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ORIGINAL ARTICLE

A high burden of late-onset sepsis among newborns admitted to the largest neonatal unit in central Vietnam

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OBJECTIVE: The objective of this study is to determine the prevalence, causes and outcome of sepsis in hospitalized neonates in the largest neonatal unit in central Vietnam.
STUDY DESIGN: A 1-year prospective cohort study of newborns admitted to the neonatal unit in Da Nang. A sepsis work-up including blood culture was undertaken before commencing antibiotics for neonates with suspected sepsis.
RESULT: Of 2555 neonatal admissions, 616 neonates had 729 episodes of suspected invasive sepsis. A pathogen was isolated from blood in 115 (16%) episodes in 106 neonates. The prevalence of early-onset sepsis (EOS) was 8 (95% confidence interval (CI): 4 to 11) per 1000 admissions, and of late-onset sepsis (LOS) was 34 (95% CI: 27 to 41) per 1000 admissions. Of 86 neonates with LOS, 69 (80%) also fulfilled the criteria for nosocomial sepsis. The commonest bacterial causes of EOS were coagulase-negative *Staphylococcus* (CoNS) and *Staphylococcus aureus*, and of LOS were *Acinetobacter,* CoNS and *Klebsiella pneumoniae*. Fungal sepsis occurred in 35 neonates of which most were nosocomial sepsis. *In vitro* resistance to multiple antibiotics was common among Gram-negative bacteria. Antibiotics were prescribed and given to 68% of all admissions, and 14% of all admissions received four or more different antibiotics. The case fatality rate for confirmed sepsis was 46%.
CONCLUSION: Late-onset, nosocomial sepsis was common and associated with a high mortality in hospitalized newborns in the largest neonatal unit in central Vietnam. These findings highlighted the need for improved infection control measures and antibiotic stewardship, which have since been implemented.

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INTRODUCTION

Sepsis is a leading cause of neonatal death globally and the burden of neonatal sepsis is greatest in the resource-poor countries.[1,2] Neonatal sepsis is due to a wide range of bacterial pathogens that challenges the development of effective antibiotic guidelines.[3] The range and relative importance of pathogens causing neonatal sepsis may vary between geographical settings and depends upon other variables such as the timing of infection (for example, perinatal or postnatal) or whether the infection was community-acquired or hospital-acquired.[3–6] There has also been an increase in the prevalence of neonatal sepsis due to multidrug-resistant pathogens, particularly common as hospital-acquired infections.[6–8] However, recent reviews highlight that data from the resource-limited setting remain sparse.[3,9,10]

The region of south-east Asia is one of the most populous regions in the world, and neonatal mortality is responsible for a large and increasing proportion of overall child mortality in this region.[2] Yet, a recent systematic review found that there are very few publications describing the causes of neonatal morbidity and mortality, and in particular the prevalence of neonatal sepsis from the south-east Asian region, especially from the resource-limited settings within that region.[11] A recent multisite study from the south-east Asia region reported the causes and outcomes for neonatal sepsis, but the sites included were neonatal intensive care units in resource-rich settings.[12]

We recently undertook a prospective study of all-cause morbidity and mortality among newborns admitted to a neonatal unit in central Vietnam.[13] Here, we report the prevalence, causes and outcome of newborns with suspected sepsis, a summary of the pathogen isolated from blood culture and antibiotic susceptibility *in vitro*.

METHODS

We conducted a prospective cohort study including all newborns admitted to the Da Nang Hospital for Women and Children, Vietnam, over a 1-year period (1 November 2010 to 31 October 2011). Final follow-up data for all study participants were collected up to 10 weeks after enrollment. Details of the study setting and overall methodology are presented elsewhere.[13]

Study setting

The neonatal unit of Da Nang Hospital is the largest neonatal unit in central Vietnam. All sick neonates born in Da Nang city are referred to the unit, as well as critically ill neonates from the surrounding provinces. Criteria for inborn admission are all newborns with birth weight <2000 g or sick neonates of any weight or gestation. The unit has a 60-cot capacity that manages ~60 to 100 inpatients each day, and is made up of a neonatal intensive care unit of 30 cots and a high dependency unit of 30 cots. The neonatal intensive care unit has a nurse:patient ratio of 1:6, has six ventilators for intermittent positive-pressure ventilation and can provide continuous positive airway pressure for an additional 15 neonates. Central catheters are used by umbilical vein or peripherally inserted central

