

Neonatal Sepsis Epidemiology in a Rural Province in Southeastern Cambodia, 2015–2017

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Abstract. Neonatal sepsis is the second most prevalent cause of neonatal deaths in low- and middle-income countries, and many countries lack epidemiologic data on the local causes of neonatal sepsis. During April 2015–November 2016, we prospectively collected 128 blood cultures from neonates admitted with clinical sepsis to the provincial hospital in Takeo, Cambodia, to describe the local epidemiology. Two percent ($n = 3$) of positive blood cultures identified were Gram-negative bacilli (GNB) and were presumed pathogens, whereas 10% ($n = 13$) of positive blood cultures identified were likely contaminants, consistent with findings in other published studies. No group B *Streptococcus* was identified in any positive cultures. The presence of GNB as the primary pathogens could help influence local treatment guidelines.

Neonatal deaths are the largest contributor to the global mortality of children aged less than 5 years, and sepsis is the second most prevalent diagnosis among neonatal deaths in low- and middle-income countries (LMICs).¹ Most evidence informing guidelines for the treatment of neonatal sepsis in LMICs come from the United States and Western Europe, where the epidemiology of this disease appears to be very different from what is observed in LMICs. There is a lack of published data on the epidemiology of neonatal sepsis in Cambodia, which has a high rate of poverty nationwide and a neonatal mortality rate of 16 per 1,000 live births as of 2016.¹ National neonatal sepsis guidelines for Cambodia recommend collecting a complete blood count, C-reactive protein, and blood culture, and administering ampicillin and gentamicin as empiric therapy in cases of suspected sepsis, as is a common practice in many Western countries. However, studies of blood culture isolates from children conducted in large, urban hospitals in Cambodia^{2,3} and neighboring countries^{4–7} have identified organisms such as *Staphylococcus aureus* and Gram-negative bacilli (GNB) in cases of pediatric sepsis. *Streptococcus suis*⁸ and *Burkholderia pseudomallei*,⁹ seen in older children and adults in Cambodia, although rarely reported in neonates, may have restricted antimicrobial susceptibility patterns and could affect antibiotic therapy choices.

No information on neonatal sepsis has been published to date from the large rural areas of the country, which could represent different epidemiology from the limited available data in more urban areas of Cambodia. We gathered blood cultures from neonates from birth to 28 days of life with clinical signs and symptoms of sepsis to define the epidemiology of bacterial causes of neonatal sepsis. This was conducted as a sub-study within a larger interventional project aiming to reduce neonatal deaths in the rural Takeo Province of southeastern Cambodia through community health worker capacity building, improved infection control practices in community health centers, and improved referral timeliness of ill neonates to local health facilities. A full description of the outcomes of this project is forthcoming.

Takeo Provincial Hospital has a large, rural population catchment area of 927,896 persons as of the 2014 census,¹⁰ with roughly 140,000 of those children aged less than 5 years. The hospital had 112–304 neonatal admissions annually between 2011 and 2014. Fifty-five to sixty-five percent of all neonatal admissions between 2011 and 2014 reported deliveries in the Takeo Provincial Hospital, with the remaining in community health centers, referral centers, private clinics, or rarely at home. Because of this, referral of infants for possible sepsis frequently comes from the community rather than infants born in the hospital. The purpose of this study was to describe for the first time the local epidemiology of the bacterial causes of neonatal sepsis in rural southeastern Cambodia.

Infants aged 28 days or less hospitalized in the Neonatal Section of the Takeo Provincial Hospital and presenting with one or more of the WHO-designated signs of possible sepsis were eligible for inclusion in the study: axillary temperature $> 37.5^{\circ}\text{C}$ or $< 35.5^{\circ}\text{C}$, convulsions, lethargy, poor feeding, subjective jaundice, sternal retractions, rapid respirations ($> 50/\text{minute}$), umbilical pus or redness, and > 10 skin pustules.¹¹

After a verbal consent process (written consent was waived by the local ethics board), study staff completed a questionnaire in Khmer, collected a 0.5- to 1-cc blood specimen using an aseptic technique with a butterfly needle, and inoculated the blood specimen into pediatric blood culture bottles using a previously published algorithm.¹² Agar is made with hair sheep blood from locally raised sheep, a sustainable source for microbiology laboratories in tropical settings.¹³ All media, including that in blood culture bottles; biochemical tube media for identification of GNB; and sheep blood, chocolate, MacConkey, and Mueller Hinton agars, are produced under strict quality control conditions with the support of the Diagnostic Microbiology Development Program's (DMDPs) microbiologists at the Central Media Making Laboratory at the University of Health Sciences, Phnom Penh. The DMDP is a global microbiology laboratory support nongovernmental organization that has worked to enhance local capacity in multiple hospitals in Cambodia since 2011. Blood cultures were processed at the Takeo Provincial Hospital microbiology laboratory; specimens were incubated for 7 days and checked once daily based on the microbiology laboratory's protocol. No automated system for incubation or monitoring is available, so organisms were identified by Gram stain and basic

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biochemical testing.¹⁴ The DMDP reviews the annual Clinical Laboratory Standards Institute (CLSI) recommendations and institutes them annually in their supported laboratories; for this study, CLSI M100 S25 and CLSI MO2 A12 were used for antibiotic susceptibility testing by the disk diffusion method. Results were then reported to the ordering physician, who was responsible for treatment decisions. The study was approved by the National Ethics Committee for Health Research, Kingdom of Cambodia, National Institute of Public Health (397NECHR), and by Tulane University Institutional Review Board (IRB) (15-812593IAA) through an IRB authorization agreement (IRB registration number 00003142, FWA00017450).

