(t_2-t_1)$ where ln=natural log, C_1 = concentration at first timepoint after baseline i.e. 2-3 hours of index dose, C_2= concentration at second timepoint after baseline i.e. 6-8 hours of index dose, t_2=time lapse between baseline and C_2 sampling (in hours), t_1= time lapse between baseline and C_1 sampling (in hours). Units of Kel (mcg per ml per hour)
b) half life ($t_{1/2}$): using formula: $t_{1/2}= 0.693 / K_{el}$ where K_{el} was elimination constant calculated in step a; 0.693 was constant
c) maximal concentration (C_{max}): using formula: $C_{max} = C_1 * e^{-kel*:\Delta t}$ where C_1 comes from step a, e=exponential, K_{el} comes from step a and Δt is difference in hours between t_1 (from step a) and t_{cmax} (0)
d) minimal concentration (C_{12h}): using formula: $C_{12h} = C_1 * e^{-kel*:\Delta t}$ where C_1 comes from step a, e=exponential, K_{el} comes from step a and Δt is difference in hours between t_{12} (12) and t_1 (from step a)
e) primary outcome variable: time above minimal inhibitory concentration2.0 (2mg/L: breakpoint for resistant *S. pneumoniae*) (T>MIC):

using formula:

Equation 1

$$K_{el} = \frac{(lnC_1 - lnC_2)}{(t_2 - t_1)} \rightarrow (t_2 - t_1) = \frac{(lnC_1 - lnC_2)}{(K_{el})} \rightarrow t_2 = \frac{(lnC_1 - lnC_2)}{K_{el}} + t_1$$

Therefore, $t_{MIC=2.0} = ((lnC_1 – lnC_{MIC2.0})/ K_{el}) + t_1$ where lnC_1 is natural log of C_1 (concentration at first timepoint after baseline i.e. 2-3 hours of index dose); $lnC_{MIC2.0}$ is natural log of 2; K_{el} as calculated in step a and t_1 is time lapse between baseline and C_1 sampling (in hours). The outcome is written as percentage of dosing interval (12hours) in Table 4

RESULTS

Of a total of 216 young infants (0-59 days) enrolled in Arm B of the SATT trial during the 11 month study period, 60 (27.8%) consented to participate in the amoxicillin PK pilot study. Sixteen (26.7%) young infants were excluded from analysis, 12 due to refusal to permit blood draws for amoxicillin assays beyond baseline, 4 from one PHC clinic due to mishandled samples. All infants who refused venepuncture beyond baseline continued to receive study medication and participate in parent trial (oral amoxicillin and intramuscular gentamicin). Refusal to allow more than one blood sample stemmed from a parental perception that consecutive venepuncture would result in pain and anemia for the infant.

Table 2 outlines descriptive data on the remaining 44 young infants. The cohort had median age 11.5 days and gestational age 38 weeks. Mean maternal age was 24.4 ± 4.3 years. Nine (20.5%) infants had concurrent vomiting, 2 both vomiting and diarrhea. One of 9 with vomiting and/or diarrhea failed treatment (11%). Mean amoxicillin levels were 11.6 ± 9.5 mg/L (n=44) at 2-3 and 16.4 ± 9.3 mg/L (n=20) at 6-8 hours of index dose. Five of 44 infants (11.4%) were clinical treatment failures (median day of failure 5:range 3.5-7.25) and were switched to parenteral therapy. One infant with treatment failure on day 5 expired on day 7 (Infant E in Table 4 and Table 5). None of these had culture proven sepsis.

Graphical plotting of individual amoxicillin serum levels versus time (since amoxicillin index dose) of 20 of 44 infants with 3 serum levels each showed classic dose-response curves in 7 of 20 (Figure 1). In remaining 13 infants, the 6-8 hour serum amoxicillin level was greater than that at the 2-3 hour timepoint. Delayed excretion was inferred due to a higher serum level at 6-8 hour timepoint unexplained by gestational age and weight (Figure 2). The remaining 24 children who did not allow sampling at 6-8 hours, had only one datapoint in the absorption phase giving predictable straight lines instead of classic dose-response curves with an ascending and descending arm. No inference could be made about excretion kinetics in these 24 infants (Figure 3). Using standard formulae (See Methods), pharmacokinetic parameters such as half life, elimination constant and trough levels (concentration at 12 hours

of inex dose) were estimated for only 7 patients showing classic dose-response curves, 2 of 7 were outliers with prolonged half life unexplained by gestational age and weight. Five of 44 (11.5%) babies had a positive blood culture with predominance of gram positive organisms.

Figure 5a gives a gross estimate of range of serum amoxicillin levels seen at two time points beyond baseline, each corresponding to the absorption and elimination curve of the drug respectively. Figure 5b shows improvement in goodness of fit (r square value 0.41 to 0.58) by removing 3 sets of outlier amoxicillin and half life values (Patient E, F and G in Table 4).

Figure 6 shows distribution of amoxicillin plasma levels versus time (since index amoxicillin dose) in all 44 young infants with >1 serum amoxicillin level. Range of time since index dose for 2-3 hour (absorption phase) and 6-8 hour (elimination phase) samplings was 0.75-4.5 and 3.4-6.2 hours respectively. Figure 7 shows distribution of amoxicillin plasma levels versus time in 44 infants based on inference of elimination kinetics from dose-response curves. Five of 7 with classic curves have plasma amoxicillin values between 25^{th} and 75^{th} quartile with two outliers. Similarly minimum and maximum plasma levels of amoxicillin in elimination phase of 13 infants with delayed excretion are higher than those with classic curves. Amoxicillin values for 24 infants in the absorption phase lie between 1-3 µg/ml.

Table 2 shows inter-group comparability of demographic, clinical and pharmacokinetic predictors of interest in 20 participants with 2 plasma amoxicillin levels beyond baseline (sub-groups based on inferred elimination kinetics: 7 classic, 13 delayed excretion). The only significant difference was in amoxicillin levels at 2-3 hours of index dose (p=0.009; 95% CI: 3.97-23.97). This difference was significant even when the comparison was extended to a third group of 24 in whom elimination kinetics could not be inferred due to absence of data on elimination curve (Table 3).

Table 4 describes the PK parameters $t_{½}$ (half life range: 1.3-31.9hr), K_{el} (elimination constant range: 0.0217 – 0.5471) and trough level (concentration at 12 hours of index dose) in 7 of 20

babies with 3 plasma amoxicillin levels (baseline, 2-3 and 6-8 hours of index dose). These estimates were calculated by help of standard formulae (See Methods). Of these, 5 (71.4%) followed expected trends (2 showed delayed excretion: $t_{½}$ 22-31.9 hr) and 7 of 7 (100%) achieved efficacy surrogate (>50% T>MIC2.0).

Table 5 describes demographic, clinical and pharmacologic data on 7 of 20 (35%) who followed a characteristic dose-exposure curve (as shown in Figure 1).

Mean time between baseline and subsequent sampling was not predictive of delayed excretion (OR: 1.023: 95% CI 0.99 – 1.02). No demographic (age in days, gestational age, body surface area/weight, gender), clinical (diarrhea, vomiting, poor feeding, number of feeds prior to blood sampling) or microbiologic (culture proven sepsis) variables showed an association with delayed excretion. As a predictor, mean amoxicillin levels at second sample (OR 1.2; 95% CI 1.09 – 1.39) was associated with delayed excretion in 13 babies. For every unit rise in amoxicillin in serum, there was a 1.2 chance of having delayed excretion.

Delayed excretion inferred in 13 babies (based on dose-response plot) was associated with mean amoxicillin serum level at 2-3 hours of 7.3 ± 6.6 µg/ml (OR: 1.2; 95%CI: 1.09 – 1.39) on univariate logistic regression. There was no association between delayed excretion of drug and historical predictors like age, gestational age, weight, vomiting, diarrhea, feed intensity before blood sample or time between index dose and sampling. Logistic regression and correlations through model building were not possible due to low sample size (n=20).

Five of 7 (71.4%) young infants with appropriate dose-response curves showed PK parameters like elimination constant (K_{el}) and half life ($t_{1/2}$) in accordance with reported priors in literature. A total of 5 (of 44) babies had culture proven sepsis (culture positivity rate of 11.4%). Of these, 4 isolates were gram positive (80%): 2 (*Streptococcus viridans*), 1 Group B Streptococcus (GBS), 1 Group A Streptococcus (GAS); 1 (20%) was gram-negative (*Campylobacter jejuni)*. None of the culture positive babies were clinical treatment failures (Annex 2).

DISCUSSION

Our study attempted to assess single dose amoxicillin pharmacokinetics in young infants following a 75-100 mg/kg/dose. More specifically, we were interested in children presenting with symptoms and signs of sepsis at primary health care centers in Karachi, where the acceptance and compliance of parenteral therapy is very low (4). We attempted to address the gap in population-specific PK estimates of a widely used, safe, low-cost antibiotic like amoxicillin in the 0-2 month old age-group where ontogeny, gestational age, birth weight and feeding intensity may influence drug disposition kinetics.

We are not aware of any published studies where pharmacokinetic (PK) data for 0-2 month old infants has been assessed for oral amoxicillin in a community-setting. The only other oral amoxicillin study of infants <2month old, assessed the efficacy of oral amoxicillin in term newborns admitted with Group B Streptococcal (GBS) sepsis. The primary difference between this study and our study is that children in the former received 48 hours of parenteral therapy prior to being switched to oral. In that study, Le-Guen et al. found serum levels at steady state to be well above desired MIC in two groups receiving doses of 200 and 300 mg/kg/day (67). This paper supports adequacy of the 200mg/kg/day dosage in our infant population.

The rate and pattern of maturation of each pharmacokinetic process may vary greatly among infants resulting in marked inter-individual variability in pharmacokinetics such that infants of similar age may exhibit differences in therapeutic efficacy (124). We see this inter-infant variability in half lives calculated for 7 participants. Our study, nested in an arm of an ongoing RCT, also assessed efficacy of a more flexible and feasible method of dosing by weight bands, whereby all infants in a weight band (eg.1.5-1.9kg) received a range of 75-100 mg/kg/dose (See Annex 3).

Most published studies on young infants have been classic pharmacokinetic studies on parenteral amoxicillin (64-66, 68, 69, 115, 119, 120, 122, 125, 126). Administering parenteral amoxicillin/penicillins in resource-constrained low-income community settings requires either

acceptance of hospital referral or the required expertise at the primary healthcare level to deliver these medications. In cases where both conditions are not met, oral antibiotics, when administered appropriately, fill a void where the only alternative is no antibiotic (22, 23). Furthermore, extrapolating oral amoxicillin pharmacokinetics for newborns and young infants from data on adults or older children is uncertain at best, largely due to immature hepatic and gastrointestinal absorption, larger volume of distributions and variable renal clearance of infants especially at early postnatal ages (<2 months) (124, 127, 128). The functional immaturity of physiological processes and organ function predispose newborns to exhibit disparate responses relative to adults.