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catheters, and total parenteral nutrition is provided for eligible preterm neonates. The high dependency unit has a nurse:patient ratio of 1:20. There is one basin for hand-washing per five cots, and bedside alcohol is provided. The median length of stay for all newborns during this study period was 6 (interquartile range 4 to 9) days.[13]

Study procedures

Data were collected on all admissions using a standardized case report form and included demographics, antenatal history, birth history, birth weight and gestational age, primary and secondary diagnoses and clinical findings. Clinical progress was closely monitored as per routine nursing care, and clinical data of progress were collected using the standardized case report until death or discharge.

A septic work-up was undertaken before commencing antibiotics for neonates with suspected sepsis on the basis of clinical investigation: such as the presence of fever, hypothermia, mottling skin, respiratory distress (which cannot be explained by respiratory conditions such as the respiratory distress syndrome or meconium aspiration), poor feeding, diminished activity and lethargy or seizures. A septic work-up included at least a venipuncture for full blood count, C-reactive protein and blood culture with additional investigations depending on the clinical presentation. The standard first-line antibiotic treatment for suspected sepsis in the neonatal unit is a combination of ampicillin and gentamicin.

For blood culture, 2 ml of venous blood were drawn and incubated in the brain–heart infusion broth at 37 °C for 7 days. If bacteria grew, Gram stains were made and the samples were subsequently cultured on blood agar and MacConkey agar to identify the bacteria. Sabouraud agar was used to isolate cases of suspected fungi. Susceptibility to antibiotics was assessed by performing a disc diffusion technique using Mueller–Hinton agar.

Confirmed sepsis was defined as a positive culture of a single pathogen from venous blood or other normally sterile sites. Early-onset sepsis (EOS) was defined as sepsis occurring before or at 72 h of age, whereas late-onset sepsis (LOS) was defined as sepsis occurring after 72 h of age. Nosocomial infection was defined as sepsis that occurred after 72 h of admission to the neonatal unit and is a subset of LOS.

Data analysis

All analyses are presented treating each admission as an independent event, ignoring the fact that occasionally there were repeat admissions in the same infant. As repeat admissions were rare ($<1\%$), this was not expected to affect the results. The prevalence of EOS and LOS is presented as the proportion of neonates with sepsis relative to the total number of admissions. Risk factors including maternal infection, gestational age and birth weight for EOS or nosocomial sepsis were explored using logistic regression, with results presented as odds ratios and 95% confidence interval. Initially, each predictor was assessed using a separate univariable model, with multivariable regression then used to identify independent predictors among those significant ($P<0.05$) in the univariable model. Variables included in the multivariable model for nosocomial sepsis included gestational age, use of central catheter, having had surgery or transfusion. Gestational age was highly correlated with birth weight, and so the birth weight was not included in multivariable analysis. We did not include invasive respiratory support into the multivariable model because respiratory support is also a consequence of infection. The numbers and proportions of pathogens were isolated and their antibiotic susceptibility were calculated.

Ethical approval

The study was approved by the Scientific Board of Da Nang Hospital and the Human Research Ethics Committee of the University of Melbourne.

RESULTS

Overall, there were 2555 neonatal admissions and 220 deaths (8.6%) during the 1-year study period. Of the admissions, 1364 (53%) were inborn, 684 (27%) were born at home and presented directly to the hospital, and 507 (20%) were referred in from outlying health facilities. There were 616 neonates having 729 episodes of suspected invasive sepsis with blood cultures performed, and of the episodes, 223 (31%) presented as suspected EOS and 506 (69%) as suspected LOS. Pathogens were isolated from blood in 115 (16%) episodes of suspected sepsis, and comprises 75 bacterial pathogens and 40 fungal pathogens. There were no pathogens isolated from other sites. There were five newborns with a clinical diagnosis of meningitis, but none had a positive culture from the cerebrospinal fluid.