A total of 128 neonates aged ≤ 28 days with at least one danger sign of sepsis were admitted to the Takeo Provincial Hospital between April 2015 and November 2016, and 125 had a blood culture obtained. More than half of the patients were male ($n = 73$, 56%), and 106 (84%) resided in Takeo Province. Neonates were delivered in various facilities, with 75 (59%) delivered in a provincial hospital, 24 (19%) in a health center, 14 (11%) in a referral hospital, 11 (9%) in a private clinic, and 1 (0.8%) at home with a trained midwife. Sixty-four (50%) had reported symptoms for less than 1 day and 49 (38%) for 1–3 days, and the remainder had symptoms for ≥ 4 days, with three missing data or refusing to respond, for an overall mean of onset of illness at 2.4 days of life. A total of 20 infants were seen by health-care professionals before evaluation at the Takeo Provincial Hospital: nine of those at a health center, six in a private clinic, three in a referral hospital, one at the National Children's Hospital, and one was missing specific data. Of 105 with documented responses, three (3%) had antibiotics administered before admission in the community.

For the WHO-designated danger signs of neonatal sepsis in infants in this group, 83 (65%) reported temperature $> 37.5^\circ\text{C}$ and 15 (12%) $< 35.5^\circ\text{C}$, and 8 (6%) had convulsions, 11 (9%) lethargy, 38 (30%) poor feeding, 23 (18%) jaundice, 19 (15%) chest indrawing, 59 (46%) fast breathing, 28 (22%) umbilical pus/redness, and 6 (5%) > 10 skin pustules. Physician-assigned source of infection was sepsis in 5 (4%) neonates, chorioamnionitis in 29 (23%), acute respiratory illness in 11 (9%), umbilical cord discoloration/infection in 15 (12%), fever in 6 (5%), skin disease in 12 (9%), multiple sources in 26 (20%), and no diagnosis in 47 (37%).

A total of 16 blood cultures obtained from infants admitted with clinical signs of sepsis were positive: three positive cultures were enteric GNB that likely represent true pathogens, two were Gram-positive rods likely representing skin contaminants, and 11 were coagulase-negative staphylococci (CoNS), which are usually considered contaminants. None of the three neonates who received antibiotics before admission had a positive blood culture. Two of the three patients with a positive culture for GNB had fever reported as the source of the infection, and one was missing the information on the data form. All three with a GNB-positive culture reported a history of fever on admission: the patient with *Escherichia coli* also reported convulsions and poor feeding; the patient with *Klebsiella pneumoniae* reported fast breathing; and the patient with *Acinetobacter* reported fast breathing, chest indrawing, and poor feeding. All three received at least two antibiotics on admission: one received ampicillin, gentamicin, and ceftriaxone, and the other two received gentamicin and ceftriaxone.

These results, although only including a small number of positive cultures and preliminary results of a larger project, identified GNB as the bacterial etiology of neonatal sepsis in

this rural Cambodian population, which is similar to findings of other studies in the region, including other areas of Cambodia, Vietnam, and Thailand.^{3,5,7} No Group B *Streptococcus* was found, which correlates with findings in other developing countries in the Southeast Asia region as well.^{15,16} The proportion of presumed true positive blood cultures at 2.3% is within the range of true positives in studies in the United States and the United Kingdom (2–12%).^{17,18} Potential contamination was found with CoNS and Gram-positive rods, but the 10% found in this study falls within what is also found in the United States (0.6–12.5%),¹⁹ although above the recommended goal of $< 3\%$. Coagulase-negative staphylococci may be considered a pathogen in low birth weight neonates as well, which complicates this distinction.²⁰ The availability of locally produced, low-cost, quality-controlled diagnostics supported by DMDP allowed for blood cultures to be performed and results to be communicated to clinicians in this setting. Overall, the small sample size and number of positive blood cultures in this study limit interpretation, but remain consistent with findings in Southeast Asia. These blood culture data add to the growing body of evidence that focusing the treatment guidelines for neonatal sepsis on GNB rather than GBS would be advisable, along with encouraging local infection control and prevention measures for enteric organisms and continued larger scale blood culture surveillance. In the future, it may be advisable to try to obtain blood culture data in the community as early as possible and repeat these surveillance activities in other geographic areas of the country to get as representative of a sample as possible to continue better informing national guidelines.

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