Currently, the WHO recommends oral amoxicillin as standard therapy for community acquired non-severe pneumonia in infants >2 months of age (36); parenteral penicillin or hospitalization is recommended for those below 2 months of age. With 10-26% of children under 2 months acquiring pneumonia/sepsis, a case fatality rate ranging from 8.8-21% (129-131), a low acceptance of hospital referral in the community (4), and the logistic difficulty in administering parenteral antibiotics in first-level healthcare facilities, it is essential to gather more information on oral alternatives in this specific age group with its PK peculiarities.

In greater than 2 month old infants, Fonseca et al found peak amoxicillin plasma levels of 10.5 ± 4.9 (day 1) and 10.6 ± 5.1mg/L (day 3) with the 25 mg/kg/dose in comparison to lower peaks with the 15 mg/kg/dose regimen; leading to a recommendation of higher doses in children (30-40 mg/kg/dose) (18). Though plasma levels correlate well with size of dose and the pharmacokinetic processes of absorption, distribution, metabolism and excretion acting upon that dose (124), this is more usual in drugs exhibiting linear kinetics. In the case of amoxicillin where absorption is saturable because of limits in the intestinal physiologic processes necessary for drug transfer, a plateau will be reached in systemic plasma concentrations which are not as intuitively correlatable with drug dose. The extent of pharmacokinetic/pharmacodynamic differences between pediatric and adult populations are still largely unknown (124). Due to the difficulty in getting permission for repeated blood

draws, and inter-patient plasma level variability between different time points we were only able to get complete results for seven infants and partial for another 13. Even though, in our case, T>MIC was estimated for only 7 of 44 patients, all 7 (100%) achieved plasma amoxicillin levels above target MIC (2 mg/L breakpoint for *S. pneumoniae*) for more than 50% of dosing interval with an index dose of 75-100 mg/kg/dose. This study also shows strong support of oral amoxicillin with gentamicin as a safe alternate for a parenteral ampicillin-gentamicin regimen by showing amoxicillin levels higher than MIC2.0 (breakpoint for resistant *S.pneumoniae*) in 88.6% (39 of 44) and 95% (19 of 20) of infants at 2-3 hours and 6-8 hours of index dose respectively.

Sub-group analysis of infants from whom 2 sera samples were collected at approximately 2-3 and 6-8 hours of index dose, showed general comparability with significant differences observed in mean amoxicillin level 2-3 hours after index dose (p value 0.009; 95% CI of mean difference 3.9-23.9) and time between baseline and 6-8 hour (of index dose) sampling (p value 0.045; 95%CI of mean difference 1.3-115.2) (See Table 2). Serum levels at 2-3 hours of index dose in the 7 with complete results were comparable to peak levels of 25.8-28 ug/L reported earlier with intravenous and oral formulations (66, 67). Serum levels at 2-3 hours ($C_{2\text{-}3hr}$) of the 13 with slow peaking and delayed excretion, were comparable to maximal concentration (Cmax) reported in older children by Ginsburg et al (4-45 months) (132), Marks et al (3.5-13 years) and Suarez-kurtz et al (133, 134). The higher 6-8 hour plasma level ($C_{6\text{-}8hr}$) in 13 with delayed excretion can be at least partially explained by delayed renal clearance due to lower age and weight in this group compared to the 7 with complete results. Low sample size may be responsible for lack of statistically significant difference in age and weight in both groups. $C_{6\text{-}8hr}$ is however reassuringly well above MIC 2.0 (breakpoint for resistant *S. pneumoniae*) in both 7 with complete and 13 with partial results. No association could be found between 13 with delayed excretion and prematurity, body surface area, culture proven sepsis, diarrhea or vomiting.

We also attempted to interpret data from 24 babies with only one plasma level beyond baseline at the 2-3 hour time point. Table 3 summarized comparability of between three groups based on inference about elimination kinetics: 7 with classic curves (1 datapoint each on the absorption/ascending and elimination/descending arms of time-concentration curve); 13 with delayed excretion (larger value of plasma amoxicillin on elimination/descending arm than ascending) and 24 where no datapoint on elimination curve disallowed any inference about kinetics. The second and third groups were similar in age, weight and mean amoxicillin level at 2-3 hours of index dose ($C_{2\text{-}3hr}$). Lower plasma levels of amoxicillin at 2-3 hours in these two groups than the first may be the result of age-related delay in gastric emptying which indirectly caused decreased rate of absorption in the small intestine. Feed intensity in all groups was reportedly comparable but was not directly observed. We could not comment on gastrointestinal first-pass effects in all three groups as age-related effect in the younger groups should have led to higher plasma levels at 2-3 hours of index dose, not lower. Renal clearance is also influenced by gestational age and birth weight and may explain the higher 6-8 hour plasma level in the second group compared to the first (124, 135, 136).

We were not able to develop predictive models for delayed excretion due to inability to infer elimination kinetics for 24 of 44 infants. However, five of seven babies with classic time-concentration curves had elimination constants (K_{el}) comparable to the K_{el} reported by Pullen et al in 10-52 day olds (-0.27 ± 0.1 1/hr) (69) (See Table 4). Delayed excretion in two patients (F and G in Table 4) with K_{el} 0.02-0.03 could not be explained by low postnatal age, prematurity or low weight at time of sampling which would indirectly effect gastric emptying time, intestinal transit time, first-pass effects and renal clearance (Table 5). There was no statistically significant difference in ethnicity (effect on PEPT1 receptor expression) or parity (related to age and weight) in both groups, however, increased gastrointestinal (PEPT1) and renal absorption (through increased PEPT2 receptor expression in the renal epithelium) may be a possible hypothetical cause of prolonged half life and trough levels in these patients. Peptide transporters have not been implicated in amoxicillin-specific pharmacodynamics yet

even though their role in metabolism of beta lactams (first generation cephalosporins, cloxacillin) has been described. Decreased protein binding as evident by clinical edema, prematurity and low birth weight did not appear to be a possible cause of higher plasma levels at 2-3 and 6-8 hours in these 2 patients (See Table 4).

Very few papers report on bioavailability of oral amoxicillin in humans. Paintaud studied absorption kinetics in healthy adults and metricised bioavailability of a dose of 500mg PO as 0.72 and a dose of 3gm as 0.45 in comparison to an IV dose of 500mg of amoxicillin (102). Adam et al reported a similar value of 0.51 ± 0.05L/h in healthy adults receiving 500mg of oral amoxicillin (105). No data is available on bioavailability of oral amoxicillin in newborns and infants. Simulation using healthy adult estimates may yield an approximation of pediatric and infant measures for bioavailability. In absence of simulation sets, plasma peak levels or PK/PD parameters such as T>MIC may be taken as an indirect surrogate for adequate oral absorption and bioavailability.

Mean total body clearance of 0.048 ± 0.075 L/hr has been estimated in infants and newborns in a few studies (64, 66, 68, 69, 103, 119, 126). In absence of simulation data, this may be an objective in a subsequent phase of this pilot study.

The population pharmacokinetic approach in contrast to classic allowed sparse sampling on the individual and exploration of drug disposition in a larger, younger population seeking care for suspected sepsis in community settings. It involved a compromise on sample error but PK/PD estimations through population pharmacokinetic approach have been found to be comparable and approximate to those through traditional/classic sampling (134). Five such studies have been done so far in infants evaluating parenteral benzyl-penicillin (137), amoxicillin (66) and flucloxacillin (84).

We chose the cut-off of >50% of time above MIC as our surrogate of efficacy. Plasma levels above MIC for more than 30-40% of the dosing interval have been studied (70) but criticized as sub-optimal in a vulnerable population like newborns and infants where immune function is still immature. Levels higher than MIC for more than 70% of dosing interval have been

advocated as more representative of efficacy in newborns (83) however, in absence of sufficient evidence, this is open to debate. This study evaluates efficacy of oral amoxicillin in sick newborns and young infants and establishes evidence for its possible utility as replacement for parenteral penicillin or ampicillin in neonatal sepsis regimens in primary care settings in developing countries. Efficacy data on oral antibiotics like amoxicillin are of special interest as oral and parenteral combinations are cost-effective, easier to administer and more acceptable due to minimal injections.

One weakness of our pilot study was the expectedly high attrition rate due to which elimination kinetics could be inferred for only 20 of 60 (33.3%) initial enrollees. Bias and generalisability were addressed by comparing participants included (n=44) and excluded (n=16) from analysis; no significant differences in demographic or clinical characteristics were found. Due to failure to determine or infer elimination kinetics on > 50% participants in this pilot study, we could not reject or fail to reject the null hypothesis that >80% young infants in our cohort achieved plasma levels above MIC for >50% of dosing intervals. Seven of seven (in whom elimination kinetics could be assessed) however, had plasma levels above MIC for >50% of the 12 hour dosing interval (See C_{12hr} in Table 4). Inter-patient variability was seen in two of seven with estimable PK parameters. A larger study sample in future studies will allow us to interpret this observed effect.