The 115 episodes of confirmed sepsis with positive blood cultures occurred in 106 neonates, which represented 4% of all admissions. There were 99 neonates with 1 pathogen isolated from a single episode. The other seven neonates had a positive blood isolate from two ($n=5$) or three ($n=2$) separate episodes, with it being two or more different pathogens in six of these. The median (interquartile range) time to detect a pathogen was 5 (refs. 4–7) days.

Pathogens and characteristics associated with onset of sepsis

Twenty neonates (19% of those with confirmed sepsis) had EOS, equating to a prevalence of 8 (95% confidence interval: 4 to 11) per 1000 admissions, and there were 8 deaths. The commonest causes of confirmed EOS were coagulase-negative *Staphylococcus* (CoNS*), Staphylococcus aureus* and *Acinetobacter* (Table 1). Eighty-six neonates (81%) had LOS, equating to a prevalence of 34 (95% confidence interval: 27 to 41) per 1000 admissions, and there were 41 deaths. The commonest causes of confirmed LOS were *Candida,* and other fungi, followed by *Acinetobacter*, CoNS and *Klebsiella pneumoniae*. Of the 86 neonates with LOS, 69 (80%) also fulfilled the criteria for nosocomial sepsis, and so the spectrum of pathogens for nosocomial sepsis was similar to that for LOS, with fungal pathogens being the causative pathogen in around half of the nosocomial sepsis cases.

Of the 75 episodes of bacterial sepsis, 19 (25%) were EOS and 56 (75%) were LOS (Table 1). Gram-positive bacteria were more commonly isolated in cases of confirmed bacterial sepsis with confirmed EOS (13/19 or 68%) than in those with confirmed LOS (15/56 or 27%) in which Gram-negative bacteria predominated. Almost all (93%) cases of fungal sepsis were among those classified as nosocomial sepsis. Fungal sepsis occurred in 35 neonates representing a prevalence of 14 per 1000 admissions. Among neonates with fungal sepsis, 69% were preterm (<37 weeks) neonates and 60% were <34 weeks' gestational age. Of the confirmed nosocomial sepsis cases, 44% occurred in very low birth weight (<1500 g) babies.

Neutropenia (white cell count $<5000\,\mathrm{mm}^{-3}$) and thrombocytopenia (platelet count $<100\,000\,\mathrm{mm}^{-3}$) were more commonly associated with episodes of confirmed sepsis compared with episodes with suspected but unconfirmed sepsis (13% versus 4% and 39% versus 14%, respectively; $P<0.001$ for both). A C-reactive protein $\geqslant 48\,\mathrm{mg\,l}^{-1}$ was also significantly more common (53%) in episodes of confirmed sepsis than for suspected but unconfirmed sepsis (53% versus 31%, respectively; $P<0.0001$).

Table 2 explores patient characteristics as predictors of confirmed EOS and confirmed nosocomial sepsis in all admissions, that is, including those with suspected but unconfirmed sepsis plus those without suspected sepsis. There was evidence that maternal infection was associated with an increased odds of confirmed EOS, but not with nosocomial sepsis. There was little evidence that gestational age or being small for gestational age were associated with confirmed EOS. Confirmed nosocomial sepsis was strongly associated with prematurity <32 weeks and with the use of transfusions and central catheter insertion. Eighty (75%) of the neonates with confirmed sepsis had invasive respiratory support at some stage during their stay in the unit. This was more common for LOS cases than EOS cases, 83% (71/86) and 45% (9/20), respectively.

Neonates with suspected but unconfirmed sepsis had a higher gestational age compared with neonates with confirmed sepsis (mean of 37 (s.d. 3) weeks versus mean 35 (s.d. 4) weeks,

respectively, $P<0.0001$) and a lower case fatality rate (14.1% versus 46.2%, $P<0.0001$). Comparison of clinical features between LOS cases with suspected sepsis that were confirmed and those that were not confirmed found that lethargy, respiratory distress, apnea and need for increased respiratory support were all significantly associated with confirmed sepsis (data not shown).