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FIGURES

Figure 1 Time-concentration curve for Patient 101

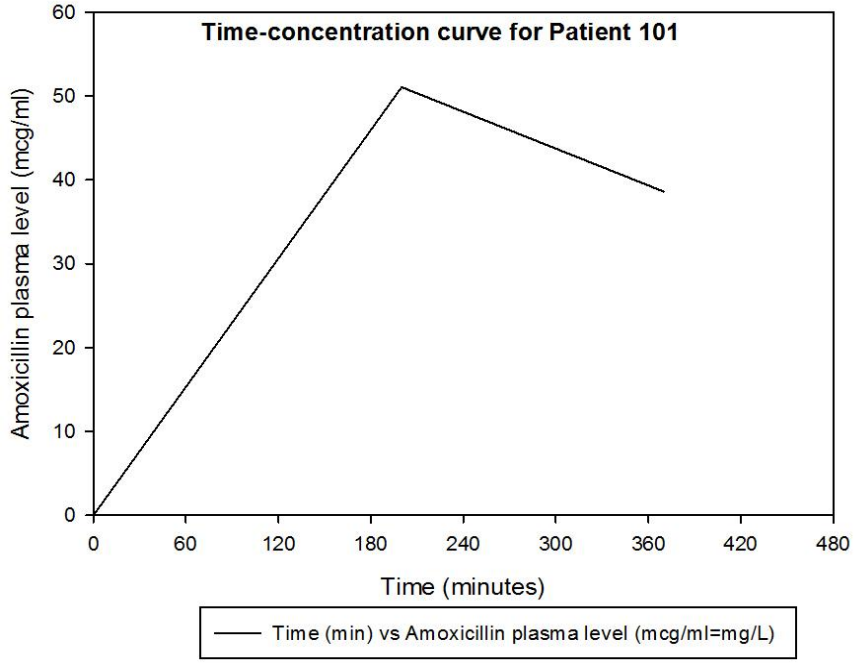


Figure 2 Time-concentration curve for Patient 216

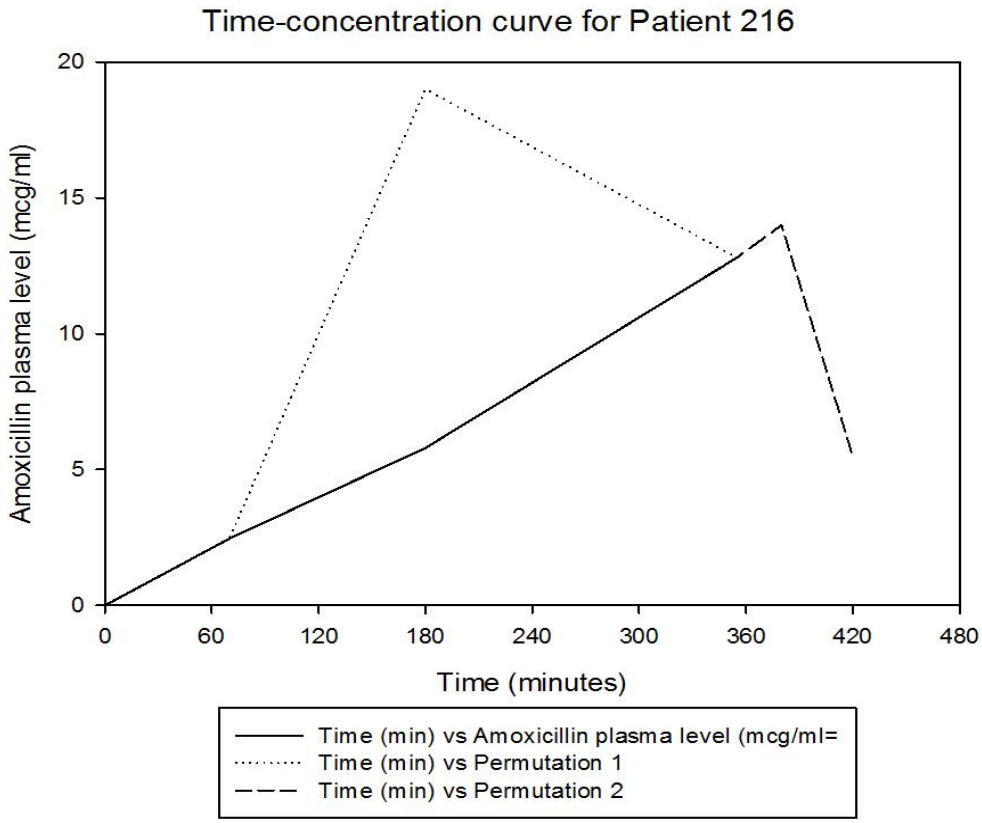


Figure 3 Time-concentration curve for Patient 225

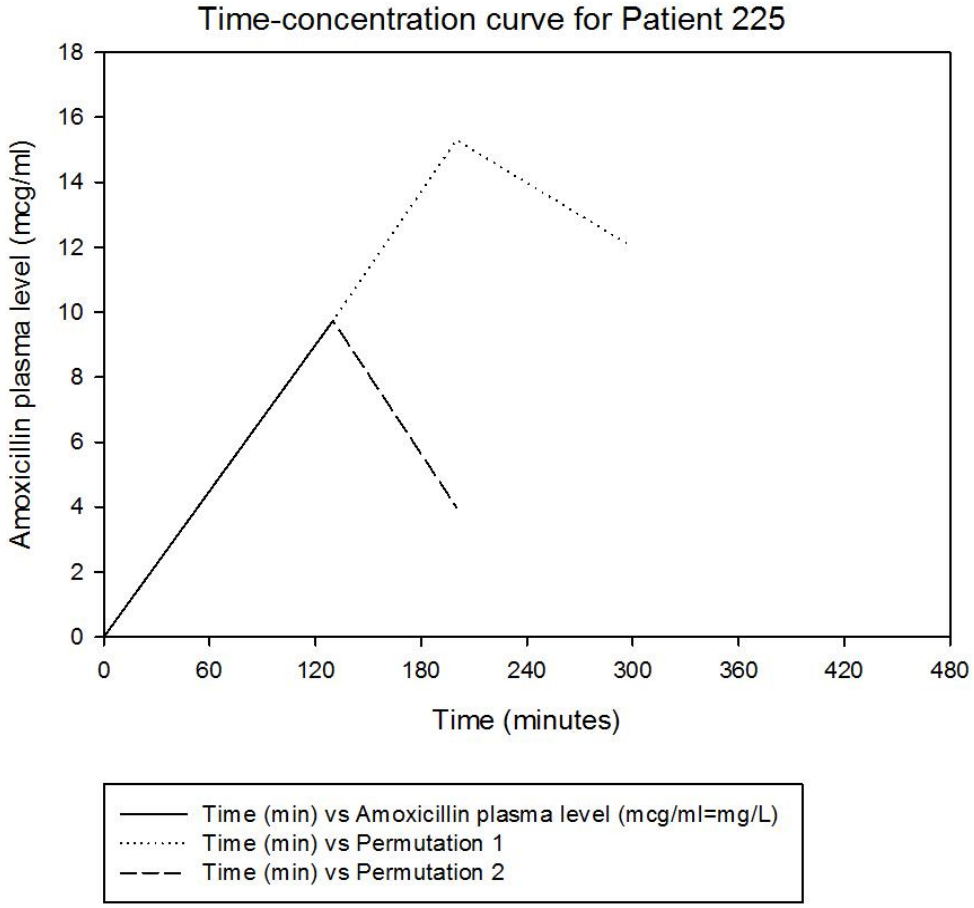


Figure 4 Standard Curve showing linearity of Amoxicillin Kinetics

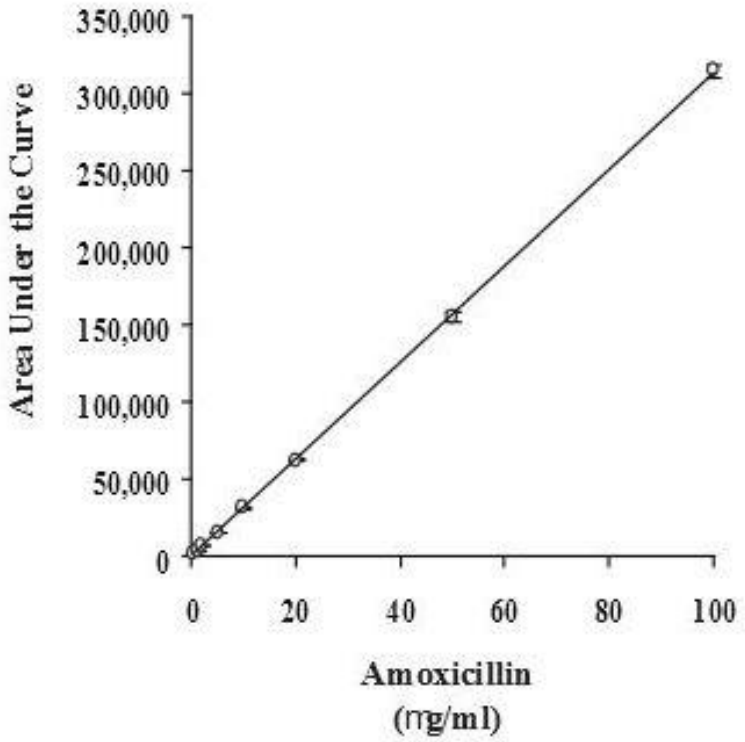


Figure 5a Composite dose concentration-time plot showing > 80% of amoxicillin concentrations at second and third sampling were above MIC2.0

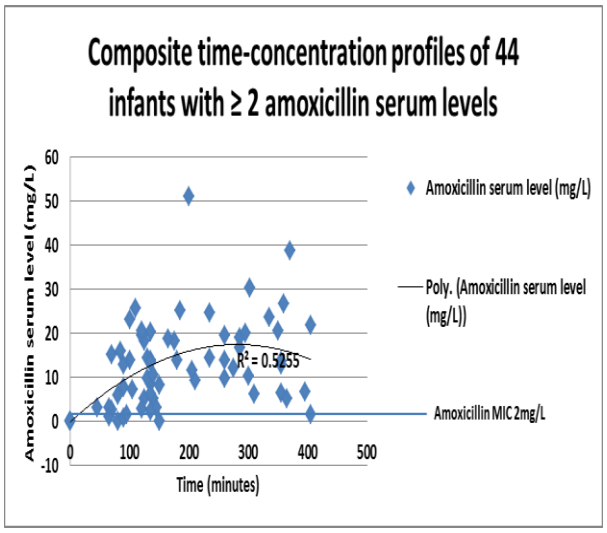
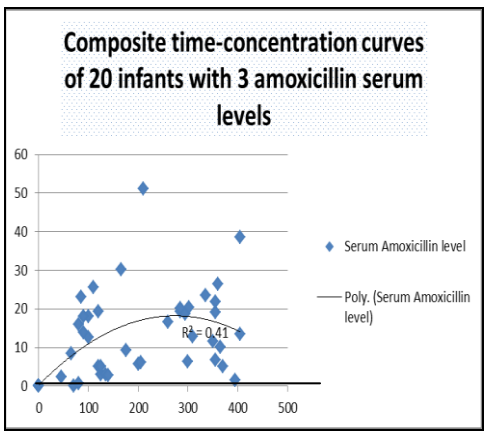


Figure 5b Minimal improvement in goodness of fit (composite dose concentration-time curve) of 20 young infants with >2 amoxicillin levels by removing outliers



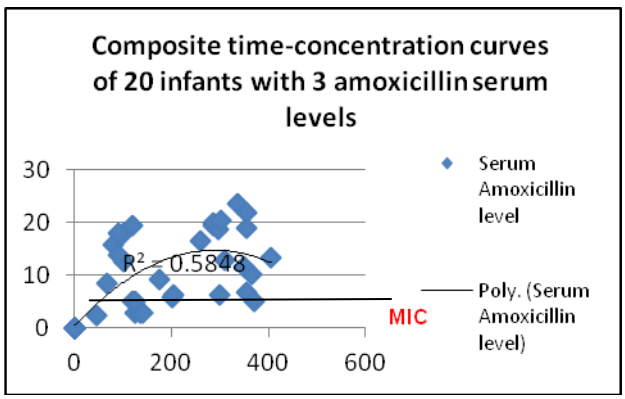


Figure 6 Distribution of time-concentration values in 44 infants with ≥ 2 and 20 infants with 3 amoxicillin serum levels (on 75-100mg/kg/dose oral amoxicillin)

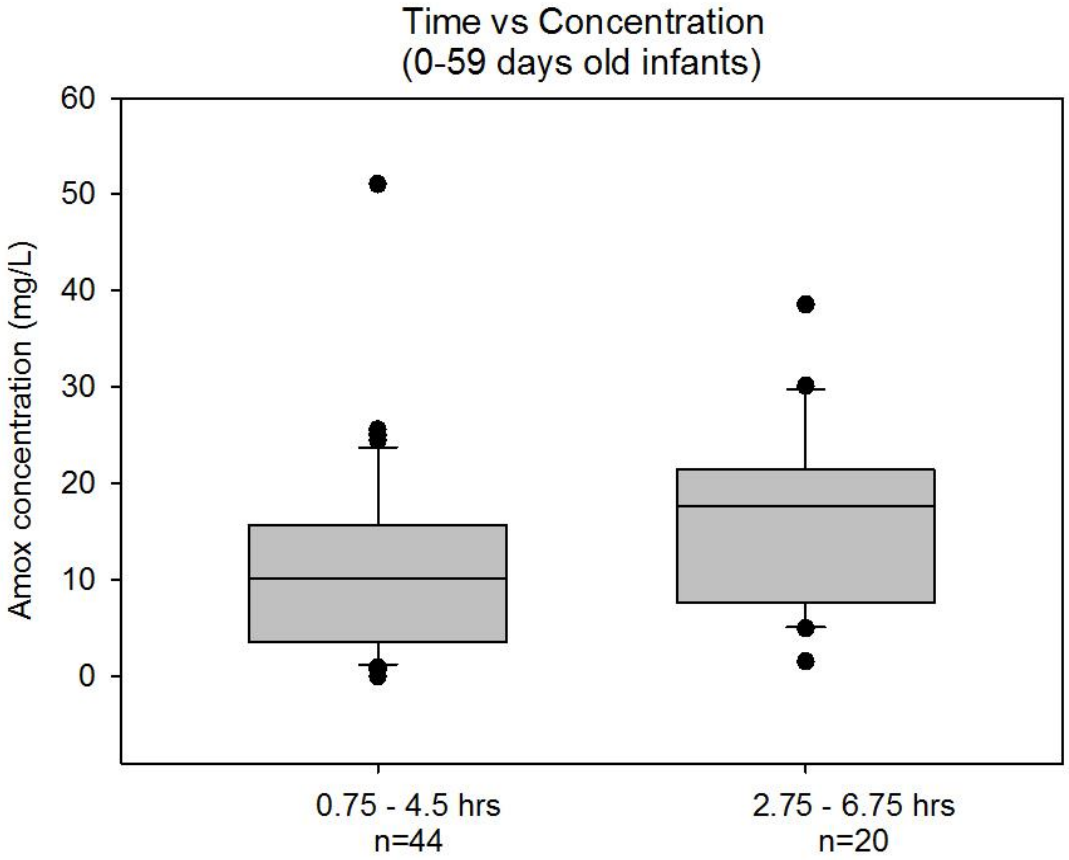


Figure 7 Range of Sampling Time and amoxicillin concentration achieved based on elimination kinetics status

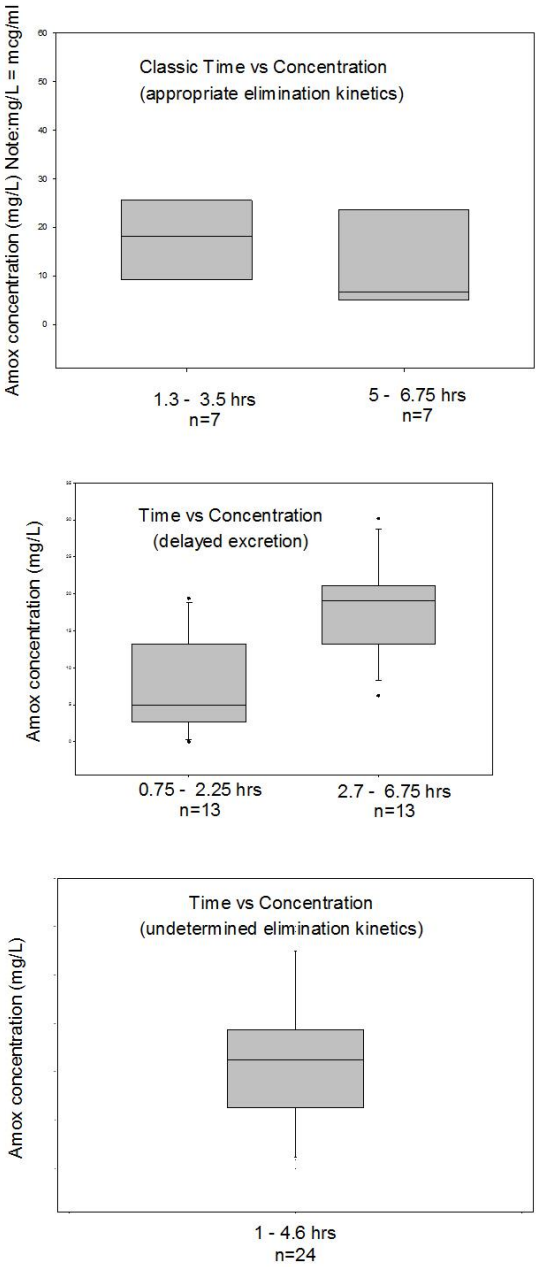
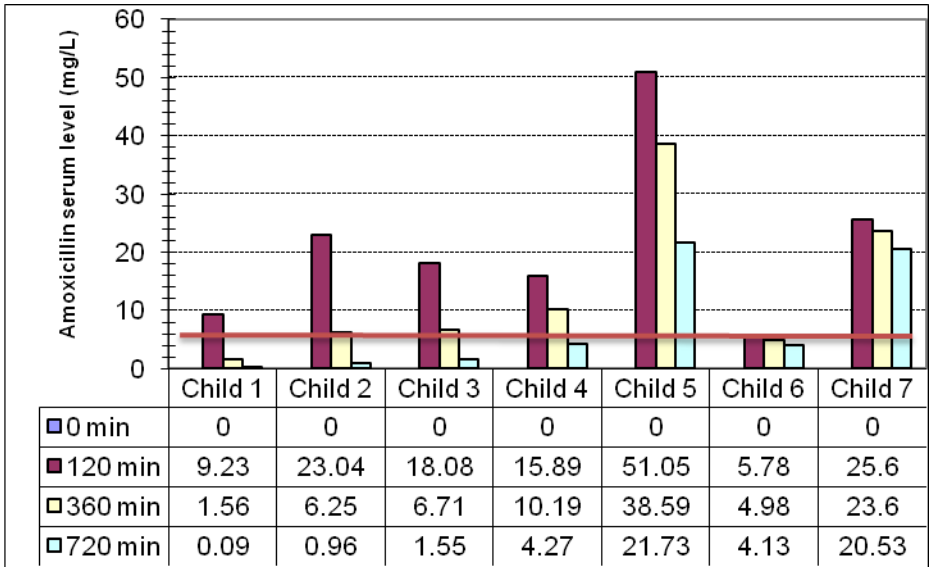
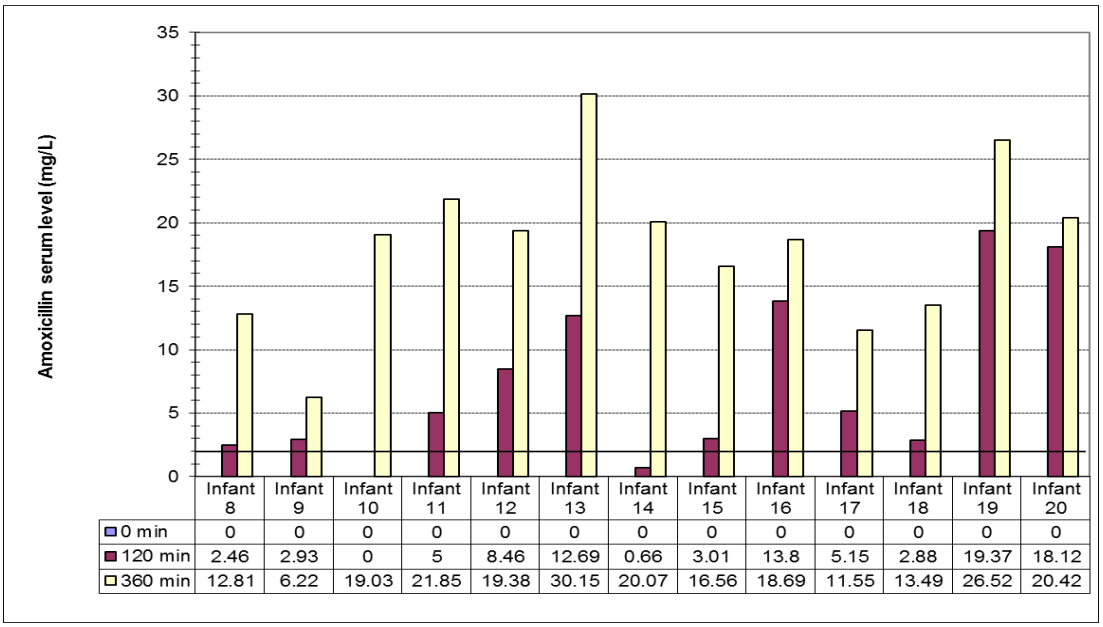


Figure 8a Serum Amoxicillin Levels in 7 young infants at 0, 2, 6 and 12 hours of index dose



	Child 1	Child 2	Child 3	Child 4	Child 5	Child 6	Child 7
0 min	0	0	0	0	0	0	0
120 min	9.23	23.04	18.08	15.89	51.05	5.78	25.6
360 min	1.56	6.25	6.71	10.19	38.59	4.98	23.6
720 min	0.09	0.96	1.55	4.27	21.73	4.13	20.53

Figure 8b Serum Amoxicillin Levels in 13 young infants at 0, 2 and 6 hours of index dose



	Infant 8	Infant 9	Infant 10	Infant 11	Infant 12	Infant 13	Infant 14	Infant 15	Infant 16	Infant 17	Infant 18	Infant 19	Infant 20
0 min	0	0	0	0	0	0	0	0	0	0	0	0	0
120 min	2.46	2.93	0	5	8.46	12.69	0.66	3.01	13.8	5.15	2.88	19.37	18.12
360 min	12.81	6.22	19.03	21.85	19.38	30.15	20.07	16.56	18.69	11.55	13.49	26.52	20.42