Table 1. Type and frequency of blood isolate in neonates with early-onset sepsis, late-onset sepsis and nosocomial sepsis

Pathogen	*Total*		*Early-onset sepsis*		*Late-onset sepsis*		*Nosocomial sepsis*[a]	
	n	%	n	%	n	%	n	%
Bacteria								
Coagulase-negative Staphylococcus	23	20	9	45	14	15	6	8
Acinetobacter	17	15	3	15	14	15	14	18
Klebsiella pneumoniae	12	10	2	10	10	11	9	12
Enterobacteria spp	6	5	0	0	6	6	5	7
Pseudomonas aeruginosa	4	4	0	0	4	4	1	1
Klebsiella ozaenae	5	4	0	0	5	5	3	4
Staphylococcus aureus	4	4	3	15	1	1	1	1
Escherichia coli	2	2	1	5	1	1	0	0
Streptococcus	1	1	1	5	0	0	0	0
Other Klebsiella spp	1	1	0	0	1	1	1	1
Fungi								
Candida	24	21	0	0	24	25	23	30
Other Fungi	16	14	1	5	15	16	14	18
Total	115		20		95		77	

[a]Nosocomial sepsis is a subset of late-onset sepsis. Early-onset sepsis was defined as sepsis occurring before or at 72 h of age, late-onset sepsis was defined as sepsis occurring after 72 h of age and nosocomial sepsis was defined as sepsis that occurred after 72 h of admission to the neonatal unit.

Table 2. Characteristics associated with the occurrence of confirmed early-onset sepsis and confirmed nosocomial sepsis

			Univariable analysis		*Multivariable analysis*	
Characteristics	*Confirmed sepsis*	*Other neonates*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
Early-onset sepsis	*N = 20*	*N = 2535*				
Maternal infection	3 (15)[a]	123 (5)	3.5 (1.0, 12.3)	0.047	3.5 (1.0, 12.3)	0.047
Gestational age, mean (s.d.) weeks	37 (4)	37 (3)	0.95 (0.8, 1.1)	0.48		
Birth weight, mean (s.d.) grams	2570 (78)	2737 (73)	0.97 (0.9, 1.0)	0.31		
Small for gestational age	3 (15)	259 (10)	1.5 (0.5, 5.3)	0.49		
Male	11 (55)	1414 (56)	1.0 (0.4, 2.3)	0.94		
Nosocomial sepsis	*N = 69*	*N = 2486*				
Maternal infection	3 (4.6)	123 (5.1)	0.9 (0.3, 2.8)	0.83		
Birth weight (g)[b]						
⩾2500	25 (36)	1662 (67)	1		[b]	
2000–2499	11 (16)	463 (19)	1.6 (0.8, 3.2)	0.21		
1500–1999	16 (23)	254 (10)	4.2 (2.2–7.9)	< 0.0001		
1000–1499	17 (25)	76 (3)	14.9 (7.7–28.7)	< 0.0001		
< 1000	0	26 (1.0)				
Male	43 (62)	1382 (56)	1.3 (0.8, 2.2)	0.27		
Small for gestational age	9 (13)	253 (10)	1.3 (0.6, 2.7)	0.45		
Gestational age, weeks						
⩾37	25 (36)	1708 (69)	1		1	
34–36	16 (23)	513 (21)	2.1 (1.1, 4.0)	0.020	1.7 (0.8, 3.3)	0.15
32–33	6 (9)	146 (6)	2.8 (1.1, 7.0)	0.026	1.0 (0.4, 2.9)	0.89
30–31	13 (19)	66 (3)	13.5 (6.6, 27.5)	< 0.0001	3.0 (1.2, 7.3)	0.017
28–29	7 (10)	26 (1.0)	18.4 (7.3, 46.3)	< 0.0001	3.3 (1.1, 10.2)	0.034
26–27	2 (3)	14 (0.5)	9.8 (2.1, 45.2)	0.004	1.6 (0.2, 10.3)	0.37
< 26	0	13 (0.5)				
Central catheter	21 (30)	52 (2)	20.5 (11.4, 36.6)	< 0.0001	5.3 (2.5, 11.3)	< 0.0001
Surgery	5 (7)	58 (2)	3.3 (1.3, 8.4)	0.014	1.0 (0.3, 3.2)	0.97
Blood transfusion	43 (62)	173 (7)	22.1 (13.3, 36.9)	< 0.0001	12.7 (7.1, 22.8)	< 0.0001

Abbreviations: CI, confidence interval; IQR, interquartile range; OR, odds ratio. [a]Data are presented as number (percentage of total for column) unless otherwise indicated. [b]Birth weight was highly correlated with gestational age, and so gestational age, and not birth weight, was included in the multivariable analysis.