TABLES

Table 1 Social demographic, clinical and laboratory profile of young infants with sepsis undergoing oral amoxicillin pharmacokinetic assessment (n=44)

Newborns and young infants	**Freq (%)**	**Mean± SD**	**Median**
Age (days) 0-6 7-27 28-59	 18(40.9) 11(25) 15(34.1)	19.7 ± 0.9	11.5
Gestational age (weeks) ≥37 weeks <37 weeks	 38(86.4) 6 (13.6)	36.8 ± 5.8	38
Weight at enrolment (kg) ≥2.5kg <2.5kg	 30(68.2) 14(31.8)	2.9 ± 0.9	2.8
Length (cm)		49.93 ± 3.04	50
FOC (cm)		34.54 ± 2.10	35
Body Surface Area ($m^{2)}$		0.201 ± 0.03	0.19
Milligram/kg/dose		90.49 ± 6.65	90.90
Gender (male)	25 (56.8%)		
Maternal age (years)		24.38 ± 4.33	23
Maternal weight (kg)		48.95± 8.68	50.53
Gravidity		3.55 ±2.71	2
Parity		3± 2.012	2
Number of feeds between baseline (C_0) and second sampling ($C_{2\text{-}3hour}$)		2.89 ± 1.912	2
Number of feeds between $C_{2\text{-}3hour}$ & third sampling ($C_{6\text{-}8hour}$)		4.78 ± 2.51	4
Time in minutes between prior feed and $C_{2\text{-}3hour}$		51 ± 50.85	30
Time in minutes between prior feed and $C_{6\text{-}8hour}$		40.95 ± 48.63	27.5
Amoxicillin dosage in milligrams per kg per dose		90.5 ± 6.7	
Vomiting (yes)	9 (20.5)		
Diarrhoea (yes)	2 (4.5)		
Temperature (ºC)		36.9 ± 1.51	36.7
Respiratory Rate (RR) (breaths per minute)		52.3 ± 12.55	52
Time interval between C_0 and $C_{2\text{-}3hour}$ (n=44) minutes		135.6 ±56.7	130
Time interval between C_0 & $C_{6\text{-}8hour}$ (n=20) (minutes)		322.9 ± 63.1	342.5
Mean amoxicillin (mg/mL) in $C_{2\text{-}3hour}$		11.62 ± 9.51	10.11
Mean amoxicillin (mg/mL) in $C_{6\text{-}8hour}$		16.43 ± 9.32	17.63
Clinical Failure	5 (11.4)		
Culture proven sepsis	7 (15.9)		
>50% Time >MIC2.0 (n=7)	6 (85.7)		
Death	1 (2.3)		

*MIC taken as breakpoint for resistant *S.pneumoniae*

Table 2 Distribution of socio-demographic, clinical and pharmacokinetic covariates by achievement of amoxicillin excretion kinetics (appropriate for age n=7) (delayed excretion n=13)

	N=7 Mean ± SD	N=13 Mean ± SD	P value	95% CI of Mean Difference
Weight (kg)	3.7 ± 1.1	2.8 ± 0.8	.050	0.002 - 1.746
Body Surface Area (m^2)	0.2 ± 0	0.2 ± 0	.062	-0.002 - 0.066
Milligrams per kg per dose	90 ± 8.7	90.5 ± 6.8	.888	-7.906 - 6.898
Frequency of feeds between baseline (C_0) and second sample ($C_{2\text{-}3hour}$)	2.8 ± 1.6	2.7 ± 1.9	.921	-2.05 - 2.25
Frequency of feeds between second ($C_{2\text{-}3hour}$) and third sample ($C_{6\text{-}8hour}$)	4.8 ± 2.8	4.7 ± 2.4	.943	-2.888 - 3.088
Time in minutes between prior feed and $C_{2\text{-}3hour}$	56.7 ± 60.5	57.3 ± 56.7	.983	-57.716 - 56.529
Time in minutes between prior feed and $C_{6\text{-}8hour}$	57.9 ± 60.7	24.3 ± 20.5	.201	-22.845 - 90.013
Age at enrolment (days)	31.4 ± 22.5	14.8 ± 16.3	.072	-1.635 - 34.954
Gestational Age	37.9 ± 1.9	37.5 ± 1.1	.627	-1.037 - 1.674
Maternal Age	25.1 ± 5	23.8 ± 3.8	.531	-3.058 - 5.707
Temperature (ºC)	37.4 ± 1.1	36.8 ± 1.3	.329	-0.631 - 1.783
Respiratory Rate (RR)	51.4 ± 7.5	48.3 ± 14.8	.610	-9.519 - 15.761
Gravidity	4 ± 4.3	3.5 ± 2.6	.765	-2.732 - 3.655
Parity	3 ± 2.6	3.2 ± 2.4	.897	-2.607 - 2.3
Length	51.5 ± 2.3	49.7 ± 2.7	.162	-0.79 - 4.359
FOC	35.1 ± 2.8	34.4 ± 1.2	.497	-1.24 - 2.462
Dose gentamicin (mg)	0.5 ± 0.2	0.4 ± 0.2	.060	-0.008 - 0.327
Dose amoxicillin (mg)	6.7 ± 2.2	5.2 ± 1.6	.088	-0.255 - 3.375
Mean amoxicillin level at 2-3 hours (mg/mL)	21.2 ± 14.9	7.3 ± 6.6	.009	3.966 - 23.968
Mean amoxicillin level at 6-8 hours (mg/mL)	13.1 ± 13.3	18.2 ± 6.3	.368	-17.473 - 7.303
Actual time range (minutes) of second sampling scheduled for 120-180 minutes after index dose	137.1 ± 56	100.4 ± 29.8	.145	-15.747 - 89.264
Actual time range (minutes) of third sampling scheduled for 360-480 minutes after index dose	360.7 ± 35.6	302.5 ± 66.2	.045	1.318 - 115.187

Table 3 One-way ANOVA to show comparability between three outcome groups

	Outcome Variable: Elimination Kinetics			
Predictor Variables	Group achieving appropriate elimination kinetics (%) 7	Group with delayed excretion (%) 13	Group with undetermined elimination kinetics (%) 24	p
Gender (male)	6(24)	10(40)	9(36)	0.017
Weight in kg	3.7	2.8	2.8	.047
Body Surface Area (mosteller)	.2	.2	.1951	.057
Milligrams per kg per dose	89.9	90.5	90.7	.974
No of feeds between baseline and second sampling ($C_{2\text{-}3hour}$)	2.8	2.7	3.1	.897
Time in minutes between $C_{2\text{-}3hour}$ & prior feed	56.7	57.3	43.8	.742
No of feeds between $C_{2\text{-}3hour}$ and third sampling ($C_{6\text{-}8hour}$)	4.8	4.7	5	.985
Time in minutes between $C_{6\text{-}8hour}$ and prior feed	57.8	24.3	57.3	.286
age at enrolment 0-6 7-27 28-59	31.4 2(11.1) 1(9.1) 4(26.7)	18.9 6(33.3) 4(36.4) 3(20)	18.9 6(54.5) 10(55.6) 8(53.3)	.172 0.662
gestational age	37.8	37.5	36.2	.704
Temperature (ºC)	37.4	36.8	36.8	.619
Respiratory Rate (per minute)	51.4	48.3	54.7	.335
Severe Chest In-drawing	4(23.5)	4(23.5)	9(52.9)	.506
Less than normal movement	0	1(33.3)	2(66.7)	.735
Poor feed and poor suck	1(16.7)	2(33.3)	3(50)	.949
Diarrhoea	0	1(50)	1(50)	.727
Vomiting	0	2(22.2)	7(77.8)	.210
Length	51.5	49.7	49.6	.334
FOC	35.1	34.5	34.4	.786
Mean Amoxicillin oncentration at 2-3 hours ($C_{2\text{-}3hour}$)	21.2386	7.2715	11.1700	.005
Mean Amoxicillin oncentration at 6-8 hours ($C_{6\text{-}8hour}$)	13.1257	18.2108	NA	.255
Time (minutes) between baseline ($C_{0)}$) and $C_{2\text{-}3hour}$	137.1429	100.3846	155.0000	.017
Time (minutes) between baseline ($C_{0)}$) and $C_{6\text{-}8hour}$	360.7143	302.4615	NA	.045
Positive Blood Culture	0	3(42.9)	4(57.1)	.400

Table 4 PK/PD Parameters for patients achieving appropriate dose concentration curves (n=7)

Pt	**t_1** **(hr)**	**C_1** **(µg/ml)**	**t_2** **(hr)**	**C_2** **(µg/ml)**	**K_{el}** **(µg/ml/hr)**	**$t_{1/2}$** **≤(hr)**	**C_{12}** **(µg/ml)**	**% dosing interval above MIC breakpoints for *S.pneumoniae***		
								Susceptible **(≤0.06µg/ml)**	**Intermediate** **(0.12-1µg/ml)**	**Resistant** **(≥2µg/ml)**
A	3.5	9.23	6.75	1.56	0.5471	1.3	0.09	100%	95.3%	52.5%
B	1.67	23.04	5.92	6.25	0.3071	2.3	0.96	100	100%	80%
C	2.92	18.08	6.58	6.71	0.2708	2.6	1.55	100%	100%	92%
D	1.42	15.89	5	10.19	0.1241	5.6	4.27	100%	100%	100%
E	3.33	51.05	6.17	38.59	0.0985	7	21.73	100%	100%	100%
F	1.33	5.78	6.08	4.98	0.0314	22 *2.5 *5.0	4.13 0.96 2.19	100% 100% 100%	100% 100% 100%	100% 78% 100%
G	1.83	25.60	5.58	23.60	0.0217 *0.2772 *0.1386	31.9 2.5 5.0	20.53 3.98 9.69	100% 100% 100%	100% 100% 100%	100% 100% 100%