Table 3. Prevalence of *in vitro* susceptibility to antibiotics among bacterial isolates[a]

	Gram-negative bacteria					*Gram-positive bacteria*	
	Acinetobacter	*Enterobacter spp*	*Klebsiella pneumoniae*	*Klebsiella ozaenae*	*Pseudomonas aeruginosa*	*Coagulase-negative staphylococci*	*Staph aureus*
Cephalothin	0 (3)[a]	0 (4)	0 (11)	0 (5)	0 (2)	36 (22)	75 (4)
Cefuroxime	33 (3)		8 (12)	0 (5)	0 (2)	30 (23)	
Cefotaxime	24 (17)	25 (4)	8 (11)	0 (5)	0 (1)	27 (22)	75 (4)
Ceftazidime	83 (8)	50 (4)	17 (12)	0 (5)	50 (4)	40 (5)	
Cefepime	65 (17)	50 (4)	18 (11)	0 (5)	75 (4)		
Gentamicin	17 (17)	60 (5)	18 (11)	0 (5)	25 (4)	77 (22)	100 (4)
Amikacin	82 (17)	0 (4)	83 (12)	100 (5)	50 (4)		
Tobramycin	17 (12)	50 (4)	25 (12)	50 (4)	50 (4)		
Ciprofloxacin	82 (17)	67 (3)	36 (11)	0 (5)	100 (4)	78 (19)	75 (4)
Ticarcillin	67 (15)		30 (10)	100 (5)	75 (4)		
Imipenem	94 (16)	100 (3)	100 (9)	100 (5)	100 (3)		
Oxacillin						43 (23)	100 (3)
Vancomycin						100 (23)	100 (4)

[a]Data are presented as percentage susceptible with number tested for that antibiotic in brackets.

It is uncertain how many of the CoNS isolates were contaminants. For example, only 2 of the 23 CoNS had a central catheter at the time of the blood culture. On the other hand, nine CoNS cases were EOS and CoNS, and would be considered an unusual cause of community-acquired EOS. Further, the use of respiratory support or transfusion was less common in those with CoNS than in those with other bacterial isolates: 35% (8/23) and 17% (4/23) versus 81% (42/52) and 60% (31/52), respectively.

Of the 40 neonates with fungal sepsis, 22 had a birth weight of <2000 g and all 22 received total parenteral nutrition. Those with fungal sepsis were more likely to have required respiratory support or had a transfusion than those with bacterial sepsis: 93% (37/40) and 70% (28/40) versus 67% (50/75) and 47% (35/75), respectively, Fisher's exact test $P=0.003$ and 0.02. However, when CoNS were excluded from this analysis, there were no significant differences for these variable between fungal and bacterial sepsis cases.

Antibiotic susceptibility and usage

Results of antibiotic susceptibility testing for all 75 bacterial isolates are listed in Table 3. *In vitro* resistance to multiple antibiotics was common among Gram-negative bacteria, especially to cephalosporins and aminoglycosides. *Klebsiella* species were commonly resistant to four different antibiotic groups, and imipenem was the only antibiotic to which most Gram-negative pathogens showed *in vitro* susceptibility. Multidrug-resistant Gram negatives such as *Klebsiella* species and *Pseudomonas* were common in LOS or nosocomial sepsis cases (Table 3). All isolates of *Staphylococcus* showed *in vitro* susceptibility to vancomycin.

Antibiotics were prescribed and given to 1732 (68%) of all admissions; 14% of all admissions received four or more antibiotics, with a maximum number of nine different antibiotics being used in one patient. Among these 1732 newborns, 350 (20%) received antibiotics to treat noninvasive umbilical or skin sepsis, 616 (36%) for suspected invasive sepsis with blood culture performed, and a further 766 (44%) cases received antibiotics for suspected sepsis but did not have blood taken for culture.

Outcomes

Overall, the case fatality rate for those with confirmed sepsis was 46% (49/106). This compares with a case fatality rate of 17% (87/510) for the newborns that had at least one episode of clinically suspected sepsis that was not confirmed from blood culture, and of 10% (74/766) for the other newborns that had at least one episode of clinically suspected sepsis but did not have blood culture taken. The case fatality rate was similar for confirmed EOS and LOS, 40% and 48%, respectively. The case fatality rate was also similar between those with confirmed bacterial sepsis, 45% (32/71), and for those with confirmed fungal sepsis, 49% (17/35). Of neonates with suspected sepsis who had blood culture performed, the case fatality rate was higher for those with confirmed sepsis compared with those with unconfirmed sepsis: 40% versus 18% for EOS ($P=0.02$) and 48% versus 14% for LOS ($P<0.001$).

Confirmed sepsis was associated with 22% (49/220) of all deaths that occurred in the neonatal unit over the 1-year study period, and there were a further 40% (87/220) of deaths where sepsis was suspected. Of deaths due to confirmed sepsis, 84% (41/49) were in neonates with LOS.

DISCUSSION

This study provides original data from the largest neonatal unit in central Vietnam, and shows that neonatal sepsis is common and is associated with a high mortality in this setting. The incidence of confirmed EOS and LOS per 1000 admissions was higher than that reported recently from a large multisite study of neonatal sepsis that included neonatal intensive care units in China, Malaysia, Thailand and Hong Kong.[12] Further, the overall case fatality rate of 46% found in our study was three times higher than that reported from the multisite study.[12] These differences are likely to reflect differences in socioeconomic indicators, available resources and practices for perinatal and neonatal care, as well as differences in the spectrum of pathogens causing sepsis and blood culture practices affecting microbiological yield.

The results of our study can be compared with findings from a 1-year audit of neonatal admissions to the largest pediatric hospital in Ho Chi Minh City to which sick newborns are referred from health facilities based both within the city and outside in the provinces of southern Vietnam.[14,15] Around two-thirds of the newborns in the Ho Chi Minh City audit were referred from outlying provinces after birth, and so represent a different population to our study where the majority were babies born in the same hospital or at home in Da Nang.[14] Of 5763 neonatal admissions in 1 year in the Ho Chi Minh City study, 2202 had blood cultures performed, and there were 399 episodes of culture confirmed sepsis from 385 neonates and 64 (17%) died.[15] The

majority had bacterial sepsis with fungal sepsis accounting for only 3% of confirmed sepsis, compared with 48% in our study. They also reported a higher proportion (84%) of LOS cases, presumably reflecting the referral pattern to that hospital. Nonetheless, their spectrum of bacterial isolates was similar to our study, with the commonest isolates being CoNS and Gram negatives such as *Klebsiella* and *Acinetobacter* species that were commonly resistant *in vitro* to first- and second-line antibiotics.

Our study has a number of limitations. There was a lack of consistency among clinicians in their approach to blood culture practice for neonates with suspected sepsis. It was observed that commencement of antibiotics without blood culture being taken was most common during the night or on weekends when fewer staff were available. However, it was noted that those who had blood culture performed had worse outcomes than those with suspected sepsis that did not, suggesting that blood culture practices may also have been influenced by clinical markers of severity. In addition, the blood culture technique that was used in our study is likely to provide a lower yield than an automated culture method such as the one that was used in the study in Ho Chi Minh City[15], which reported a higher yield of bacterial isolates from blood culture of 18% as compared with 10% in the current study. Blood culture practice and methodology may also influence yield by biasing against the isolation of more fastidious bacteria such as group B *Streptococcus*. While a major focus of prevention and management of neonatal sepsis in resource-rich settings, Group B *Streptococcus* is often not found to cause neonatal sepsis in resource-limited settings, as was the case in our study.[3–5,16] Group B *Streptococcus* was also not found in the recent study from Ho Chi Minh City, but that study mainly reported causes of LOS.[15] Group B *Streptococcus* has been reported from resource-limited settings as a cause of EOS, including in studies from the Asian region.[12,17]