*Elimination constants assuming half life 2.5 and 5.0 hours

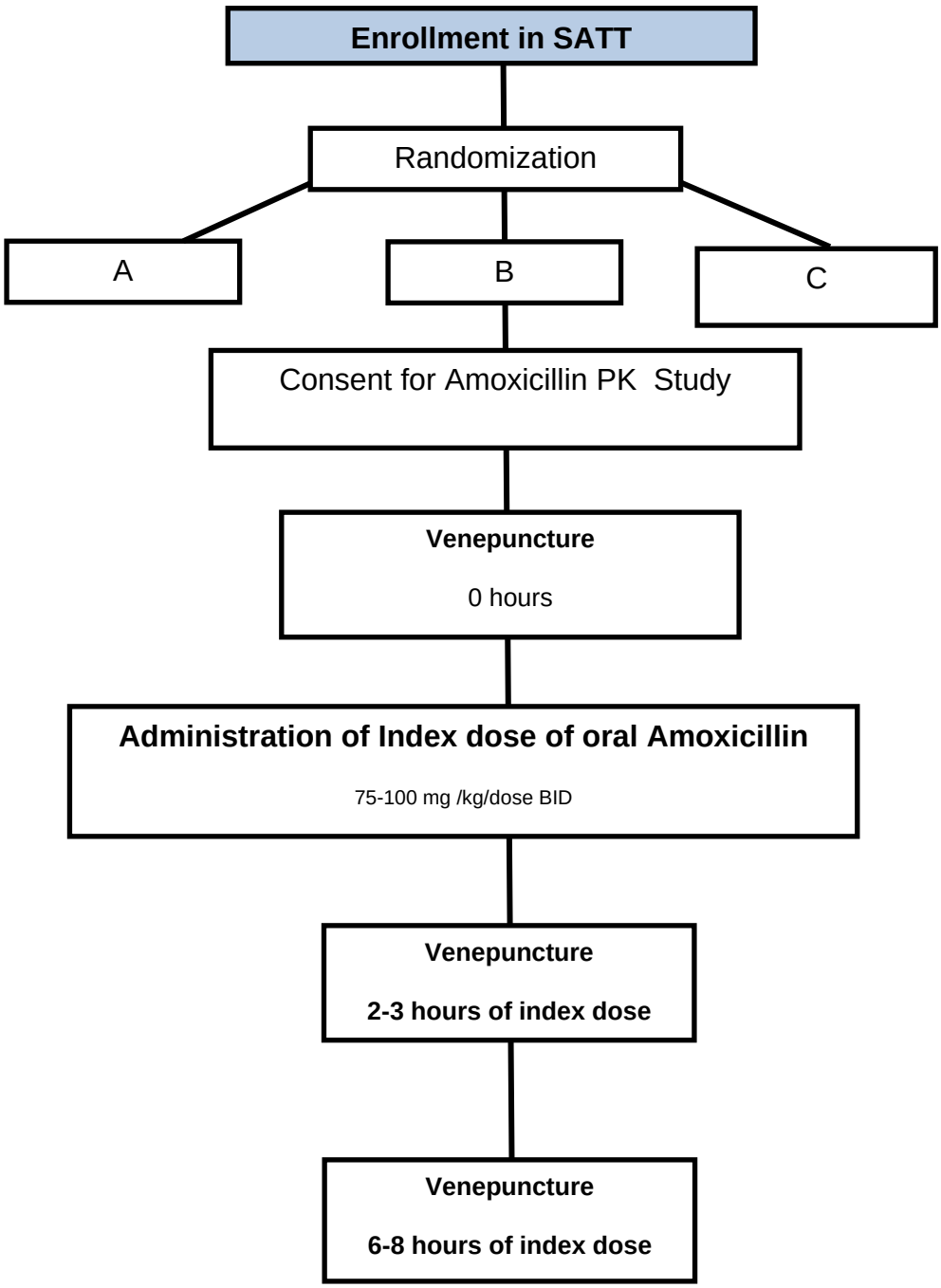
Table 5 Predictors of delayed excretion among 7 achieving dose-concentration – time curves

Pharmacokinetics	A	B	C	D	E	F	G
Half life (hours)	1.3	2.3	2.6	5.6	7	22*	20.5*
Elimination constant (1/hr)	0.55	0.31	0.27	0.12	0.10	0.03*	0.02*
Demographics							
Age (days)	52	53	25	4	5	50	36
Gender (male)	-	-	-	-	yes	yes	yes
Gestational Age (weeks)	40	38	38	38	34	39	38
Weight (kg)	4.7	5	3.5	3.8	1.8	4	3.1
Length (cm)	53	54.5	51.5	51.2	47	52	52
FOC (cm)	37	36.4	35.5	35	29	37	35
BSA (m2)	0.26	0.28	0.22	0.23	0.15	0.24	0.21
Maternal Age (years)	28	25	22	22	29	20	35
Clinical							
Poor Feeding	-	-	-	-	yes	-	-
Tachypnea (>60breaths/min)	-	-	yes	-	-	-	-
Severe lower chest indrawing	yes	yes	yes	-	-	yes	-
Hypo/hyperthermia	-	-	-	yes	-	-	yes
Clinical treatment failure	-	-	-	-	yes	-	-
Death	-	-	-	-	yes	-	-
Pharmacologic							
Infant medication in prior week	-	-	-	-	-	yes	-
Amoxicillin mg per dose (BID)	400	500	300	300	150	400	300
Amoxicillin mg per kg per dose	85.1	100	85.7	78.9	83.3	100	96.8
% T>MIC **(≤0.06µg/ml)**	100	100	100	100	100	100	100
% T>MIC **(0.12-1µg/ml)**	95.3	100	100	100	100	100	100
% T>MIC **(≥2µg/ml)**	52.5	80	92	100	100	78-100	100

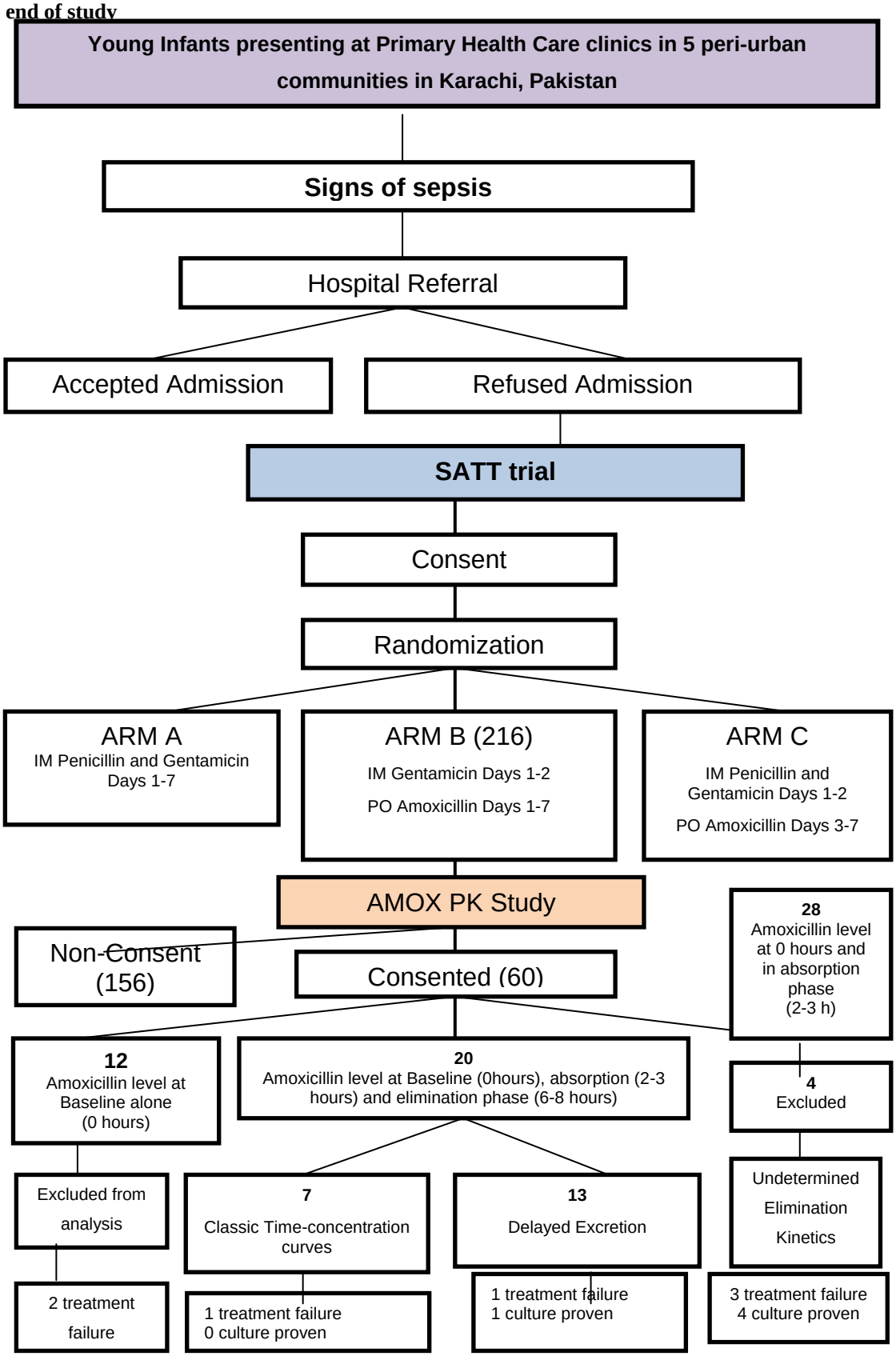
*outliers

ANNEXURES

Annex 1 Flowchart showing sequence of venepuncture for determining amoxicillin kinetics in young infants with sepsis



Annex 2 Flowchart showing progress of Amoxicillin PK study participants from enrolment to end of study



Annex 3 Antibiotic Dosage for Neonatal Sepsis Trials

Gentamicin – desired range 4-5 mg/kg/day in 0-6 days and 6.5mg/kg in 7-59 days (40mg/ml injection; single daily injection)

Weight band	Amount per dose	Daily dose	Lower Limit (mg or units/kg/d)	Upper Limit (mg or units/kg/d)
1.5-1.9 kg	0.20 ml	08 mg	4.2	5.3
2.0-2.4 kg	0.25 ml	10 mg	4.2	5.0
2.5-2.9 kg	0.30 ml	12 mg	4.1	4.8
3.0-3.9 kg	0.45 ml	18 mg	4.6	6.0
4.0-4.9 kg	0.65 ml	26 mg	5.3	6.5
5.0-5.9 kg	0.80 ml	32 mg	5.4	6.4

Amoxicillin – desired range 75-100 mg/kg/day (25mg/ml)(125mg/5ml); twice daily orally

Weight band	Amount per dose	Daily dose	Lower Limit (mg or units/kg/d)	Upper Limit (mg or units/kg/d)
1.5-1.9 kg	3.0 ml	150 mg	75.4	100
2.0-2.4 kg	4.0 ml	200 mg	80.3	100
2.5-2.9 kg	5.0 ml	250 mg	83.6	100
3.0-3.9 kg	6.0 ml	300 mg	75.2	100
4.0-4.9 kg	8.0 ml	400 mg	80.2	100
5.0-5.9 kg	10.0 ml	500 mg	83.5	100