Nosocomial sepsis was commonly found in the neonatal unit in Da Nang. As would be expected, nosocomial sepsis was associated with very low birth weight, invasive procedures, prolonged hospital stay and a high mortality. The causes were fungi or Gram-negative bacteria with a high prevalence of *in vitro* resistance to multiple antibiotics. These findings are consistent with many previous studies including that in the Asian region.[6–8,12,15,18,19] Lubell *et al.*[20] reviewed susceptibility of community-acquired pathogens in neonates in low- and middle-income countries in Asia and Sub-Saharan Africa from 2002 to 2009, and identified that ~70% of Gram-negative bacteria were resistant *in vitro* to ampicillin, 55% were resistant to gentamicin and 30% were resistant to ceftriaxone. Fungal sepsis is also a major nosocomial infection in low birth weight neonates in many studies.[21–24] The frequent usage of a large number of antibiotics such as reported in our study is recognized as a risk factor for fungal infections.[25,26]

The high burden of LOS, mainly nosocomial, emphasizes the need to improve infection control in the unit. Since the current study, a number of measures have been introduced that aim to reduce the risk of nosocomial sepsis in the neonatal unit in Da Nang. There has been training and implementation of practices that improve infection control and reinforce hygienic practices, such as hand washing, aseptic techniques for invasive procedures, improved skin care and the introduction of kangaroo mother care for preterm newborns.[27] Given the findings on antibiotic usage in our study, there has also been efforts to improve adherence to antibiotic guidelines and reduce the overuse of broad-spectrum antibiotics in the unit. A recent study from Indonesia demonstrated marked reductions in nosocomial infections and inappropriate antibiotic usage in a pediatric setting by introducing infection control procedures and antibiotic stewardship.[28] An audit of recent data (November 2013 to March 2015) from the neonatal unit in Da Nang shows a marked fall in antibiotic usage from 64 to 16% of admissions. Further, the overall case fatality rate in the unit over the same period has been $<4\%$ overall compared with 8.6% at the time of this study[13] (unpublished data).

In summary, neonatal sepsis was common and associated with a high inpatient mortality in the largest neonatal unit in central Vietnam. The finding that nosocomial sepsis due to fungi or Gram-negative bacteria is resistant to multiple antibiotics highlights the need for the implementation of improved infection control measures in the unit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

HTT and SMG conceived the study. HTT was the principal investigator, led the protocol development and study design, supervised data collection, performed data entry and analysis. NMD was the research nurse coordinator, overseeing the day-to-day running of the project and supervision of data collection. SMG, LWD and KJL provided supervision and input into study design, data collection and analysis. HTT and SMG wrote the first draft of the manuscript, and all authors contributed and approved the final draft.

REFERENCES

1 Lawn JE, Wilczynska-Ketende K, Cousens SN. Estimating the causes of 4 million neonatal deaths in the year 2000. *Int J Epidemiol* 2006; **35**: 706–718.
2 Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE *et al.* Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; **379**: 2151–2161.
3 Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis. *Arch Dis Child* 2013; **98**: 146–154.
4 The WHO Young Infants Study Group. Bacterial etiology of serious infections in young infants in developing countries: results of a multicenter study. *Pediatr Infect Dis J* 1999; **18**: S17–S22.
5 Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. *Pediatr Infect Dis J* 2009; **28**: S10–S18.
6 Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. *Lancet* 2005; **365**: 1175–1188.
7 Thaver D, Ali SA, Zaidi AKM. Antimicrobial resistance among neonatal pathogens in developing countries. *Pediatr Infect Dis J* 2009; **28**: S19–S21.
8 Litzow JM, Gill CJ, Mantaring JB, Fox MP, MacLeod WB, Mendoza M *et al.* High frequency of multidrug-resistant gram-negative rods in 2 neonatal intensive care units in the Philippines. *Infect Control Hosp Epidemiol* 2009; **30**: 543–549.
9 Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: a review of evidence from community-based studies. *Pediatr Infect Dis J* 2009; **28**: S3–S9.
10 Seale AC, Mwaniki M, Newton CR, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. *Lancet Infect Dis* 2009; **9**: 428–438.
11 Tran HT, Doyle LW, Lee KJ, Graham SM. A systematic review of the burden of neonatal mortality and morbidity in the ASEAN region. *WHO South-East Asia J Public Health* 2012; **1**: 239–248.
12 Al-Taiar A, Hammoud MS, Cuiqing L, Lee JK, Lui KM, Nakwan N *et al.* Neonatal infections in China, Malaysia, Hong Kong and Thailand. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F249–F255.
13 Tran HT, Doyle LW, Lee KJ, Dang NM, Graham SM. Morbidity and mortality in hospitalised neonates in central Vietnam. *Acta Paediatr* 2015; **104**: e200–e205.
14 Kruse AY, Phuong CN, Ho BT, Stensballe LG, Pedersen FK, Greisen G. Identification of important and potentially avoidable risk factors in a prospective audit study of

neonatal deaths in a paediatric hospital in Vietnam. *Acta Paediatr* 2014; **103**: 139–144.
15 Kruse AY, Thieu Chuong do H, Phuong CN, Duc T, Graff Stensballe L, Prag J *et al*. Neonatal bloodstream infections in a pediatric hospital in Vietnam: a cohort study. *J Trop Pediatr* 2013; **59**: 483–488.
16 Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: an international perspective. *Arch Dis Child Fetal Neonatal Ed* 2005; **90**: F220–F224.
17 Gray KJ, Bennett SL, French N, Phiri AJ, Graham SM. Incidence and serotype distribution of invasive Group B streptococcal infection in infants in Blantyre, Malawi. *Emerg Infect Dis* 2007; **13**: 223–229.
18 Wu JH, Chen CY, Tsao PN, Hsieh WS, Chou HC. Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. *Pediatr Neonatol* 2009; **50**: 88–95.
19 Huang Y, Zhuang S, Du M. Risk factors of nosocomial infection with extended-spectrum beta-lactamase-producing bacteria in a neonatal intensive care unit in China. *Infection* 2007; **35**: 339–345.
20 Lubell Y, Ashley EA, Turner C, Turner P, White NJ. Susceptibility of community-acquired pathogens to antibiotics in Africa and Asia in neonates - an alarmingly short review. *Trop Med Int Health* 2011; **16**: 145–151.
21 Huang YC, Li CC, Lin TY, Lien RI, Chou YH, Wu JL *et al*. Association of fungal colonization and invasive disease in very low birth weight infants. *Pediatr Infect Dis J* 1998; **17**: 819–822.
22 Ariff S, Saleem AF, Soofi SB, Sajjad R. Clinical spectrum and outcomes of neonatal candidiasis in a tertiary care hospital in Karachi, Pakistan. *J Infect Dev Ctries* 2011; **5**: 216–223.
23 Rueda K, Moreno MT, Espinosa M, Saez-Llorens X. Impact of routine fluconazole prophylaxis for premature infants with birth weights of less than 1250 grams in a developing country. *Pediatr Infect Dis J* 2010; **29**: 1050–1052.
24 Xia B, Tang J, Xiong Y, Li XH, Mu DZ. Peripherally inserted central catheters and the incidence of candidal sepsis in VLBW and ELBW infants: is sepsis increased? *World J Pediatr* 2010; **6**: 154–157.
25 Avila-Aguero ML, Canas-Coto A, Ulloa-Gutierrez R, Caro MA, Alfaro B, Paris MM. Risk factors for Candida infections in a neonatal intensive care unit in Costa Rica. *Int J Infect Dis* 2005; **9**: 90–95.
26 Benjamin DK Jr., Stoll BJ, Gantz MG, Walsh MC, Sánchez PJ, Das A *et al*. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. *Pediatrics* 2010; **126**: e865–e873.
27 Borghesi A, Stronati M. Strategies for the prevention of hospital-acquired infections in the neonatal intensive care unit. *J Hosp Infect* 2008; **68**: 293–300.
28 Murni IK, Duke T, Kinney S, Daley AJ, Soenarto Y. Reducing hospital-acquired infections and improving the rational use of antibiotics in a developing country: an effectiveness study. *Arch Dis Child* 2014; **100**: 454–459.