Annex 4 Amoxicillin pharmacokinetic parameter estimates derived from the medical literature

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
Paintaud et al 1992	adults	6	Amox	500mg	adults	PO				0.72		1.23		25	14.9	8.8	
Paintaud et al 1992	adults	6	Amox	3g	adults	PO				0.45		1.27		91	14.9	26.8	
Ahmed et al 2001	adults	7	Amox	250mg	PNA: 23-35y	PO	57-75		0.6± 0.03			1.18 ±0.05					
Spyker et al 1977	adults	8	Amox	250	PNA: 18-32 y	IV vs PO	57-98	1.23	2.65		1.25 ±0.41	1.2	0.29	9.8	0.32	3.8	
Spyker et al 1977	adults	8	Amox	500	PNA: 18-32 y	IV vs PO	57-98	0.9	1.68		1.25 ±0.41	1.2	0.46	18.8	0.32	5.9	
Spyker et al 1977	adults	8	Amox	1000	PNA: 18-32 y	IV vs PO	57-98	0.66	1.56		1.25 ±0.41	1.2	0.49	36.2	0.34	10.2	
Gordon et al 1972	adults	8	Ampic	500	adults	PO vs PO						1.3± 0.33		269.5		2.04	0.5 (6h), 0.19 (8h)
Gordon	adults	8	Am	500	adults	PO						1.13		269.5		7.5	0.5(6h),

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
et al 1972			ox			vs PO						±0.56					0.19 (8h)
Suarez-kurtz et al 2001	adults	48	Amox	cap	PNA: 25.5±4.4 y	PO	62± 11.3		0.63 ±0.14		1.54 ±0.42	1.16 ±0.27		13.4±4.8		5.4±2.2	
Suarez-kurtz et al 2001	adults	48	Amox	susp	PNA: 25.5±4.4 y	PO	62± 11.3		0.62 ±0.16		1.33 ±0.49	1.21 ±0.22		11.7± 3		5±1.5	
Adam et al 1982	adults	12	Amox	500mg	PNA: 26.1 ± 2.5y	PO		1.68 ±0.36	NA	0.51 ±0.05	1.3± 0.11	1.1± 0.05	NR	19.4±1.3			
Alexander et al 2008	children		Amox	45	children	IV	<40										
Bolme et al 1995	children		Amox			IV						unchanged	decreased in malnourished children		decreased clearance in all malnourished		
Zerihun et al 1991	children		Amox			PO											
Muller et al 2007	neonates	20	Pen G	50,000 q12h	PNA: 3d, GA: <32 wk	IV						3.9	central 0.359±0.06; peripheral 0.152±0.03	0.103 ±0.01			
Ahsman et al	neonates		Amox		neonates	IV											

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
2009																	
Huisman et al 1995	neonates	17	Amox	25 q12h	PNA:3d, GA: 29±1.9 wk	IV	1.2 ±0.28	NA	NA	NA	NA	6.7± 1.7	0.67±0.12	NR	0.06 ± 0.02	53.6±9.1	16±4.9
Charles et al 1997	neonates	40	Amox	50 qd	PNA: 1-3d, GA: ≤32 wk	IV	<1.5					6.9 (with gent)	0.68±0.365		0.0685 (with gent)	71	4.1
Charles et al 1997	neonates	40	Amox	20 q12h	PNA: 1-3d, GA: ≤32 wk	IV	<1.5					5.2 (without gent)	0.68±0.365		0.0904 (without gent)	28	6.9
Weingartner et al 1977	neonates	16	Amox	50	PNA: 1-3 d, GA: >37 wk	IV	?									38	13
Weingartner et al 1977	neonates	7	Amox	50	PNA: 1-3 d, GA: <37 wk	IV	?									59	19
Autret et al 1988	neonates	21	Amox	40		PO & IV						4.28 +/- 2.4				81 +/- 32	
Tazi-lakhassi et al 1985			Amox			PO & IV											
Peskine et al 1982	neonates	26	Amox	33	GA: Term	IV amox		NA	NA	NA	NA	5.1± 3.4	1.4±1.13	NR	0.211 ±0.277		
Peskine et al	neonates	6	Amox	33	GA: 34-37wk	IV amo		NA	NA	NA	NA	5.1± 3.4	1.4±1.13	NR	0.211 ±0.27		

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
1982						x									7		
Peskine et al 1982	neonates	1	Amox	33	GA: 28wk	IV amox		NA	NA	NA	NA	5.1±3.4	1.4±1.13	NR	0.211±0.277		
Gras-le Guen et al 2007	neonates	222	Amox	300	PNA: 0-27d, GA: 39.3±1.5 wk	PO following IV	3.4±0.53									31.15	
Gras-le Guen et al 2007	neonates	222	Amox	200	PNA: 0-27d, GA: 39.3±1.5 wk	PO following IV	3.4±0.53									25.8	
Pullen et al 2006	neonates & young infants	150	Amox	50 or 100	PNA:0.76±1.57 mo; GA: 34.6±4.9 wk	IV	2.29±1.08	NA	0.1±0.03	NA	NA	5.2±1.9	0.67±0.12 (GA<32 weeks); 0.62±0.23 (>=32 weeks GA)	NR	0.096±0.036	48.8±11 (range: 25.6-80.9)	21.4
Pullen et al 2006	neonates & young infants	32	Amox	50-100	PNA:10-52 d, GA: 26-41 wk	IV	2.3		0.27±0.1			3±1.3	0.66±0.27		0.004±0.23		
Pullen et al 2006	neonates & young infant	55	Fluclox		PNA: 0-44 d, GA: 26-42 wk	IV			0.27±0.1			3±1.3	0.54±0.17		0.004±0.23	80.1±37.9	10.2±8.4

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
	s																
Vargas et al 2004	neonates & young infants	11	Amox	30 q8h	PNA: 3-60d	IV	3.6 (<5)					3.16 ±0.29	0.4		0.0003	n/a	n/a
Hibberd et al 2004	older infants and children		Amox vs Pen		PNA: 3-59mo	PO vs IM											
Garrison et al 2004	older infants and children		Amox	80-90 vs 40-45	PNA: >3mo	PO (HD vs SD)	<=18										
Fonseca et al 2003	older infants and children	66	Amox	25 BID	PNA: 3-59 mo	PO		NR	NR	NR	NR	2	NR	44.1± 24.6		10.5±4.9	
Fonseca et al 2003	older infants and children	66	Amox	15 TID	PNA: 3-59 mo	PO		NR	NR	NR	NR	1.5	NR	28.59± 14.4		6.9±3	
Ginsburg et al 1979	older infants and childr	24	Amp	25 (fasting)	PNA: 4-45 (27)mo	PO vs PO	4.4-21(12.7)					1.1		16		5.4 ±0.76	0.6-0.25

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
	en																
Ginsburg et al 1979	older infants and children	24	Amox	15 (fed)	PNA: 4-45 (27)mo	PO vs PO	4.4-21(12.7)					1.8		14		3.2±0.48	0.7±0.11
Ginsburg et al 1981	older infants and children	12	Amox	15	PNA: 4-39 (17.3)mo	PO	6.6-15.4 (11.2)					1.2		18		7.3±1.2	0.42±0.09
Pichichero et al 2008	older infants and children	11	Amox	475 sprinkle	PNA: 6mo-4y	PO vs PO		0.53	0.529	NR	2.5	1.98	NR	39.3		8.65	
Pichichero et al 2008	older infants and children	12	Amox	775 sprinkle (fed)	PNA: 5-12y			0.47	0.456	NR	2	1.6	NR	34.4		8.93	
Pichichero et al 2008	older infants and children	12	Amox	775 sprinkle (fasting)	PNA: 5-12y			0.48	0.481	NR	1.5	1.51	NR	34.2		10.3	
McCracken et al 1983	older infants and children	75	Amox	25 (fasting)	PNA: 3-59mo		4.4-21					1.2		24		8.9	0.6

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
McCracken et al 1983	older infants and children	75	Amox	25 (fed)	PNA: 3-59mo		4.4-21					1.2		24		7.9	0.7
McCracken et al 1978	older infants and children	106	Amox		PNA: 2-46 mo							1.3		18		6.4±2.6	
McCracken et al 1978	older infants and children	106	Amox		PNA: 2-46 mo							1.5		25		5.8±2.8	
Marks et al 1978	older infants and children	20	Amox	12.5	PNA: 3.5-13y	PO								18.97		6.6	0.5
Marks et al 1978	older infants and children	20	Amox	25	PNA: 3.5-13y	PO								26.49		7.2	1.1
Hazir et al 2007	older infants and children	876	Amox	45 vs 90	PNA: 2-59 mo	PO											
Jones et	older	15	Am	50/kg	PNA: 2-14	IV		N	N	1	N	0.9±	0.47±0.0	130.1±1			

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
al 1990	infants and children		ox		y			A	A		A	0.14	8	8.9			
Muller et al 2009	pregnant women	44	Amox	1000-2000 total dose	adults	IV					0.5		6.4±0.6		19.7±0.99	88.7	
Muller et al 2009	cord blood	44	Amox	1000-2000 total dose	adults	IV					0.06		11.9		19.7±0.99	8	
Muller et al 2008	pregnant women	34	Amox		adults	IV							8.7±6.6		21.1 ± 4.1		
Muller et al 2008	women in labour	34	Amox		adults	IV							11.8±7.7		21.1 ± 4.1		
Muller et al 2008	postpartum women	34	Amox		adults	IV							20.5±15.4		21.1 ± 4.1		
Andrew et al 2007	first trimester	16	Amox		PNA: 29.3±4.3 y	PO	70.1 ±12.2			CL/F: 35.5±8.5		1.2± 0.5			24.8± 6.7		
Andrew et al 2007	second trimester	16	Amox		PNA: 29.3±4.3 y	PO	70.1 ±12.2			CL/F: 34.5±5.9		1.3± 0.2			24±3.9		

Author	Pop	n	Drug	Amox Dose (mg/kg/d)	Age Range	Route	weight (kg)	Ka	Ke (/hr)	F (l/h)	Tmax	t1/2 (hr)	VD (L/kg)	AUC (mg/L*hr)= (mcg/ml/hr)	Ttl body clearance L/hr)	mean peak (mcg/ml)=(mg/L)	mean trough (mcg/ml) =(mg/L)
Andrew et al 2007	peri-partum	16	Amox		PNA: 29.3±4.3 y	PO	70.1 ±12 .2			CL/F: 28.8±16		1.6± 0.2			15.3± 2.6		
Canafax et al 1998			Amox			PO										9.5 (range 0-20.6)	

Annex 5 Clinical Studies with amoxicillin use in children derived from the medical literature

Author	Center	Year	Journal	Research question	Drug	dose	regimen	mode of admin	design	sample size	PNA	Illness	Outcome Variable 1	Outcome Variable 2	Outcome Variable 3	outcome variable 4
CATCHUP Study Group	WHO, Islamabad	2001	Arch Dis Child	non severe pneumonia: equivalency of cotrim and amox	cotrim	4mg/kg	BID for 5 d	PO	RCT, double blinded	734	2-59m	pneumonia	TXFAIL: 18.9%			
CATCHUP Study Group	WHO, Islamabad	2001	Arch Dis Child	non severe pneumonia: equivalency of cotrim and amox	amox	25 mg/kg	BID for 5 d	PO	RCT, double blinded	725	2-59m	pneumonia	TXFAIL: 16.1%			
Fonseca	WHO, Ceara Brazil	2003	AAC	pneumonia: PK and efficacy of 2 dosages of amox	amox	25 mg/kg	BID for 5 d	PO		27	3-59m	pneumonia	T>MIC2.0: 48.2±12.8	Cmax:10.5±4.9	AUC: 54.7±60.2	t1/2:2.0±1.9
Fonseca	WHO, Ceara Brazil	2003	AAC	pneumonia: PK and efficacy of 2 dosages of amox	amox	15 mg/kg	TID for 5 d	PO		31	3-59m	pneumonia	T>MIC2.0: 56.4±16.1	Cmax:6.9±3.0	AUC: 24.9±9.6	t1/2:1.5±0.6
Bang	Gadchiruli	1998	Lancet	neonatal sepsis: impact of home care packages on mortality	cotrim and gent	wt bands	BID for 7 d	PO IM	39 intervention villages; 47 control	913	NB	pneumonia	mortality Rate: 27.5-6.6%	Δ20.9 (76%)		

Author	Center	Year	Journal	Research question	Drug	dose	regimen	mode of admin	design	sample size	PNA	Illness	Outcome Variable 1	Outcome Variable 2	Outcome Variable 3	outcome variable 4
Abucejo-Ladesma	Phillipines	2007	Scand J Infect Dis	meninigitis: aetiology					cohort	989	0-59m	meningitis	37% hib, 18%spneumo	all strains sensitive to cpl, cotrim, amp		
Addo-Yobo, E.	8 countries	2004	Lancet	severe pneumonia: is PO amox equivalent to parenteral penicillin	amox			PO	randomised, open-label equivalency study	875	3-59m	pneumonia	TXFAIL: 19%			
Addo-Yobo, E.	8 countries	2004	Lancet	severe pneumonia: is PO amox equivalent to parenteral penicillin	pen			IM		845	3-59m	pneumonia	TXFAIL: 19%			
Adeyemo,	Nigeria	1993	Ann Trop Paediatr	neonatal sepsis: klebiella outbreak report and AMR in nosocomial												

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
Al Zwaini	Iraq	2002	East Mediterr Health J	neonatal sepsis: aetiology					hosp cohort case series	112	NB	nn sepsis	39%S.aureus, 30% kleb, 21% ecoli	resis to amp and gent		
Arrieta, A.	California, US	2003	Antimicrob Agents Chem other	OM: equivalency of azithromycin and amoclav	Azithromycin	20 mg/kg	QD for 3 d	PO	double-blind, double-dummy	300	1-5 y	AOM	tx success: at day 12-16 (84% and 86%)	tx success: better in azithromycin at d 28-32 (85% vs 79%)		
Arrieta, A.	California, US	2003	Antimicrob Agents Chem other	OM: equivalency of azithromycin and amoclav	Amox clav	90 mg/kg	BID for 10 days	PO	double-blind, double-dummy							
Aurangzeb	Peshawar Pk	2003	J Coll Physicians Surg Pak	neonatal sepsis: aetiology and abx susceptibility patterns					hosp cohort case series	67/112 pos cx	NB	nn sepsis	77%ecoli, 8.9%Pseud/kleb, 4.4% s.aureus	GN: 79.3% amp, 74.6% amox, 71.6% ceftaz, 55% cefotax,	gen 43%, amik 22%,imip 23%, 11.9 % cipro	GP: 75%S.aureus to amox

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
Baqui	Sylhet, Bangladesh	2009	Trop Med Int Health	neonatal sepsis: comm-based validation of CHW assess of NB illnesses									classified severe disease sens 91%, spec 95% K 0.85	sens> 60% spec 97-100% recognition signs and symptoms		
Baqui	Sylhet, Bangladesh	2008	Lancet	neonatal sepsis: impact of home care packages on mortality					cluster randomized				mortality rate: dec by 34% (adj RR 0.66; 95% CI 0.47-0.93)			
Bartlett	Guatemala	1991	Pediatr Infect Dis J	neonatal sepsis: infections in nb cohort in indigenous rural community					cohort study	329			38 episodes seriosu illness, 35 sepsis or pneumonia			
Bhandari		1996	Lancet	young infant sepsis: Treating sick young infants in urban slum												

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
				setting												
Bolme	ethiopia	1995	Pharmacol Toxicol	Malnourished: penicillin pk in malnourished children									decreased CL and VD in malnourished.F was decreased if given non fasting			
Canafax	minnesota, US	1998	Pediatr Infect Dis J	OM: amox pk in AOM	amox	40 mg/kg		PO					MEF cx positive 23 of 40 ears (57.5%) before tx; txfail:4 of 38 ears (10.5%)			
Dagan	Israel	2003	Int J Infect Dis	OM:increase dose of penicillin for penicilin reisstant GP organisms	amox-clav	90/kg	BID	PO	open-label, non-comparative study			OM	high doses of penicillin can be used to tx penicillin reisstant s.pneumoniae			
Daniel		1993	J Clin Invest	ADME amox:									amino-penicillins	penicillins are		

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
				affinity for oligopeptide/H+ symporter									like amox (alpha-amino group in the phenylacetamido moiety) had low affinities	transported by a less discriminative system		
Fu	Washington, US	2006	Pediatrics	severe pneumonia: hypoxemia as predictor of amox failure	amox vs penicillin					857	3-59m	pneumonia	oximetry at 0,12 and 24 hours improved predictive ability			
Ganapathy		1995	J Biol Chem	ADME amox: differences in b lactams recognition at PEPT1 &2 receptors	cefedroxil vs cyclacillin								Cyclacillin was 9-fold more potent in competing with glycylsacosine for uptake via PEPT 1.cefadroxil was 13-fold more potent in			

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
													competing with the dipeptide for uptake via PEPT 2.			
Garrison		2004	Ann Pharmacother	OM: High-dose versus standard-dose amoxicillin for acute otitis media		45/kg vs 90 mg/kg		PO	double blinded randomzied				abx fail rate with SD & HD (95% CI -1.5% to 3.4%; p = 0.78); mean duration of illness same in both gps			
Gatchalian	Phillipines	1999	Pediatr Infect Dis J							35/873	0-90 d	sepsis	meningitis: Acineto(4), S.pneum(2), Ecoli (2), Enterobac (1), P.aeru (1), H.flu(1) S.aureus (1)	sepsis: Salm(6), Enterob (3), S.pyog(3), GN(8), S. pneum(1), S.aureus (2)		
Ghuyse	Belgiu	1991	Annu													

Author	**Center**	**Year**	**Journal**	**Research question**	**Drug**	**dose**	**regimen**	**mode of admin**	**design**	**sample size**	**PNA**	**Illness**	**Outcome Variable 1**	**Outcome Variable 2**	**Outcome Variable 3**	**outcome variable 4**
n	m		Rev Microbiol													
Giachetto	Montevedeo, Uruguay	2004	Pediatr Infect Dis J	Pneumonia: amp and pen in serum and pleural fluid	amp	400 mg/kg/d				17			serum concentrations were >4 microg/ml for >40% of the interdose interval			
Giachetto	Montevedeo, Uruguay	2004	Pediatr Infect Dis J	Pneumonia: amp and pen in serum and pleural fluid	pen	200000 IU/kg/d	QID			13			serum concentrations were >4 microg/ml for >40% of the interdose interval			
Gordon	Australia	2005	Cochrane Database Syst Rev	neonatal sepsis: Abx regimens for late onset sepsis in NB infants						24			one study comparing blactam with blactam and aminoglycoside	no diff mortality (RR 0.17, 95% CI 0.01 to 3.23) or tx fail (RR 0.17, 95% CI		

Author	Center	Year	Journal	Research question	Drug	dose	regimen	mode of admin	design	sample size	PNA	Illness	Outcome Variable 1	Outcome Variable 2	Outcome Variable 3	outcome variable 4
														0.01 to 3.23).		
Gratten		1991	P N G Med J	neonatal sepsis: aetiology of meningitis and pneumonia									73% h.flu and s.pneumo, 85% blood, 92% CSF	pen resis in 75% of all carriage strains, 52% and 22% of blood and CSF isolates		
Guibert		1985	Chem iotera pia	single and multiple dose PK of LAMP and AMPC									no measurable accum no remarkable variations btw the single- and multiple-dose PK			
Herngren		1987	Eur J Clin Pharm	neonatal sepsis: Pk free and												

Author	Center	Year	Journal	Research question	Drug	dose	regimen	mode of admin	design	sample size	PNA	Illness	Outcome Variable 1	Outcome Variable 2	Outcome Variable 3	outcome variable 4
			acol	total fluclox in NB infants												
Jehan	pakistan	2009	Bull World Health Organ	nenatal sepsis: in a community cohort					pop-based cohort study				23% neonatal mortality due to infection			
Jones		1990	J Antimicrob Chemother	tonsillectomy: cefaclor vs amoxicillin									recovery same in both groups.			
Kacet		1992	Pediatr Infect Dis J							28	0-28					
Lazzerini		2011	Bull World Health Organ	SAM: review evidence supporting abx use in SAM					lit review	18 studies			1RCT: cro and amox equally effective; amp&gent dec mortality OR: 4.0; 95% CI: 1.7-9.8;			
Litzhow		2009	Infect Control Hosp Epide										358 (19.6%) of 1831 became bacteremi	20% resis imip, 41% to TMP.S		

Author	Center	Year	Journal	Research question	Drug	dose	regimen	mode of admin	design	sample size	PNA	Illness	Outcome Variable 1	Outcome Variable 2	Outcome Variable 3	outcome variable 4
			miol										c	MX; 52% amik and 63%amp-sulb		
Marks		1978	J Clin Pharmacol	amp and amoxil												
Adam		1982			PO Amox in healthy adults			PO Amox		12	26.1 ±2.5 y					
Averono	Italy	2010	Int J Pediatr Otorhino	amox levels in plasma and tissue of tonsillectomy pts				PO Amox		33						